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12th Congress of the European Hematology Association
Vienna, Austria, June 7 - 10, 2007

ABSTRACT BOOK

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12TH CONGRESS
JUNE 7 - 10, 2007
VIENNA

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EUROPEAN HEMATOLOGY
ASSOCIATION

VIENNA, AUSTRIA,
JUNE 7-10, 2007

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12TH CONGRESS
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Words of welcome

On behalf of the Scientific Program Committee, we would like to welcome you to Vienna and to the 12th Congress of the European Hematology Association. We have put together an exciting programme with, we hope, an interesting balance of clinical and scientific topics ranging across the haematology disciplines. We invite you to participate in as many of the high quality Education Hematology-in-Focus Science-in-Progress and Plenary sessions as you can and to join in the challenging Lunch Debates and Clinical Trial Updates. We also have a number of highly expert 'Meet-the-Expert' sessions and encourage you to register early to grasp the opportunity to exchange information in a very informal and friendly atmosphere. As a new feature this year we have organized a Molecular Hematopoiesis Workshop on Friday June 8th. This will present cutting edge science in a new and exciting way and we hope to particularly attract scientists and young haematologists interested in a scientific career- please come along !

From the record number of abstracts we have also put together an interesting programme of simultaneous oral sessions and poster sessions. The 6 best abstracts will be presented during the Presidential Symposium. This year's joint EHA-ASH Symposium will take place on Saturday, June 9th and will address the very challenging and controversial topic of stem cell banking which is increasingly an issue in the lives of many haematologists- experts from the US (David Scadden) and Europe (Wim Fibbe) will provide stimulating and informative insight into practice on both sides of the Atlantic. A number of meetings of EHA Scientific Working Groups will be held on Thursday evening, which we hope will be of interest to many of you.

In addition, 26 Satellite Symposia will run on Super Thursday, covering the State-of-the-Art in experimental and clinical haematology. The Joint Symposium of the European School of Hematology (ESH) and EHA will again take place on Friday.

We would also like to welcome you to the Opening Ceremony which will take place on Friday, June 8th, directly followed by the presentation of the José Carreras Lecture by Professor Rogier Bertina. In the same session, the winners of the EHA-José Carreras Young Investigators Fellowship and the additional EHA Fellowships will be presented.

The congress program is accredited for continuing medical education (CME) by the EHA-CME System. The scientific program of the 12th Congress of the EHA has also been reviewed and approved for accreditation by the American Medical Association (AMA).

From a social point of view, we have the privilege to be here in Vienna with its rich historical and cultural attractions. On behalf of the EHA Board and the Scientific Program Committee of the 12th Congress: we are pleased to welcome you to this beautiful city and hope that this top hematology congress in Europe will provide you with fruitful and enjoyable interactions with your peers and induce new creative ideas for your work!

Irene Roberts
Chair Scientific Program Committee

Heinz Ludwig
Congress President

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POSTER SESSION I

Acute lymphoblastic leukemia - Biology

0001

IDENTIFICATION OF PML AS NOVEL PAX5 FUSION PARTNER GENE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. PAX5 encodes the B-cell lineage specific activator protein (BSAP) and is required for B-cell development and lineage maintenance. Pax5 fulfills a dual role by activating B-cell specific genes and simultaneously repressing lineage-inappropriate genes. In acute lymphoblastic leukemia (ALL) PAX5 is fused to ETV6 with a dic(9;12)(p13;p13) and is juxtaposed to the IGH locus in B-cell non-Hodgkin's-Lymphoma with t(9;14)(p13;q32). Recently, it has been shown that PAX5 can also fuse to FOXP1 (3p13), ZNF521 (18q11), and to ELN (7q11). *Aims and Methods.* In order to determine the overall incidence of PAX5 rearrangements in childhood ALL dual-color split-apart FISH assays using BAC clones flanking the PAX5 gene as well as PAX5 exon-specific cosmid probes were used. Novel fusion partner genes were identified by narrowing down the breakpoints on the respective partner chromosomes utilizing a panel of locus-specific FISH probes, and the presence of specific fusion genes was verified by RT-PCR. *Results.* In a retrospective study, 332 patients enrolled in the Austrian ALL-BFM 2000 study were screened for PAX5 rearrangements. Three patients showed a separation of the PAX5 FISH probes and 3 showed PAX5-3' deletions indicating PAX5 rearrangements. One of these cases has been previously identified as dic(9;12)(p13;q13)/PAX5-ETV6 positive, a fusion associated with a PAX5-3' deletion. In one patient cytogenetics revealed a 46,XX,add(9)(p13) and a separation of the PAX5 probes was observed. Subsequent 24-color FISH revealed a t(9;15), and the breakpoint was narrowed down to 15q24 by chromosome walking with appropriate FISH probes. Hybridization with a PML-specific probe resulted in a split signal providing compelling evidence that PAX5 was fused to PML. In a second case of childhood ALL (not included in the ALL-BFM 2000 series) a similar aberration, namely a t(9;15)(p21;q25) was detected. In both patients fusion gene-specific RT-PCR detected a chimeric PAX5-PML transcript and sequence analyses identified the same in-frame fusions between PAX5 exon 6 and PML exon 2. PAX5 consists of an N-terminal paired DNA-binding domain and a C-terminal proline-serine-threonine-rich region that harbors a transactivation domain. In the central region an octapeptide capable of recruiting corepressors and a partial homeodomain functioning as a protein-protein interaction motif are presented. PML is fused to RARA in the t(15;17)(q22;q12) in acute promyelocytic leukemia and is implicated in various cellular mechanisms. The PML protein consists of a *really interesting new gene* (RING) domain, followed by two zinc fingers (B Boxes), and a α-helical coiled-coil motif mediating protein-protein interaction. The putative chimeric PAX5-PML protein fuses the paired domain, the octapeptide and the partial homeodomain of PAX5 to almost the entire PML protein lacking only the 5' proline-rich region. *Conclusions.* Out of the 332 patients, three (0,9%) showed a PAX5 rearrangement including one with a PAX5-PML fusion, and two with yet unknown PAX5 partners. In addition, three (0,9%) displayed a PAX5-3' deletion, indicating fusion to a partner gene, among these one PAX5-ETV6 positive case. Although the incidence of PAX5 rearrangements seems rather low, the emerging variety of PAX5 fusion partners nevertheless underlines its important role in leukemogenesis.

0002

ANALYSIS OF T(9;22) BREAKPOINTS INDICATES THAT P210 AND P190 BCR-ABL ARE FORMED BY DISTINCT MECHANISMS

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Chromosomal translocations that produce oncogenic fusion genes are common in haematological malignancies but the mechanism by which they are formed is incompletely understood. Analysis to date has indicated that translocations result from non-homologous end joining following two double stranded DNA breaks. In lymphoid disorders, DNA breaks may be a consequence of normal or aberrant RAG activity, whereas in myeloid disorders the reasons for breakage are unknown. The paradigm for fusions genes in leukaemia is BCR-ABL, produced as a consequence of the t(9;22). In chronic myeloid leukaemia (CML), breaks within BCR are located in the 5.8kb major breakpoint cluster region and a region of at least 200kb in ABL, resulting in a p210 BCR-ABL protein. In Philadelphia chromosome positive acute lymphoblastic leukaemia (Ph^+ ALL), however, the breaks within BCR are frequently located further upstream in the 70kb minor breakpoint cluster region, resulting in a smaller p190 protein. To determine if the mechanism giving rise to the Ph chromosome is different in CML vs ALL, or p210 vs p190, we have developed a FISH and long range PCR strategy to amplify and sequence t(9;22) genomic breakpoints. Thus far, forward breakpoints have been characterized for 19 cases with p190 ALL, 25 with p210 ALL and 32 with p210 CML with reciprocal breakpoints identified in a subset of these cases. Preliminary analysis has revealed some differences between the p190 and p210 breakpoints: perfectly balanced translocations occur in 26% of p190s but only 2% of p210s (p=0.001). Small insertions and/or deletions at the breakpoint occur in 63% of p190s compared to 35% of p210s (p=0.03) whereas large insertions and/or deletions occur in 11% of p190s vs 63% of p210s (p<0.001). This data supports the hypothesis that p190 and p210 are formed by distinct mechanisms.

0003

WNT16 AND BETA-CATENIN HAVE INDEPENDENT AND DISTINCT ROLES IN E2A-PBX1 POSITIVE PRE B ALL CELLS

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Background. The t(1;19) chromosomal translocation is observed in approximately 5-6% of childhood early B-lineage acute lymphoblastic leukaemia (ALL). It results in the expression of the fusion protein E2A-PbX1, which has been shown to activate expression of Wnt16. Inhibition of Wnt16 may induce apoptosis in ALL-cells by blocking canonical Wnt signalling (Mazieres J *et al.,* 2005). Apart from this, little is known about Wnt16 and Wnt signalling pathways in ALL cells. *Aim.* We want to elucidate the roles of Wnt signalling pathways and β-catenin in pre-B ALL cells with activated Wnt16 expression. A particular focus is on interaction between leukaemia cells and the bone marrow stromal cells. *Methods.* PCR, proliferation studies, Western blotting, luciferase reporter assay, confocal microscopy, flow cytometry, siRNA transfection and adhesion assays. *Results.* We demonstrate that the cell lines 697 and RCH-ACV, both harbouring the t(1;19) translocation, express high levels of Wnt16B as well as β-catenin. We further found that high doses of the soluble Wnt inhibitor sFRP1 gave only a small reduction of proliferation, but no induction of apoptosis in these cells. However, we were not able to replicate this finding by treatment with the Wnt inhibitors Dkk1, Dkk4 or a specific Wnt16 antibody previously shown to induce apoptosis (Mazieres J *et al.,* 2005). Therefore, we suggest that Wnt16 does not have an important role in proliferation or survival of pre-B ALL cells. To study a possible link between canonical Wnt signalling and the high levels of Wnt16 and β-catenin, we measured the reporter gene (TOPflash) activity. Compared to other leukaemia cell lines and to induction with Wnt3A, the TOPflash acitivity in the Wnt16 expressing cell lines was

low. This was further confirmed by immonofluorescence demonstrating that β-catenin was localized in the cell membrane and not in the nucleus. In the cell membrane, β-catenin co-localized with N-cadherin. As we have shown that stromal cells protect several pre-B ALL cell lines from apoptosis during cytostatic treatment, we are currently investigating whether β-catenin in the cell membrane participates in the interaction between leukaemia cells and stroma cells. Using sFRP1 we are able to demonstrate a small reduction of β-catenin in Wnt16 expressing cells as well as a reduced protective effect of stromal cells during cytostatic treatment. The role of β-catenin, however, may be independent of Wnt16 and canonical Wnt signalling. These issues are currently being studied using siRNA and functional assays. *Conclusions.* Pre-B ALL cells harbouring the t(1;19) translocation express high levels of Wnt16 and β-catenin, but do not have constitutively active canonical Wnt signalling. The proposed role of Wnt16 in cell survival and proliferation of pre-B ALL cells was not confirmed. β-catenin is localized in the cell membrane, not in the nucleus of these cells, where it co localizes with N-cadherin and is likely to participate in cell-cell interactions. We are currently investigating whether the β-catenin level is independent of Wnt16 and other possible signalling pathways initiated by Wnt16.

0004

IGH, TCR GAMMA AND TCR DELTA GENE USAGE, FRAME CODING AND MUTATIONAL STATUS ANALYSIS IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Clonal antigen (Ag) receptor gene rearrangements are detected in ~75% of adult ALL patients and represent good targets for minimal residual disease monitoring. Association of specific Ag receptor gene rearrangement with outcome has been suggested by some investigators, at least in childhood ALL. *Aims*. The aims of this study were to investigate: 1) IgH, TCRg and TCRd gene usage in B and T lineage adult ALL; 2) the occurrence of in-frame (IF) and out-of-frame (OF) sequences and rate of mutation; 3) the correlation of these parameters with overall clinical outcome. *Patients and methods.* DNA from 176 *de novo* adult ALL patients (65F/111M) were obtained from diagnostic PB or BM samples. 312 clones were identified using primers for IgH, TCRg and TCRd loci in precursor B-ALL (242 clones from 133 patients) and T-ALL patients (37 clones from 24 patients). Median WBC was 18.1×10^9/L (range $0.25\text{-}653\times10^9$/L). Sixty three (70%) patients are, to date, in complete clinical remission (median follow up: 13.2 months; range: 0.49-158.3 months), 24 (26.7%) patients have relapsed (median time: 10.97 mo; range: 2.46-36.9 mo) and 3 (3.3%) patients showed resistant disease. Patients carrying the t(9;22) (no:39) and t(4;11) (no:14) translocations and those who received an allogeneic stem cell transplantation (no:59) were excluded when calculating relapse free survival to avoid bias towards bad and good performance, respectively. *Results*. 125 IgH clones from 97 patients, 117 TCRg clones from 92 patients and 70 TCRd clones from 59 patients were identified. In precursor B-ALL, usage of VH3, Vd2 and Vg2 followed by Vg9 and VH1 predominated. In T-ALL Vg10, Vg4 and Vd1 were predominantly used. In precursor B-ALL Vg4, Vg8 and Dd2 were associated with good prognosis while VH5 was associated with poor prognosis. In T-ALL Vg1, Vg2, Vg4, Vg5, Vg11, Vd2 and Dd2 were associated with good prognosis. When Ag receptor gene usage was analysed in the t(9;22) and t(4;11) ALL subgroups compared to non-translocation group, patients with t(9;22) used VH and TCRg genes across the length of respective loci while t(4;11) patients used downstream J proximal V genes (VH6, Vg10 and Vg11 genes) more frequently; indicative of prominent lineage immaturity of the latter. Majority of IgH (60%) and TCRg (64.9%) rearrangements were OF while 75% of Vd1 rearrangements were IF, with no association with clinical outcome in either types. DNA V-gene mutations ≥2% were observed in 15-20% of IgH rearrangements, 2-4% of TCRg and 0% of TCRd rearrangements without any impact on outcome compared to un-mutated germline rearrangements. *Conclusions.* In both adult precursor B-ALL and T-ALL specific IgH and TCR genes correlated with outcome. Gene usage pattern differs in t(9;22) and t(4;11) patients in line with cell origin of the two subgroups. Unlike observations made in other leukemias such as CLL, V gene mutations ≥2% does not appear to affect clinical outcome in our data set (p=ns).

0005

CELLULAR CHOLESTEROL MODULATES VEGFR-1 FUNCTION ON ACUTE LEUKEMIA CELLS

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Vascular endothelial growth factor (VEGF) and its receptors play a crucial role in malignancy and in disease, regulating the survival, proliferation, and migration of several cell types, such as endothelium and also leukemia cells. Following our recent report on the role of VEGFR-1 (FLT-1) in ALL (Fragoso R *et al.,* 2006), in the present study we analyzed the molecular mechanisms whereby it modulates acute leukemia cell migration in response to VEGF/Placental Growth Factor (PLGF). First, we observed the formation of cell protrusions on ALL cells after VEGF/PLGF stimulation, with evidence for polymerized actin and FLT-1 co-localization (as determined by phalloidin, immunofluorescence staining, and confocal microscopy). Next, we investigated the mechanisms whereby FLT-1 and actin co-localize at the cell *leading edge* (protrusions), after VEGF/PLGF stimulation, and the relevance of such co-localization for cell migration. We addressed this question by impairing the formation of lipid rafts/caveolae using drugs that either sequester (nystatin) or deplete (methyl-β-ciclodextrin, MβCD) total cholesterol. Accordingly, co-treatment of leukemia cells with nystatin or MβCD and PLGF/VEGF blocked cell migration, an effect that was associated with a decrease in FLT-1 polarization and co-localization with actin filaments. Instead, FLT-1 was now found mostly in the cell cytosol. Given that leukemia cells have an increased rate of cholesterol up-take we sought to understand if increased cholesterol levels affected FLT-1 function in leukemia cells. Cholesterol enrichment enhanced leukemia migration in response to VEGF/PlGF (about 3 folds). This significant increase was associated with an increase in FLT-1 protein expression that resulted from increased protein translation in response to cholesterol enrichment. In order to address the *in vivo* importance of the cholesterol in modulating FLT-1 expression during leukemia onset and progression, we performed experiments using Nod-Scid mice subjected to high fat diet (that results in increased cholesterol levels in the BM and in the spleen) inoculated (or not) with leukemia cells. This metabolic condition worsened the disease, and significantly decreased the survival of leukemic mice. Very interestingly, this increased mortality was not associated to the early onset of extramedullary disease but rather to increased BM engraftment. This increased engraftment of leukemia in high fat diet was diminished by treating the mice with cholesterol lowering drugs (statins) and also with a FLT-1 neutralizing Ab, and was almost impaired by the synergistic use of the two drugs. These results shows clearly the importance of the BM micro-environment in modulating leukemia engraftment and progression and implicate for the first time the cholesterol metabolism in this process, which may be used to establish novel therapeutic approaches.

0006

IDENTIFICATION OF A NEW MECHANISM OF RESISTANCE TO IMATINIB AND DASATINIB IN PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: OVEREXPRESSION OF ABERRANTLY SPLICED ONCOGENIC IKAROS ISOFORMS

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Background. Pre-mRNA splicing is an important determinant of the protein repertoire in human cells but it is also a natural source of cancer-causing errors in gene expression. Spliced isoforms of Ikaros, a critical regulator of haematopoiesis, which lack the DNA-binding domain act as dominant negatives by binding long isoforms through the C-terminal zinc-finger domain, which allows for the homo-or heterodimerization of the proteins. Human leukemia has been shown to be heterogeneous for the pattern of Ikaros isoforms. Forced expression of short isoforms alters the differentiation capacities of haematopoietic progenitors and arrest lineage commitment. Despite imatinib or the second generation tyrosine kinase inhibitors induce a high complete remission rate in ALL patients carrying a BCR-ABL rearrangement, the disease often relapses due the occurrence of resistance. *Aim.* We sought to determine if the expression of aberrantly spliced oncogenic Ikaros isoforms could contribute to the resistance in Ph^+ acute lymphoblastic leukaemia (ALL) patients treated with imatinib and the novel dual Src/Abl inhibitor,

dasatinib. *Methods*. We studied Ikaros gene expression in bone marrow and peripheral blood samples from 29 patients with Ph+ ALL: 5 adult *de novo* patients, 16 patients resistant to imatinib and 8 resistant to dasatinib after imatinib failure. Reverse transcription-polymerase chain reaction (RT-PCR) using primers specific for exon 1 and exon 7 of Ikaros and nucleotide sequencing were performed to identify the specific isoforms. Genomic sequencing of the regions surrounding the predominant splice donor and acceptor sites at the exon-intron splice junctions was performed in search for mutations. BCR-ABL transcript levels were monitored in each patient by real-time quantitative PCR (RQ-PCR). *Results.* We detected expression of the full-length Ik1, Ik2 DNA binding isoforms in cells from healthy volunteers and in 3 (10%) Ph+ ALL patients (2 resistant to dasatinib and 1 to imatinib). In the 26/29 (90%) remaining patients the Ik6 isoform lacking all DNA-binding domains was detected and in 13/26 (50%) it was the predominant isoform, demonstrating its dominant activity. In 5/26 patients we detected also the Ik4 isoforms. Genomic sequencing of splice junction regions demonstrated no mutations. We confirmed the identification of a SNP that affects 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 Ikaros isoforms suggested that trans-acting factors were 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. The majority of patients with Ik6 expression had also point mutations in the ABL kinase domain. *Conclusions.* The Bcr-Abl oncoprotein may induce the expression of aberrantly spliced oncogenic Ikaros isoforms which arrest the leukemic cells at the pre B-cell stage and contribute to the tyrosine kinase inhibitor resistance interfering with proteins in pathways that are normally regulated by the full-length Ikaros protein.

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0007

MLL TRANSLOCATION IN A MULTIPOTENT PROGENITOR CAUSING ACUTE LYMPHOBLASTIC LEUKAEMIA - TWO-STEP MODEL OF THE DISEASE

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Background. Leukaemias with MLL gene rearrangement are usually considered prognostically unfavourable and the clinical symptoms typically follow the translocation formation rapidly. MLL rearrangement is thus thought to be a major hit in leukaemogenesis that is either sufficient to cause the disease or it is a very strong and rapid inducer of the subsequent hit(s) required for the malignant transformation. *Methods and Results*. We report an unusual presentation of secondary acute lymphoblastic leukaemia (sALL) with MLL rearrangement. Our patient was diagnosed originally with acute myeloid leukaemia (AML-M3) characterised by PML/RARα fusion and an internal tandem duplication of FLT3 (FLT3/ITD). After 30 months of complete remission of AML, she developed sALL with MLL/FOXO3A fusion gene. Bone marrow (BM) samples taken during AML therapy were analysed for the presence of these aberrations. Both the PML/RARα fusion and FLT3/ITD disappeared shortly after AML onset and did not reappear. However, FISH and quantitative RT-PCR showed the presence of the MLL/FOXO3A fusion 20 months before the diagnosis of sALL, present in 10-90% of BM cells. Morphological examination showed no blast infiltration of the BM at this time. Experiments combining FISH and morphology confirmed the presence of an MLL rearrangement in myeloid as well as lymphoid cells, indicating that the fusion arose in a multipotent progenitor. In order to identify potential secondary genetic events precipitating sALL in this patient, we used Affymetrix 50K single nucleotide polymorphism (SNP) array analysis on DNA from the diagnostic sALL sample versus the *preleukaemic* (remission AML) sample taken 16 months before. This analysis revealed a 10 Mb amplification on 19q13.32 in the sALL sample, not present in the preleukaemic sample: this was confirmed by FISH with a BAC from the amplified region. A difference between the preleukaemic and leukaemic cells is also demonstrated by the incomplete rearrangement of IgH gene (DH1/JH) present only at the diagnosis of sALL. There are about 450 genes in the amplified region on 19q and several of them might be involved in deregulation of the preleukaemic cell if overrepresented (e.g. FLT3 ligand, interleukin 11, Ras interacting protein 1, Stem cell growth factor, Aurora C). *Summary and conclusions.* The long latency period prior to the onset of the secondary leukaemia in our case resembles the mouse model of MLL/FOXO3A. However, in contrast to the animal model and also to the previous reports of MLL/FOXO3A patients (2 cases described so far, both secondary AMLs after Hodgkin's disease), our child developed leukaemia from the lymphoid lineage. Taken together, these results indicate that the MLL/FOXO3A fusion alone is not sufficient to cause leukaemia and that second hit is required to the onset of the disease. A responsible gene is possibly located on the telomeric part of the 19q.

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0008

V(D)J-MEDIATED TRANSLOCATIONS MORE EXCEPTION THAN THE RULE?

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Background. Translocations of proto-oncogenes to the B-cell or T-cell antigen receptor loci (BCR and TCR) usually are accompanied by an overexpression of the proto-oncogene involved which leads to the development of lymphoid neoplasms. The mechanisms held accountable for these translocations are either V(D)J- or class switch recombination, depending on the receptor region the oncogene is translocated to. Arguments for one or the other mechanism range from a simple statement with no additional comments to very detailed discussions why the authors think that a particular mechanism is responsible for the translocation or not. In most cases, however, arguments are rather indiscriminating and do not take into account mechanistic definitions characteristic for the recombination mechanism. *Aims.* To assess the potential of cryptic sites in the proto-oncogenes LMO2/RBTN2, LCK/p56, HOX11/TLX1, and E2A/TCF3 to undergo illegitimate V(D)J recombination. LMO2, LCK, and HOX11 translocations to the TCR occur in 7, 4 and 1% of T-ALL, respectively. The E2A-PBX or E2A-HFL fusions are found in 25% of pre-B cell leukemias and in 5% of childhood and adult ALL. While not rearranged to one of the antigen receptor loci, cryptic sites have been found close to the breakpoints at E2A. *Methods.* An *ex vivo* recombination substrate assay was used to assess potential and efficiency of the cryptic sites to undergo V(D)J-recombination with a genuine TCR element. *Results.* LMO2 cryptic site, a site 1,3 kb downstream of a cryptic site that had been proven to function as a 12-RSS, appeared to be a 23-RSS target for V(D)J-recombination. Interestingly, this region contained additional cryptic sites which also engaged in V(D)J-recombination. While not as attractive a target as the 12-RSS site, these sites showed high recombinogenic efficiency in our assay. For LCK, two sites have been reported to be involved in translocations to the TCRβ locus. LCK site #1 functioned as a 12-RSS with high efficiency. Site #2, situated 30 kb upstream of site #1, only showed unspecific breaks scattered over the region used in our assay, indicating that it was not a direct target for V(D)J-recombination. The breakpoints of more than 6 patients were found close to a putative 23-RSS cyptic site 300 bp upstream of HOX11 exon 1. Recombination in our assay, however, resulted in unspecific breaks scattered over the region tested indicating that, as for LCK site #2, it was not a direct target for V(D)J-recombination. Finally, E2A contains two putative heptamers close to the breakpoint region found in translocations to PBX and HLF. These heptamers did not function as direct targets and recombination was unspecific in our experiments. *Conclusions.* While being potentially dangerous due to the initiation of DNA breaks, V(D)J-recombination is a tightly controlled mechanism that keeps the percentage of directly V(D)J-mediated chromosomal aberrations as low as possible. Other factors are involved causing doublestrand breaks which result in DNA ends that subsequently are incorrectly ligated to one of the immune receptor loci thus leading to overexpression of a proto-oncogene and the development of leukemia.

0009

REFINEMENT OF THE REGIONS OF MINIMAL DELETION AT 6Q IN CHILDHOOD T-ALL BY BAC TILING PATH ARRAY CGH

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Background. T-cell acute lymphoblastic leukemia (T-ALL) is a high-risk malignancy of thymocytes, and accounts for 10-15% of pediatric ALL cases. In T-ALL genetic analyses have elucidated an enormous heterogeneity in genetic abnormalities including chromosomal translocations, deletions, amplifications, and mutations. In particular, quantitative changes seem to play a crucial role in the pathogenesis and progression of the disease. Among these, deletions of the long arm of chromosome 6 have been found in 10-15% pediatric T-ALL. However, owing to the heterogeneity in the extent and the location of the 6q deletions, only one potential tumor suppressor gene, GRIK2, contributing to the pathogenesis of T-ALL has yet been identified in this region. *Aim and Methods.* This study was aimed at the refinement of the regions of minimal deletion (RMD) at 6q in childhood T-ALL by means of BAC tiling path array comparative genomic hybridization (CGH) with the final goal to identify novel genes possibly implicated in the development or progression of T-ALL. *Results.* Genomic DNAs extracted from 70 diagnostic bone marrow samples of childhood T-ALL patients were subjected to BAC tiling path array CGH. In 7 (10%) of the cases a del(6q) was detected, which is in accordance with formerly published incidences. Comprehensive data analysis allowed to determine two different potential RMDs on chromosome 6q14.1-15 and 6q16.2 corroborating the notion that not all cases with a del(6q) share a common RMD. The RMD at 6q14.1-15, which encompassed approximately 12 Mb was deleted in 6 of the cases, and apart from several other genes contained the previously described putative tumor suppressor gene GRIK2. The second, much smaller potential RMD at 6q16.2, spanned roughly 270 kb, and was also consistent with a common deleted region in 6 of the cases. This MRD contained only two genes, namely POU3F2 and FBXL4. POU3F2 (homeobox/POU domain protein 2) is a transcription factor, which is mainly expressed in fetal brain and melanocytes, and its expression is known to be altered in melanoma. The second gene, FBXL4, encodes a protein, which is a member of the F-box protein family and is probably involved in the phosphorylation-dependent ubiquitination of proteins, has so far not been shown to be implicated in any type of cancer. *Conclusions.* Analysis of hematopoietic diseases by BAC tiling path array CGH is an important tool to refine minimal regions of deletions and to delineate genes, which potentially play a critical role in leukemogenesis. Both genes, in particular FBXL4, located in the smallest region of RMD at 6q in T-ALL defined so far, may be crucial for tumor development or progression and warrant further investigations to elucidate their possible implication in T-cell disease.

0010

IDENTIFICATION OF NOVEL RECURRENT GENOMIC ABERRATIONS BY ARRAY-CGH IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In acute lymphoblastic leukaemia (ALL), genomic aberrations like translocations t(4;11) or t(9;22) are associated with an adverse prognostic impact and play an important role in the development of risk adapted treatment strategies. So far, chromosomal banding analysis is the standard cytogenetic procedure for routine diagnosis. Genomic array-comparative genomic hybridization (aCGH) has become a useful technique for genomic screening allowing a more precise delineation of small genomic aberrations. *Methods.* We analyzed 54 patients with ALL, which were treated within the GMALL trials, by aCGH analysis using a genomic 2,8K chip. *Results.* Forty-seven patients had a B-precursor-ALL including 14 patients with t(9;22) and 4 patients with t(4;11); 7 patients had a T-ALL. We found genomic aberrations in 37 out of 54 patients (69%). The most frequent aberrations were deletions on chromosome bands 9p21 (12 cases; 22%), 7q35 (9 cases; 17%) and 13q14 (8 cases; 15%); as well as gains on chromosome X, on chromosome arms 1q and 11q 13 (5 cases each, 9%). The consensus region of the 9p21 deletion was narrowed down to a size of 420 kilobasepairs. This region covers 3 different genes with potential tumor suppressor gene (TSG) function: p14ARF, p15INK4B/p10 and p16INK4A. Further consensus regions could be delineated which contains candidate gene of possible biologic importance: gains on chromosome bands 8q24 (C-MYC), 9q34 (ABL-1) and 11q13 (CyclinD1), deletions on chromosome bands 12p13.1 (CDKN1B) and 16q22 (TRADD). In the group of B-precursor-ALL, there was a tendency to a higher number of aberrations per case in the standard risk group (that means no t(9;22) and t(4;11)) in contrast to the t(9;22) positive group (2.2 vs. 1.2 aberrations per case). Deletions on 9p21 and 12p13 seems to be more frequent in the standard group than in the t(9;22) group (9p21: 10/27 vs. 1/14, p=0.06; 12p13: 5/27 vs. 0/14; p=0.14, Fisher´s exact test). *Conclusions.* aCGH analysis is useful for the identification of recurrent genomic aberrations which are not detectable using standard cytogenetic techniques.

0011

INV(11)(Q21Q23) FUSES MLL TO THE NOTCH CO-ACTIVATOR MASTERMIND-LIKE 2 IN SECONDARY T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Development of a secondary leukemia after chemotherapeutic treatment for childhood acute leukemia is associated with rearrangements of the MLL gene on chromosome 11q23 and characteristically results in acute myeloid leukemia (AML). We identified the NOTCH co-activator Mastermind-like 2 as novel MLL fusion partner in two cases of pediatric therapy-related leukemia. The MLL-MAML2 fusion results from a cryptic inv (11)(q21q23). With a latency of twenty months after initial diagnosis of MLL negative AML and five years after precursor B acute lymphoblastic leukemia, respectively, both patients developed a therapy-related T-cell acute lymphoblastic leukemia. MAML2 is the first MLL fusion partner involved in human NOTCH signaling and was only recently identified as recurrent translocation fusion partner in a subset of salivary gland tumors. The genomic MLL breakpoint shows similar localization and sequence features described for etoposide induced t-AML. The growth dynamic between primary and secondary disease was quantified using the individual genomic fusion sequence and Ig/TCR rearrangements as clonal markers. Whole genome expression profiles demonstrated involvement of target genes downstream of NOTCH, which suggests a modulatory role of the MAML2 transcriptional activation domain in MLL leukemogenesis and lineage assignment induced by the MLL-MAML2 fusion protein.

0012

CHARACTERISTICS OF MESENCHYMAL STROMAL CELLS FROM BONE MARROW OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The effect of several chemotherapeutic agents on mesenchymal stromal cells (MSC) has been tested *in vitro* and it was found that MSC are sensitive to different cytotoxic drugs. In the only existing *in vivo* study assessing MSCs properties from bone marrow (BM) of adult patients under chemotherapy though, such an effect was not observed. *Aim.* To detect a) any effects of the acute lymphoblastic leukemia on the morphology and functional characteristics of MSCs in children, b) alterations in MSC characteristics that could be attributed to chemotherapy and c) the possible bone marrow microenvironment restoration, if damaged, and the time point that this occurs. *Material and Methods.* BM mononuclear cells (MNC) from children with ALL [diagnosis (ALL_d, n=12), 15th day (d15, n=6), 33rd day of therapy (d33, n=9), maintenance (n=26) and end of therapy (ALL_end, n=11)] were cultured under conditions that favor MSC growth, for six passages (1-6). The cell doubling time (DT), the development of CFU-F colonies and surface antigen expression were evaluated in each treatment phase and in P2, P4 and P6. Moreover, apoptosis by 7AAD staining (flow cytometry) was assessed. Selected samples from each group were cultured under the appropriate conditions for adipocyte, osteocyte and chondrocyte differentiation.

Expression of the oncogene H-Ras and the tumor suppressor genes p53, p16 and Rb was assessed in both MSC_P2 and P6 and MNC of diagnosis. *Results.* MNC of diagnosis required more days to reach confluency (23.9±2.09) compared to MNCs from the groups corresponding to different stages of treatment, which required 16.9±0.6 days. DT, which at diagnosis had the highest value, (4.26±0.88 vs 2.34±0.32 d15, 2.95±0.45 d33, 3.52±0.54 remission, 2.94±0.31 ALL_end) increased with the progression of passages. The immunophenotypic analysis showed a homogenous cell population with MSC characteristics appearing as early as P1 (CD73, CD105, CD146>90%) and simultaneous absence of hemopoietic markers. The CD95 expression was found to be high (95.8±2.7% ALL_d, 95.6±3.6% ALL_end) whereas the rate of apoptosis was low (<3%) in serial passages, indicating a cell population able to survive in a long term culture. The CFU-F /105 MNC count was lower at diagnosis (1.4±0.4), compared to the rest phases of therapy 3.5±0.9 d15, 6.4±0.6 remission and 9.3±0.8 ALL_end ($p<0.05$) and remained lower in all passages. In addition, the CFU-F decreased with progression of passages in all groups. At all the studied phases, MSCs had the ability of trilineage differentiation. MSCs at P2 expressed higher levels of p53, Rb and H-Ras and lower levels of p16 in comparison to MNCs in the group used as control, but in ALL samples the expression levels were similar between MNCs and MSCs at P2. No differences were observed in expression levels between passages P2 and P6. *Conclusions.* ALL affects the clonogenic and proliferative potential of MSCs and this could be attributed to BM blast infiltration and/or qualitative differences in the BM microenvironment. Chemotherapy does not seem to have any effect as MSC characteristics are re-established early in the course treatment and remain stable throughout treatment and after the end of it.

0013

IDENTIFICATION OF POTENTIAL THERAPEUTIC TARGETS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA BY PROTEIN KINASE GENE EXPRESSION PROFILE AND *IN VITRO* FUNCTIONAL TESTS

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Background. Rational drug discovery and tailored targeted therapies represent a promising alternative in the treatment of adult ALL. In this context, PK have emerged as one of the most attractive targets since they often display an aberrant activation in cancer. Aims. We sought to assess if PK genes showed an altered expression pattern and might be regarded as potential therapeutic targets in adult ALL. Methods. We studied the gene expression profile of PK in 133 adult ALL samples with newly diagnosed ALL by oligonucleotide arrays (HGU133 Plus 2.0, Affymetrix). Ninety-one leukemia samples were of B-lineage affiliation and included, according to molecular assessments, 4 subgroups: BCR/ABL$^+$ (40 pts), ALL1/AF4$^+$ (5 pts), E2A/PBX$^+$ (3 pts) and B-lineage ALL without known molecular abnormalities (43 pts), defined as B-NEG. Leukemic cells from the remaining 42 samples were of T-cell origin. The comparison between the 5 ALL subgroups was primarily performed by ANOVA, using a list of 1324 probesets coding for PK; then the genes identified were further selected to focus on distinguishing PK genes. Real-time quantitative PCR (Q-PCR) was used to validate the oligonucleotide array expression values of 13 genes identified by ANOVA. *in vitro* proliferation experiments were performed on cell lines harboring the BCR/ABL, ALL1/AF4 and E2A/PBX transcripts - namely, SUP B15, RS4;11 and ACV - as well as on the Jurkat T-cell line after treatment with increasing doses, from 0.01 μM to 100 μM, of the TK (tyrosine kinase) inhibitors imatinib and dasatinib. *Results.* ANOVA provided a list of 290 probesets differentially expressed among the 5 ALL subsets considered. The stringent selection criteria applied highlighted the PK genes significantly distinctive of each group. T-ALL and E2A/PBX+ ALL showed the highest number of specific PK genes, namely 18. In particular, ZAP-70, LCK, ITK, EPHB6, FGFR1 and RYK distinguished the T-ALL group. The E2A/PBX+ samples were characterized by high expression levels of MERTK, BLK, TNK2 and ROR1. In the ALL1/AF4$^+$ group 15 genes were specifically overexpressed, including FLT3, ILK and LTK. Only 8 genes satisfied the selection criteria in the BCR/ABL$^+$ set; apart from ABL, FYN was selected. A consistent number of PK genes was overexpressed, though not exclusively, in B-NEG samples. Q-PCR analysis confirmed the array results, with a median Pearson correlation coefficient of -0.73. As expected, *in vitro* experiments showed a marked proliferation decrease of the BCR/ABL$^+$ cell line when exposed to TK inhibitors. Furthermore, we observed a significant and dose-dependent reduction in the proliferation of Jurkat and ACV cells following treatment with TK inhibitors, even at the lowest concentrations used. On the other hand, the RS4;11 proved insensitive. *Conclusions.* These data document a distinctive PK signature for different ALL subgroups. T-ALL, E2A/PBX$^+$ and B-NEG cases show a prevalence of TK. Thus, TK inhibitors may be effective not only in BCR/ABL$^+$ patients, but also in the aforementioned ALL subsets, as corroborated by preliminary *in vitro* experiments on leukemic cell lines. Functional studies are currently ongoing on primary cells to establish the role of the available TK inhibitors in the management of adult ALL.

0014

IDENTIFICATION AND CHARACTERISATION OF MLL-AF4-REGULATED CELLULAR PROCESSES

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Background. The translocation t(4;11)(q21;q23) marks an infant acute lymphoblastic leukaemia with a particularly dismal prognosis. We have recently shown that the der11, MLL-AF4, is indispensable for maintaining the leukaemic phenotype in part by interfering with apoptotic programmes. *Aims.* We examined the effects of RNAi-mediated depletion of MLL-AF4 on cell proliferation and gene expression in t(4;11)-positive leukaemia cell lines. *Methods.* t(4;11)-positive cell lines SEM and RS4;11 were electroporated with preformed siRNAs. Changes in gene expression levels were analysed by real time RT-PCR and by immunoblotting. Proliferation and apoptosis was examined by cell counting, MTT assays, cell cycle analysis, CFSE, and annexin V staining. DNA methylation and binding was studied by bisulfite sequencing and chromatin immunoprecipitation. *Results.* In previous experiments we showed that transient suppression of MLL-AF4 by siRNAs impairs clonogenicity, but not proliferation in suspension culture. However, prolonged MLL-AF4 depletion by repetitive siRNA transfections abolishes cell expansion. This inhibition is mainly caused by the induction of apoptosis and not so much by the inhibition of cell proliferation, as CFSE labelling studies indicate ongoing proliferation in spite of prolonged MLL-AF4 suppression. On the molecular level, two classes of putative target genes can be distinguished. The first class consisting mainly of homeotic genes such as HOXA7 is affected within 48 hours after MLL-AF4 siRNA transfection. The expression levels of a second class containing genes such as FGFR1, CDH2, DNMT1, DNMT3A, TERT (all downmodulated), DAAM1 and SEPT4 (ARTS, all upregulated) are only substantially affected by prolonged MLL-AF4 depletion. Inhibition of apoptosis by the general caspase inhibitor zVAD does not prevent changes of SEPT4 and TERT expression, suggesting that caspase-dependent apoptotic processes are not responsible for the observed expression changes. Furthermore, the DNMT inhibitor Aza-dC causes similar changes in TERT and SEPT4 expression as MLL-AF4 siRNAs, which is also reflected by very similar changes in the DNA methylation pattern of the TERT promoter. Current experiments address the role of these MLL-AF4 target genes in the MLL-AF4-dependent regulation of apoptosis. Furthermore, we are examining how MLL-AF4 controls these putative target genes. *Conclusions.* We have identified several MLL-AF4 target genes, which may contribute to the regulation of cell survival by MLL-AF4. The analysis of target gene functions may open up new treatment options for this therapy resistant infant leukaemia.

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0015

EFFECTIVE CELL KILLING OF PRIMARY ACUTE LYMPHOBLASTIC LEUKEMIA CELLS BY THE BCL-2 ANTAGONIST ABT-737

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Background. Studies on the treatment of adult acute lymphoblastic leukemia (ALL) have shown only modest improvements over the last two decades, with the actual cure rate remaining in the range of 30-40%. Novel treatment modalities have been directed toward inappropriately activated cell-signaling pathways associated with the abnormal proliferation and/or apoptosis escape of leukemic blasts. Further studies on cell signaling may allow the identification of novel therapeutic targets for adult ALL. Proteins in the B cell leukemia/lymphoma 2 (Bcl-2) family are important regulators of the commitment to apoptosis at the mito-

chondrion level and are frequently found aberrantly expressed, particularly in lymphoid malignancies. The role of Bcl-2 overexpression in tumorigenesis and chemoresistance prompted us to investigate whether the inhibition of the antiapoptotic function may result also in ALL in an attractive therapeutic strategy. *Aims.* We investigated the cell cycle and apoptotic effects of the ABT-737 (kindly provided by Abbott Laboratories), a Bcl-2 (BH3) inhibitor, on primary adult and childhood LAL primary cells. *Results.* The lymphoid leukemia cell line CEM was exposed to increasing concentration of ABT-737 (from 0.1 to 1 μM) up to 72 hours. A dose- and time-dependent cell growth arrest and induction of apoptosis was found. In fact, measuring the subG0/1 peak at 48h, levels of apoptosis increased from 14.1% in DMSO to 34.4%, 64.5%, 86.5% and 98.6% at ABT-737 concentrations of 0.1, 0.25, 0.5 and 1 μM, respectively. The effects of ABT-737 were then examined on primary blasts from 6 untreated ALL patients (4 adults and 2 pediatrics). Bone marrow aspirates with a blast percentage infiltration (>70%) were obtained from patients broadly characterized for clinical and biological parameters, as well as therapeutic response. ALL cells were cultured *in vitro* with ABT-737 (at increasing concentrations from 0.01 to 1 μM) for 24 hours. A significant decrease in viability was observed at 0.01 μM (p=0.039) with a remarkable dose-dependent increase of apoptosis. In fact, Annexin V-positive cells increased from a mean baseline value of 16.3±9.9% up to 39.1±21.4% (p=0.04), 69.1±25.45% (p=0.005), 70.3±26.9% (p=0.05), 74.6±18.9% (p=0.03) and 80.8±11.8% (p=0.0001) in the presence of ABT-737 at 0.01, 0.1, 0.25, 0.5 and 1μM, respectively. A significant cell killing was demonstrated in 6/6 samples, including Ph-positive leukemias. No significant cell cycle changes were instead detected even at higher concentration of ABT-737. *Summary.* This study shows, for the first time at our knowledge, in primary adult and childhood ALL samples a potent growth-inhibitory and pro-apoptotic activity of the Bcl-2 antagonist ABT-737 at nanomolar concentrations. These results prompt to further extend pre-clinical studies in the different biologically-defined subset of ALL and suggest a potential clinical development of ABT-737 in this disease.

Acute lymphoblastic leukemia - Clinical

00016

E2A/PBX, T(1;19) IS THE PREDOMINANT TRANSLOCATION IN PRECURSOR- B PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN EGYPT WITH NO SPECIAL ASSOCIATION WITH PRE B PHENOTYPE

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Background. Molecular genetics of pediatric ALL is essential for risk stratification. Egyptian data in this field are sporadic. *Aim.* To determine the relative incidence of the 4 most common fusion genes of pediatric precursor B-ALL in a cohort of Egyptian patients and study their possible impact on clinical outcome. *Patients and Methods.* The study comprised 134 children (<18 years) newly diagnosed precursor B ALL patients presented to the Pediatric Oncology Department, NCI, Cairo University in the period 2003-2004. Cases were diagnosed according to standard methods including morphology, cytochemistry and immunophenotyping. Accordingly they were classified as CALL (74 patients, 55.2%), Pre B ALL (47 patients, 35.1%) and Pro B ALL (13 patients, 9.7%). Patients were risk stratified according to age, total leucocyte count, immunophenotyping, and early response to therapy (BM day 14). Patients were tested for the 4 major fusion gene transcripts frequently associated with ALL namely t(1;19) E2A/PBX1, t(12;21)TEL/AML1, t(4;11) AF4/MLL and t(9;22) BCR/ABL p190 and p210. Fusion genes were identified by both conventional and real-time RT-PCR using cell lines as positive controls. *Results.* t(1;19) was encountered in 24 patients (17.9%) while t(12;21) was encountered in 13 patients (9.7%). t(9;22) and t(4;11) were encountered in 2 patients each (1.5%). One case (CALL) showed double translocation t(1;19) and t(12;21). The remaining 93 patients (93/134, 69.4%) were negative for all fusion genes tested. None of the fusion transcripts correlated with any particular immunophenotype. t(12;21) was found in CALL and pre B subtypes while t(1;19) was distributed among the 3 precursor B subtypes including 11 CALL cases, 10 pre-B and 3 pro-B. The 2 cases with t(9;22) were CALL. The 2 cases with t(4;11) included one pro-B and one pre-B. Myeloid marker expression was encountered in 1/13 cases with t(12;21). Nor was there any significant association with DNA ploidy. However the majority of cases (11/13) with t(12;21) showed 2N DNA; the other 2 cases showed DNA index of 1.1 and 1.21. No statistically significant correlation was encountered between t(1;19), t(9;22) or t(4;11) on one side and any of the prognostic parameters on the other side. However, 9/13 (69.2%) cases with t(12;21) were classified as low risk (p 0.019) . The other 4 would have been classified as low risk if the translocation status was known upfront. The disease free survival (DFS) and event-free survival (EFS) at 30 months of all studied patients was 88.5±3.5% and 70.6±5.2%, respectively. DFS and EFS at 30 months of all groups together after excluding t(4;11) and t(9;22) was 100% and 90.9±8.7% for t(12;21), 90.9±8.7% and 69.3±10.7% for t(1 ;19), and 85.8±4.4% and 67.9±6.3% for cases without translocations (p=0.42. *Summary and conclusions.* Our study reports, probably for the first time, the incidence of the 4 specific fusion genes occurring in pediatric precursor B ALL Egyptian population in the same cohort of patients. t(1;19) proved to be the most common among our series followed by t(12;21); another feature in our patients that is different from western countries.

0017

ROLE OF ERYTHROPOIETIN RECEPTOR IN T(12;21) POSITIVE LEUKEMIA

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Background. The chromosomal translocation t(12;21)(p13;q22) resulting in the TEL/AML1 (also known as ETV6/ RUNX1) fusion gene is the most frequent translocation in childhood B cell precursor (BCP) ALL. This particular type of ALL is, like the other genetic subtypes, associated with a specific pattern of differentially expressed genes, among which

is the gene for the erythropoietin receptor (EpoR). So far, it is not known, which effects EpoR overexpression has on the t(12;21) positive leukemia, nor which signaling pathways, potentially different from those used in erythroblasts, are activated. *Aim.* The aim of this study was to evaluate the effects of EpoR signaling on survival and proliferation in t(12;21) positive leukemias and to investigate the apoptosis-modulatory potential of Epo. We further planned to explore relevant signaling pathways, linked to the respective effects and thereby elucidating mechanisms that might be essential for cell survival. *Material and Methods.* As model system for TEL/AML1 positive ALL the only available human BCP ALL cell line REH was used. Controls included the t(12;21) negative leukemic cell lines Nalm6, K562 and the Epo-dependent cell line UT7. Proliferation was measured by 3H-Thymidine incorporation assays. Cell viability and apoptosis rates were evaluated by MTT assay and Annexin V/ propidium iodide staining, respectively. Activated members of signaling pathways were detected by Western blotting using appropriate anti-phospho-antibodies either after immunoprecipitation or from whole cell lysates. *Results.* REH cells exhibited a dose dependent increase in proliferation when cultured with Epo (10, 50, 100U/mL) for 72 hours. Upon addition of blocking anti-EpoR antibodies these effects were negated. Epo-induced proliferation was also abolished when cells were co-cultured with AG490, a JAK2 inhibitor. We could, however, not detect an Epo-induced phosphorylation of proteins involved in the EpoR/JAK2/STAT5 cascade. This observation has been already described in several non-hematopoietic cancers and suggests that other members of the JAK/STAT pathway compensate for this specific lack of activation. REH cells further showed an impaired glucocorticoid (GC)-induced apoptosis in the presence of Epo. We thus evaluated whether signaling pathways implicated in survival of tumor cells exposed to apoptotic stimuli play a role in this rescue mechanism. Preliminary data indicate that the NF-kB as well as the PI3K/Akt pathway is triggered by Epo. Since cell lines may have intrinsic changes which could affect their signaling pathways, we are currently evaluating whether the observed results can also be reproduced in primary leukemic cells. First results suggest that also primary t(12;21) positive ALL may exhibit a superior survival and reduced apoptosis rate to GC in the presence of Epo. *Summary and Conclusions.* Our data indicate that in t(12;21) positive leukemias binding of Epo to its receptor leads to enhanced survival *in vitro* and negatively affects the sensitivity to GCs. andrea.inthal@ccri.at

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0018

FLOW CYTOMETRY ANALYSIS OF THE CO-EXPRESSION OF T- AND B-CELL MARKERS IN BLASTS FROM PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Analysis of genes encoding Ig- and T-cell receptors (TCR) in acute lymphoblastic leukemia (ALL) demonstrated frequent lineage infidelity of blast cells. Rearranged Ig-genes were found in 50-95% cases of T-cell ALL, and rearranged T-cell receptor genes were found in 70-82% cases of B-cell ALL. It remains, however, unclear whether the infidelity of lymphoblasts may be defined at the study of antigenic properties of blasts. We used multiparameter flow cytometry to test the expression of CD3/TCR ε'chain and B-cell receptor α-chain (CD79a) in the cytoplasm of electronically separated (gated) blasts from 51 pediatric and 16 adult patients with ALL. T-ALL was diagnosed in 16 pediatric and 5 adult patients, and B-ALL in 35 pediatric and 11 adult patients. Two arbitrary cutoff thresholds, above the 10% and above the 20% of positive cells within gated lymphoblasts, were used to define the co-expression of T- and B-cell markers in blast cells. Statistical analysis of data was performed using the Fisher exact probability test. At 10% cutoff threshold, the expression of CD79a was determined in 7 of 21 cases (33%) of T-ALL with CD3ε+ blasts. The expression of CD3ε was found in 13 of 46 cases (28%) of B-ALL with CD79a+ blasts. Totally the blasts with co-expression of T- and B-cell markers were found in 30% patients with ALL. Thirteen from 20 patients (65%) with CD3ε+/CD79a+ double-positive blasts had either pre-B or late cortical subtype of ALL. At 20% cutoff threshold for positive cell count, double-positive blasts were identified in 8 patients (12%) with ALL. Two of these patients had pre-B, and 5 had late cortical subtype of ALL, indicating more frequent finding of biphenotypic blasts in patients with these immunologic subtypes of ALL than in patients with other subtypes of ALL (p=0.01). The proportion of cases with biphenotypic blasts was higher in patients with T-ALL than in patients with B-ALL (p<0.01). The study of intracellular expression of VpreB (CD179a) fragments of surrogate light chains in 3 cases of T-ALL with CD79a+ blasts showed a strong expression of CD179a in blast cells. Blasts from one patient with CD3ε+ T-ALL displayed high level of intracellular TCRβ, CD79a, and CD179a markers. The patients with T/B biphenotypic lymphoblasts demonstrated a slightly lower rate of residual disease during induction therapy than the patients without biphenotypic blasts. Obtained data showed that T/B biphenotypic blasts may be found in 12-30% cases of ALL. Biphenotypic appearance of lymphoblasts is apparently associated with relatively mature immunologic subtypes of T- and B-ALL. Analysis of intracellular expression of CD179a and TCRβ seems to be useful for the detection of ALL with T/B biphenotypic lymphoblasts. Clinical significance of ALL with biphenotypic blasts remains unclear and requires further investigation.

0019

EPIGENETIC THERAPY WITH 5-AZACITIDINE IN A TREATMENT SCHEDULE OF FIVE DAYS EVERY 4 WEEKS INDUCES HEMATOLOGIC RESPONSES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA REFRACTORY TO OR NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY

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Epigenetic alterations such as DNA methylation within gene promoter regions and histone modification are prevalent in cancer. Reversal of epigenetically inactivated genes by the nucleoside analogue 5-azacitidine is an effective treatment in MDS. In the current study, the safety and efficacy of azacitidine in patients with AML refractory to or not eligible for chemotherapy were assessed at the University of Leipzig. *Patients and Methods.* Since August, 2005, 25 patients with AML (14m/11f), median age was 69 (range 32-80) years, received a 5 days treatment schedule with 75 mg/m^2 azacitidine sc every 4 weeks mostly in an outpatient setting. Secondary AML was present in 15 (60%) patients. Abnormal cytogenetics were found in 9 (36%) patients [8 with complex aberrations and one with trisomy 8]. Azacitidine was given as first-line therapy in 10 (40%) patients not eligible for chemotherapy. The remaining 15 (60%) were treated because of relapsed or refractory AML including 6 patients who relapsed after allogeneic hematopoietic cell transplantation (HCT). Median pre-treatment marrow blasts, WBC, ANC and platelets were 24% (range 21-88%), 5.7 (range 0.5-82)×10^9/L, 1 (range 0-27.7)×10^9/L and 32 (range 3-1520)×10^9/L respectively. Hematological response was assessed according to the International Working Group Criteria for AML. For non-hematologic toxicity, the NCI CTC and for hematologic toxicity the NCI CTC hematology criteria for leukemia studies or bone marrow infiltrative/myelophthisic processes were used. *Results.* To date, 92 treatment cycles with a median of 3 (range 1-8) cycles/patient were applied. Leukopenia and thrombocytopenia > grade III occurred in 9 (36%) and 12 (48%) patients respectively. ANC and platelet nadirs occurred day 21 (range 5-23) and 14 (range 10-27) respectively. Non-hematologic side effects were constipation grade I, n=7, liver toxicity grade I, n=7, nephrotoxicity grade I, n=2, parasthesia grade I, n=1, fever grade II, n=1. Hospitalisation because of grade IV liver toxicity, arthritis grade III, and pneumonia were required in three patients. After a median follow-up of 15 (range 3-78) weeks, 18 (72%) patients responded [CR, n=7 (39%), PR, n=2 (11%), hematologic improvement, n=4 (22%), stable disease, n=5 (28%)]. Two (22%) complete cytogenetic remissions were achieved. Responses occurred after a median of 6 (range 2-16) weeks after treatment. Median response duration amounts to 36 (range 3-not reached) weeks. Median survival time in responding patients is 57 (range 9-not reached) weeks compared to 16 (range 4-29) weeks in refractory patients (p<0.0001). Two Patients in CR received HCT after RIC [200cGy TBI+ fludarabine 30 mg/m^2 on 3 days] from a MUD. Engraftment with 50% marrow donor T-cell chimerism day 28 after HCT occurred in both patients. Azacitidine given to patients with relapsed AML after HCT was also feasible with no negative impact on donor chimerism. *Conclusions.* Azacitidine applied in a 5 days schedule every 4 weeks is well tolerated and induces remarkable hematologic responses even in patients with refractory AML. HCT after RIC seems feasible after azacitidine therapy. Azacitidine could also be safely given in relapsed patients with AML after HCT. A phase II study is currently underway to confirm these data.

0020

MRD BY MULTIPARAMETER FLOW CYTOMETRY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: PROGNOSTIC IMPACT OF EARLY RESPONSE PARAMETERS ON THE MRD-STATUS AT THE END OF INDUCTION PHASE I

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Background. Multiparametric flow cytometry (MFC) offers the unique possibility of quantitative monitoring of therapy response at early stages of treatment. *Aims.* In the present study we analyzed the predictive impact of early blast reduction kinetics measured by MFC on the minimal residual disease (MRD) status of the BM at day 33, an important time point for later therapy stratification. *Methods.* Standardized MFC-MRD quantification was performed for 197 patients within the AIEOP-BFM ALL MRD study. Relation between BM MRD status at d33 and blast reduction rates at day 8 in pb (BRRd8) and day 15 in bone marrow (BRRd15) was investigated using a a binary logistic regression model. Cut-off values for BRR were chosen according to ROC-curve calculations. Crosstable calculations were used for estimation of odds ratios. *Results.* BRR at d8 and d15 were significantly lower in patients with a positive FCM-MRD status than in patients with a negative FCM - MRD status on day 33. Furthermore, BRRd8 and BBRd15 were significantly contributing variables relating to the MRD status with a specificity of 83.5%. The incidence for a positive MRD status was higher for patients with a BRRd8<96% compared to a BRRd8 > 96% (44.9% vs. 23.4%; odds ratio, 2.67; 95 percent confidence interval [CI], 1.43-4.9; p=0.002) and for patients with BRRd15<99.5% (incidence, 37.7% vs.10.9%; odds ratio, 4.9; 95% CI, 2.4 -10.3; p<0.001). Combination of both blast reduction parameters defines two subgroups of patients with a high and low incidence of a positive MRD- status (incidence, 75% vs. 25%; odds ratio, 8.9; 95% CI, 3.2-24,6 p<0.001). *Summary.* Quantitative MFC assessment of early response parameters, in particular their complementary application, has a predictive impact on the MRD status after induction phase I of the AIEOP-ALL-BFM 2000 protocol.

0021

CYTOREDUCTION RATES ARE ASSOCIATED WITH BCL-2 PROTEIN EXPRESSION IN ALL BLASTS PERSISTING DURING INDUCTION THERAPY

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Background. In childhood acute lymphoblastic leukemia (ALL), leukemic cell reduction rate during therapy is of crucial prognostic significance. Recently, we investigated gene expression changes during the first week of induction glucocorticoid (GC) therapy and identified a common set of genes differentially expressed in blasts at day 8 (d8) and at diagnosis (d0). The hallmark of mRNA expression changes in d8 cells was a downregulation of BCL-2, the antiapoptotic factor critically involved in the regulation of both normal B cell lymphopoiesis and GC-specific apoptosis. Moreover, BCL-2 is frequently overexpressed in ALL cells at diagnosis, but its expression levels do not correlate with rates of cytoreduction. *Aims.* Investigation of expression changes of BCL-2 protein during induction therapy in a large series of ALL patients and evaluation of its impact on leukemic cell reduction. *Methods.* Blood samples (d0; d8) from patients with B cell precursor ALL enrolled in the multicenter ALL-BFM 2000 study were acquired prospectively (n=100). BCL-2 expression in leukemic blasts was assessed using multiparameter flow cytometry. BCL-2 expression in ALL cell lines was downregulated by transfection with BCL-2 specific siRNAs. *Results.* BCL-2 protein expression decreased after 1 week of therapy in the majority of cases (86%, mean fold change 0.6, p<0.001). Comparison of BCL-2 reduction rates with therapy-induced cytoreduction revealed a tendency towards positive correlation (p=0.059), and absolute levels of BCL-2 at d8 (but not at d0) inversely correlated with the cytoreduction (p=0.002). In experimental cell systems (B-lineage ALL cell lines), downregulation of BCL-2 was a common event in GC sensitive lines and a pre-requisite for GC-induced apoptosis. Downregulation of BCL-2 alone was sufficient to induce apoptosis in the absence of GC treatment. Moreover, cytoreduction rates and expression levels of BCL-2 protein inversely correlated in BCL-2 siRNA transfected cells (p=0.05). *Conclusions.* BCL-2 acts as an antiapoptotic threshold in ALL blasts which is targeted by GC treatment. An inability of GCs to downregulate BCL-2 or an insufficient decrease of BCL-2 levels results in a worse therapy response.

0022

DIFFERENTIAL EXPRESSION OF VALOSIN-CONTAINING PROTEIN (VCP, P97) IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS WITH AND WITHOUT GLUCOCORTICOID TREATMENT

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Background. The response to initial glucocorticoid therapy in childhood acute lymphoblastic leukaemia (ALL) reliably predicts the response to multiagent chemotherapy. Patients resistant to glucocorticoids (prednisone poor responders (PPR)) have a poorer event-free survival compared to glucocorticoid-sensitive patients (prednisone good responders (PGR)). In a case control study to investigate differential protein expression in leukaemic blasts, catalase, RING finger protein 22 α, VCP and a G-protein coupled receptor (family C, group 5, member D (GPRC5D)) were overexpressed in PPR. *Aims.* In this study the functional consequences of glucocorticoid treatment of ALL cell lines on the RNA and protein expression of the valosin-containing protein (VCP) was investigated with respect to VCP expression on the RNA and Protein level. Moreover, the study was extended to primary bone marrow samples of patients with childhood ALL. *Methods.* Human B cell precursor leukaemic cell lines MHH cALL 2 (PPR) and MHH cALL 3 (PGR) were studied after induction with glucocorticoids (3 µg/mL Solu-DecortinH®) for 96 hours, according to one doubling time of the cell lines. Incubated cells were sampled every 24 hours for protein isolation, RNA extraction and immunfluorescence. Western blot analysis using an anti-p97 antibody was performed on whole cell lysates, nuclear, membrane and cytosolic fractions separated by differential detergent fractionation with the Qproteome Cell Compartment Kit (Qiagen, #37502). The amount of expressed VCP RNA was analysed by Lightcycler analysis after for each time point. The results were further evaluated using mononuclear cells from bone marrow samples from five PGR and five PPR patients with childhood ALL in a matched-pair design. *Results.* After glucocorticoid induction for 24h, 48h and 72h, VCP RNA expression was increasing. However, in the PPR cell line VCP was upregulated 2.8 fold compared to the PGR cell line after 96h of glucocorticoid induction. In agreement with these findings, VCP protein expression was also overexpressed in the PPR cell line compared to the PGR cells . The results of the immunfluorescence analysis supported these findings, but revealed no differences in the localization of the target protein. On average, VCP RNA expression was increased about 0,56 times in three of five PPR samples (5,1'6,5) in comparison to the PGR (3,1-3,3). Results were normalized with the housekeeping gene TBP. VCP protein expression was slightly increased about 0,71 in the PPR group in comparison to the PGR group. *Conclusions.* The results of this study indicate that the deregulation of the proteasome-ubiquitin degradation pathway with valosin-containing protein (VCP) as part of it may determine multi-agent chemotherapy resistance and treatment outcome in childhood ALL patients.

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0023

SAFETY AND TOLERABILITY OF INTRATHECAL LIPOSOMAL CYTARABINE AS CNS PROPHYLAXIS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Meningeal recurrence in acute lymphoblastic leukaemia (ALL) occurs in up to 15% of patients and depends on intensity of front line systemic and CNS directed therapy and subtype of ALL. Meningeal recurrence not only adds significant morbidity for the individual patient but also is frequently associated with poor outcome. Liposomal Cytarabine (DepoCyte) is a sustained release formulation of Ara-C with a more homogeneous distribution throughout the neuroaxis and a prolonged half life maintaining cytotoxic concentrations in the CSF for more than 14 days. *Aims.* The purpose of our study was to evaluate the efficacy and

safety of a slow-release liposomal formulation of cytarabine for intrathecal (IT) meningeal prophylaxis in patients suffering from ALL. *Patients and Results.* From 7/2004 to 01/2007 17 patients aged 20 to 65 years (median= 37) were preventively treated with a total of 44 (range:1-6) single doses containing 50 mg of liposomal cytarabine on a compassionate use basis. Diagnoses consisted of Pro-B-ALL (n=6), c-ALL (n=2), T-ALL (n=6), and Pre-B-ALL (n=3). All patients were treated according to the risk adapted German Multicenter Protocol/ GMALL 07/2003 including 24 Gy irradiation to the neurocranium as constituent component of CNS directed therapy. 4 patients expressed the bcr-abl fusion oncogen and were concomitantly treated with imatinib mesylate as outlined in the study protocol. All patients received dexamethason for 3'5 days in order to prevent chemical arachnoiditis. Except for headache grade 2 in one patient no specific toxicity attributable to IT liposomal cytarabine application was noted. So far, after an observation period of 11 months (1-27) 6 patients died, 5 of disease recurrence and one of sepsis. 2 patients show a refractory course of disease. 9 patients are in complete remission, one after successful salvage therapy of first relapse and 5 after allogeneic stem cell transplantation. Only one patient experienced a combined medullary - leptomeningeal disease recurrence 6 month after primary diagnosis. None of the surviving ALL patients developed neurological symptoms or unexpected long term neurological side effects. *Conclusions.* IT liposomal cytarabine therapy at a dose of 50 mg with concomitant dexamethasone appears to be feasible and well tolerated. However, since all patients received concurrent systemic chemotherapy and CNS directed irradiation the efficacy of liposomal cytarabine cannot be assessed separately. Further studies are warranted to determine the exact schedule for IT prophylaxis in adult patients with ALL.

0024

GENE EXPRESSION PROFILING IN E2A-PBX1-SILENCED T(1;19)+ LEUKEMIA CELLS

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Background and Aims. The translocation t(1;19)(q23;p13) is one of the most common ones in childhood pre-B acute lymphoblastic leukemia and usually results in the chimeric gene E2A-PBX1. Through extensive studies, E2A-PBX1 has been shown capable of transformation in fibroblast, myeloid, and T lineage cells, but its role during leukemogenesis is not yet fully understood (Fu X, Kamps MP. Mol Cell Biol 1997; Kamps MP, Baltimore D. Mol Cell Biol 1993; Dedera DA, *et al.* Cell 1993). We initially silenced E2A-PBX1, achieving an efficient downregulation of this fusion in several pre-B leukemia cell lines and obtaining as consequence a strong downregulation of the Wnt16b and EB-1 genes. The presented project aims to clarify the mechanism of E2A-PBX1 in leukemia cells also through the elucidation of its interactions with a panel of gene involved in tumorigenesis and transformation, based on differential gene expression array in pre-B acute lymphoblastic leukemia cell lines (in normal conditions as well as during RNAi silencing). *Methods.* The transfection of small interfering RNAs (siRNAs) was monitored by fluorescence microscopy and FACS analysis. The specific mRNA expression of the fusion gene was measured using TaqMan real-time quantitative PCR, while the reduction of the correspondent protein levels was assessed by Western Blot. To better understand the role of E2A-PBX1 in human pre-B cell leukemogenesis, the study focused also on genes whose expression invariantly accompanies the t(1;19) translocation, and their transcripts were detected by SYBR Green PCR. The expression profiles were obtained through PCR arrays, considering eighty-four genes representative of different biological pathways. *Results and Conclusions.* The downregulation induced by anti-E2A-PBX1 siRNA in the fusion gene expressing cells reduced the specific transcript expression by 85-90% (ABL-normalized levels were measured 24 hours after transfection). In particular, the fusion gene depletion downregulated other two genes: EB-1, which encodes for a protein that could contribute to the transformed phenotype of pre-B ALL, and Wnt16b (not the Wnt16a isoform of the Wnt16 gene); their aberrant expression may therefore be a key-step in leukemogenesis in t(1;19)-positive pre-B leukemia. The roles of E2A-PBX1 target genes was characterized through differential gene expression PCR arrays, investigating genes involved in tumorigenesis, transformation, growth regulation, proliferation, and allowed to focus on the most significant pathways and on the interactions of E2A-PBX1 with specific signal trasduction molecules, transcription factors and target genes.

0025

TOLERANCE TO METHOTREXATE THERAPY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA: IMPORTANCE OF GENETIC POLYMORPHISMS IN FOLATE METABOLIC PATHWAY

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Background. Present days, approximately 80% of children diagnosed with acute lymphoblastic leukemia (ALL) are cured. Almost all therapeutic protocols use methotrexate (MTX) as antifolate agent and this drug is administred in high doses (5 g/m^2) during interval therapy. It has been observed that only some patients experience therapy-related toxicity, developing intolerance to MTX such as hepatic toxicity and/or mucositis. MTX targets several critical aspects of folate metabolism inhibiting enzymes in the folate cycle and leading to the decrease of folate pool. The folate metabolic pathway is crucial in purine and pyrimidine synthesis, as well as in the provision of methyl groups for DNA, RNA and protein methylation. SNPs in genes encoding enzymes involved in methotrexate metabolism have been implicated in relapse or toxicity in ALL patients. *Aims.* We hypothesized that toxicity to MTX could be influenced by genetic polymorphisms in the folate-metabolizing pathway because MTX induces a low folate state. In this work we investigated how seven genetic polymorphisms in five genes encoding enzymes involved in folate metabolism (Methylenetetrahydrofolate Reductase - MTHFR, Thymidylate Synthase - TYMS, Methionine Synthase - MS, Methionine Synthase Reductase - MTRR and Serine Hydroxymethyltransferase - SHMT), influence the response to MTX in childhood ALL therapy. *Methods.* Using PCR/RFLP and direct sequencing based methods, we characterized DNA sample from 34 children diagnosed with ALL, and retrospectively analyzed their clinical histories. All children were following the same protocol for ALL therapy. Intolerance to MTX was defined as the occurrence of hepato-toxicity (increase in liver transaminases - TGO/TGP) and mucositis. *Results.* The MTRR C524T variant was found to be significantly associated with a decrease of mucositis during MTX administration and an over-representation of the MTHFR C677T allele in the group of patients who developed mucositis was also observed although not reaching statistical significance. *Conclusions.* While these results seem to indicate that pretherapeutic testing of MTRR and MTHFR may predict toxicity reactions to MTX treatment in children undergoing ALL therapy, enlargement of sample sizes (which is currently ongoing) is needed to validate these preliminary observations.

0026

DASATINIB INDUCES RAPID AND DURABLE RESPONSES IN PATIENTS WITH PH+ ALL RESISTANT OR INTOLERANT TO IMATINIB: UPDATED RESULTS FROM CA180015 (START-L) TRIAL

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Background. Relapsing patients with Ph$^+$ ALL who have previously received treatment with imatinib and/or chemotherapy have a particularly poor prognosis. Dasatinib is a novel multi-targeted kinase inhibitor of BCR-ABL and SRC family kinases, with *in vitro* potency some 325-fold greater than imatinib against BCR-ABL and proven efficacy in this patient population. *Aims.* This study was designed to assess the efficacy and safety of dasatinib. *Methods.* Phase-II, open-label, international, multicenter study where patients with Ph$^+$ ALL with resistance or intolerance to imatinib were treated with dasatinib 70 mg BID, administered orally. Dose escalation to 100 mg BID was allowed for inadequate response, and dose reduction to 50 mg or 40 mg BID for adverse events. All patients provided written informed consent. *Results.* From January through July 2005, 46 patients (median age 48 years; 59% male) enrolled and received treatment, 96% of whom were imatinib-resistant; 46% had received doses of >600 mg/d per day, and 52% had received imatinib therapy for ≥12 months. Median time from initial diagnosis of Ph$^+$ ALL was 18 months and 37% of patients had undergone prior stem-cell

transplantation (SCT). BCR-ABL mutations were present at baseline in 78% of patients. With follow-up extending to 18.5 months, the overall major hematologic response rate was 41%. Major cytogenetic response (MCyR) was achieved for 57% of patients; this was complete in all but one of these patients (54%). Response rates were consistent irrespective of pre-existing BCR-ABL mutations. Responses were durable with a positive impact on survival; median overall survival (OS) was 8.0 months and 22% of patients remained alive and progression-free after 1 year of treatment. Outcome was favorable for patients with prior SCT: median OS of 9.0 months (5.8 months for patients without prior SCT). Grade 3-4 thrombocytopenia and grade 3-4 neutropenia both occurred in 78% of patients. Most frequent non-hematologic side-effects included diarrhea in 33% of patients (grade 3-4, 9%), pleural effusion in 24% (grade 3-4, 7%), nausea in 22% (no grade 3-4), and pyrexia in 22% (grade 3-4, 2%). The dasatinib dose was reduced in 30% of patients and interrupted in 43%, primarily due to non-hematologic toxicities. The average median daily dose was 143 mg. *Summary and conclusions.* Dasatinib continues to show impressive efficacy in this difficult-to-treat population post-imatinib failure.

0027

OUTCOME OF ADULT PATIENTS WITH RELAPSED ACUTE T-LYMPHOBLASTIC LEUKEMIA (T-ALL) CAN BE IMPROVED WITH SEQUENTIAL SALVAGE THERAPIES AND SCT IN SECOND CR

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Outcome of newly diagnosed T-ALL which was formerly an unfavourable subgroup has improved considerably. With current regimens CR rates >80% and an overall survival (OS) >50% can be achieved in adults by chemotherapy and risk adapted stem cell transplantation (SCT). However few data on outcome of relapsed T-ALL are published and prognostic factors at relapse are unknown. This is a retrospective analysis of 155 pts with 1st relapse of T-ALL. All pts had been treated at first diagnosis in the German Multicenter Study Group (GMALL) studies 05/93 (N=88) and 06/99-07/03 (N=67). At relapse treatment decision was at the discretion of the physician and generally consisted of intensive salvage regimens e.g. modified induction, HDMTX or HDAC combinations. Since treatment approaches are different for CNS and BM relapse the outcome analysis will focus on BM relapse (N=117). Patient characteristics did not differ significantly (sign.) between both studies (Table 1).

Table 1. Paient Characteristics and outcome of relapsed T-ALL.

		Study 05/93	Studies 06/99-07/03
N		88	67
Age	Median (Range)	28 (15-63) yrs	31 (17-62) yrs
Subtype	Early	22%	12%
	Mature	34%	15%
	Thymic	44%	70%
Time to relapse	Median (Range)	12 (1-47) mo	11 (1-35) mo
	Early (< 18 mo)	70%	73%
Localisation	BM ± Other (no CNS)	76%	75%
	CNS ± Other	19%	13%
	Other only	5%	12%
CR rate		39%	30%
Survival		6%	24%

The CR rate with 1st approach was 35% (33% in early and 39% in late relapse). OS was 12% and significantly higher in study 06 (24%) compared to 05 (6%) (p=0.0001). Overall 56% of the patients were referred to SCT, more in study 06-07 (72%) versus 05 (43%). Survival after any SCT vs no SCT was 9% vs 4% in study 05 and 30% vs 0% in study 06-07 (p<0.0001). Results were sign. better for SCT performed in 2nd CR compared to SCT in relapse. In study 06-07 more pts were treated with several subsequent approaches incl. experimental drugs. Thus a CR-rate of 41% was achieved with Nelarabine in 94 pts with refractory relapse of T-ALL. 1 of 5 patients achieved a CR with MabCampath monotherapy. The most sign. prognostic factor for OS ' also confirmed in multivariate analysis - was achievement of CR with first attempt (23% vs 6%; p=0.0001) and realisation of SCT (19% vs 3%; p=0.0001). Immunophenotype was a prognostic factor for CR-rate and survival, with better results for thymic compared to early/mature T-ALL (24% vs 0%; p=0.04). Duration of first remission and relapse localisaton had no sign. impact on survival. In this large cohort of pts with relapsed T-ALL with first intensive therapy approach the CR rate was 35% and the OS 12%. Achievement of 2nd CR, realisation of SCT and subtype were sign. prognostic factors. Survival improved in the recent study 06-07 which may be partly attributed to more intensive attempts to achieve CR even with experimental approaches, higher SCT rates and improved outcome after SCT. Since pts with relapsed T-ALL are generally young several subsequent attempts to achieve CR and perform SCT in CR are reasonable. The GMALL has activated sequential studies with T-cell specific drugs including Nelarabine, MabCampath and Forodesine. If proven successful the next step is integration of these drugs in front-line therapy.

0028

L-ASPARAGINASE HYPERSENSITIVITY IN CHILDHOOD ALL - ISRAEL EXPERIENCE

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Asparaginase (ASP) is an effective antileukemic agent. However, hypersensitivity and allergic reactions are reported in 2.5-45% of recipients and are a major limitation in its clinical use. The risk of developing sensitivity to ASP increases with higher doses, *E. coli* preparation, IV (vs. IM), continuous and repeated treatment after a prolonged abstinence. We surveyed the rate of hypersensitivity in pediatric ALL in Israel, while trying to identify factors of significance. *Methods.* Data gathered from medical records of 245 children diagnosed with ALL during the years 2000-2005 from 4 Pediatric Hematology-Oncology wards comprising 70% of all children treated. Drugs were uniformly administered in all children on the Israel National leukemia Study, modified from BFM protocols *E. coli* L-ASP was to be substituted with PEG or Erwinase in case of allergy or hypersensitivity. The definition and severity of allergy was defined by the professional opinion of the treating medical team. *Results.* Forty-eight (19.6%) of patients developed an allergic reaction. A wide range was noticed between the treating centers (6.4%-29.4%). A significantly higher number of patients with allergic reactions were treated on the high-risk arm (39.1% vs. 14.6% in the overall group), including 10 with severe reactions. A higher incidence was noticed at the start of the second cycle, occurring after 8 initial doses and a 4 month break in ASP treatment with 15 (31.2%) of the allergic patients developed the reaction to ASP on the second cycle's first dose, and 8 (16.6%) on the second dose, 3 days later. PEG ASP had significantly more allergic reactions vs. E. coli L-ASP treatments (8/145; 5.5% vs. 37/2610; 1.4%). PEG was also associated with allergies of higher severity, (8/9; 88.9% vs. 43.6%). Ewrina product was discontinued during the study period. A careful analysis revealed no differences between the allergenic and non-allergenic groups in relation to sex, age, mortality and/or chromosomal abnormality. *Conclusions.* The overall allergy rate (with noticed wide variations) and the high incidence at the beginning of the second cycle are within the described literature. The findings regarding the PEG were unexpected. We report here a relationship between the higher risk group and the development of the allergy and the severity of the allergy. We believe that the differences observed between treating wards are due to unclear definitions of reaction or severity .We have noticed a wide variability in executing the supporting treatment. We recommend that the ASP treatment should be preceded with a period of steroids at the beginning of all treatment cycles.

0029

OUTCOME OF THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA USING A PEDRIATRIC PROTOCOL

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Background. Adult acute lymphoblastic leukemia (ALL) still have poor outcome compared with childhood ALL, with an expected long time survival of less than 40%. Adolescents aged from 15 to 20 years have improved complete remission (CR) and event free survival (EFS) rates when treated with pediatric protocol instead of adult one. *Aims.* In a pilot study, we have tested the feasibility and the efficiency of the French pediatric protocol FRALLE 2000 to treat adult ALL aged up to 55 years. *Methods.* 20 consecutive adults Philadelphia negative ALL aged from 15 to 55 years received treatment courses according to the French pediatric protocol FRALLE 2000 from 2001 to 2006. After a prednisone prephase and a four-drugs induction (prednisone, daunorubicin, vincristine and 9 infusions of L-asparaginase), patients in CR received a consolidation course, two delayed intensifications with L-asparaginase separed by an interphase, a CNS irradiation and a maintenance chemotherapy during two years. Results were compared with the outcome from 20 consecutive patients treated in our institution with the historic EORTC ALL-4 adults protocol from 1998 to 2001. Median age, WHO performans status, white blood count at diagnosis, phenotype and cytotogenetics risk groups were statistically similar in both groups. *Results.* Cortico-sensitivity and chemo-sensitivity was assessed for 15 and 17 patients respectively and CR rate was 85% after FRALLE induction, and 100% after a salvage therapy with high doses cytarabine. All patients who achieved cortico and chemo-sensitivity were in persistant CR with a median follow-up of 26 months. Minimal residual disease (MRD) study was available for 12 patients, using IgH/TCR rearrangement or E2A/PBX1 transcript. Among the 10 patients with an indetectable MRD at D90, no one experienced relapse. Overall, with a median follow-up of alive patients of 26 months, the 4-years EFS and overall survival are 80±10% vs 47±12%, (p=0.04) and 87±9% vs 35±16%, (p=0.03) respectively. This better outcome is not explained by significant differences in patients characteristics nor by a better CR rate but rather by a lower relapse rate in the pediatric treatment group (more than two-fold lower). This indicates a major role of the drugs indication and the dose intensity, especially in L-asparaginase administration. No treatment related mortality and no severe side effect were observed during treatment with supportive cares including parenteral nutrition, granular growth factor, infectious prophylaxis, and antithrombine III infusions. *Summary and conclusions.* This pilot study shows that adults up to 55 years with Ph negative-ALL have a dramatically better outcome when there are treated with childhood ALL protocol without any major side effect. This therapeutic strategy has to be confirmed by the current prospective study performed by the EORTC group.

Acute myeloid leukemia - Biology I

0030

EXPRESSION OF ANGIOPOIETINS AND VASCULAR ENDOTHELIAL GROWTH FACTORS AND ITS CLINICAL SIGNIFICANCE IN ACUTE MYELOID LEUKEMIA: ANG-2 EXPRESSION IS AN INDEPENDENT PROGNOSTIC FACTOR FOR OVERALL SURVIVAL

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Background and Aims. Concerted expression of angiopoietins, their receptor Tie2 and vascular endothelial growth factor (VEGF) family, most specific inducers of angiogenesis secreted by leukemia blasts, are known to play an essential role in normal and pathologic angiogenesis. We therefore evaluate the clinical implication of angiogenic factors in patients with acute myeloid leukemia (AML). *Methods and Patients.* We investigated the RNA expression of genes encoding angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), the receptor Tie2, VEGF-A and VEGF-C by real-time quantitative polymerase chain reaction (RQ-PCR) in a cohort of 126 patients with newly diagnosed de novo AML at National Taiwan University from June 1995 to Feb 2006. The results were correlated with clinical features and outcome of the patients. *Results.* Expression of Ang-1, Ang-2 and VEGF-A was significantly higher and that of VEGF-C was lower in AML patients than in normal controls. RNA expression level correlated well with the results of immunocytochemical staining in the selected patients studied. Patients with high Ang-1, Ang-2, or Tie2 expression had significantly shorter relapse-free survival than those with low expression (8 months versus 14 months, p=0.029; 9 months versus 14 months, p=0.01; 8.5 months versus 14 months, p=0.01, respectively) by univariate analysis, but the significance disappeared by multivariate analysis. On the other hand, high expression of Ang-2 and Tie2, but not other angiogenic factors, was correlated with a shorter overall survival (15.7 months versus 38 months, p=0.005; 14 months versus 26 months, p=0.043, respectively). In multivariate analysis, only karyotype and Ang-2 expression were independent prognostic factors, with a hazard ratio of 2.19 (95%CI, 1.27-3.77, p=0.005) and 2.05 (95%CI, 1.20-3.52, p=0.009), respectively. Furthermore, the prognostic relevance of Ang-2 expression became even more pronounced in the patients with intermediate-risk karyotype (p=0.004). Subgroup analysis showed that the prognostic impact of Ang-2 expression was only evident in the patients with low Ang-1 or low Tie2 levels, but not those with high levels, and in the patients high VEGF-A or high VEGF-C levels, but not those with low levels. *Conclusions.* These results provide evidence that high pre-treated levels of Ang-2 in the bone marrow indicate an unfavorable prognosis in AML. Further research into interaction of concerted angiogenic factors in AML is warranted.

0031

MUTATED NUCLEOPHOSMIN (NPM1) IS ASSOCIATED WITH INCREASED SPONTANEOUS APOPTOSIS AND FAVORABLE PROGNOSIS IN FLT3-ITD NEGATIVE ACUTE MYELOID LEUKEMIA (AML)

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Nucleophosmin (NPM1) is a multifunctional protein that interacts with p53 and its regulatory molecules (ARF, Hdm2/Mdm2), thus controlling cell proliferation and apoptosis (Falini et al, 2007). Mutations in NPM1 occur in up to 50% to 60% of normal karyotype acute myeloid leukemia (AML) patients and are associated with more favorable response to therapy. Moreover, genes and proteins involved in apoptosis have been shown to be relevant in response to treatment and prognosis in AML (Del Poeta *et al.,* 2003). We analysed NPM1 mutational status and the expression of apoptosis proteins (bcl-2 and bax) in 188 pts affected by de novo non-M3 AML. Median age was 61 yrs (range 21-82) and patients received treatment according to the GIMEMA-EORTC cooperative groups protocols. The aims of our study were: 1) to correlate the NPM1 status with bax/bcl-2 ratio levels, as a measure of spontaneous apoptosis, and 2) to assess the prognostic significance of NPM1 and FLT3 status in relation to the bax/bcl-2 ratio. Bcl-2 and bax proteins were determined by multicolor flow cytometry and bax/bcl-2 ratio was obtained by dividing mean fluorescence intensity (MFI) of bax/MFI bcl-2. The threshold of positivity was set at the median value >0.3. NPM1

mutations and FLT3-ITD were detected by multiplex PCR and capillary gel electrophoresis. One hundred-one/188 (54%) pts were bax/bcl-2 ratio positive. NPM1 and FLT3 mutations were detected in 21.3% and in 20.9% of cases, respectively. There was a significant correlation between higher WBC counts (>100×10^9/L) and NPM1 mutations (p=0.001). A normal karyotype was found in 25/32 (78%) NPM1 mutated pts (p=0.003) and 35/40 NPM1 mutated cases were CD34 negative (p<0.0001). A positive bax/bcl-2 ratio was significantly associated with NPM1 mutations (28/40; p=0.02) and 15 of 21 (78%) NPM1+FLT3-ITD negative cases showed higher bax/bcl-2 ratio. As concerns clinical outcome, significantly lower complete remission (CR) and overall survival (OS) rates were found in NPM1+FLT3-ITD+ vs NPM1+FLT3-ITD negative pts (27% vs 63%; p=0.04 and 0% vs 36% at 9 months; p=0.006, respectively). Bax/bcl-2 ratio and NPM1 showed additive prognostic impact, since higher bax/bcl-2 ratio plus mutated NPM1 in absence of FLT3-ITD identified AML pts at better prognosis with regard to disease free survival ([DFS] 76% vs 0% at 4 months; p=0.015). Of note, within the poor prognostic subgroup NPM1+FLT3-ITD+, higher bax/bcl-2 ratio was able to distinguish pts at better prognosis with regard to CR (60% vs 0%; p=0.04) and OS (50% vs 0% at 3 months; P=0.017). There were no significant differences between NPM1+ and NPM1 negative pts with regard to CR, OS and DFS. In multivariate analysis, only bax/bcl-2 ratio and WBC counts resulted to be independent prognostic factors with regard to OS (p=0.0001 and p=0.03, respectively) and DFS (p=0.009 and p=0.001, respectively). Therefore, we confirm that NPM1 mutations in the absence of FLT3-ITD identifies a subgroup of pts with favorable prognosis and in pts with both mutations FLT3-ITD dominates the leukemic phenotype conferring a poor outcome. We demonstrate that NPM1-mutated/FLT3-ITD negative pts exhibit high levels of spontaneous apoptosis, thus explaining their more favorable response to therapy.

0032

ACUTE MYELOID LEUKEMIA IS PROPAGATED BY A LEUKEMIC STEM CELL WITH LYMPHOID CHARACTERISTICS IN A MOUSE MODEL OF CALM/AF10 POSITIVE LEUKEMIA

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Background. The demonstration that most, if not all cancers are sustained by a small population of malignant clones within the tumor with stem cell properties has rendered the identification of cancer stem cells in leukemia critical to the understanding of the pathogenesis of this disease. *Aim.* The aim of this study was to identify the leukemic stem cells (LSCs) in acute myeloid leukemia (AML) associated with the CALM/AF10 (C/A) fusion gene. *Methods.* Mice (n=13) were transplanted with BM cells retrovirally engineered to express the C/A fusion gene. For human C/A$^+$ patient samples, IGH DJ analysis, immunostaining, FISH and CFC assays were performed. *Results.* We demonstrate that AML can be propagated by LSCs with lymphoid characteristics (Deshpande et al. Cancer Cell, Nov 2006). All mice injected with C/A$^+$ cells succumbed to AML showing IGH DJ rearrangements. In the leukemic mice only a minor fraction, (avg. 6.7%) cells in the BM displayed the B220 lymphoid antigen and lacked myeloid markers (avg. 9.4%). The majority of cells expressed myeloid markers (avg. 82.9% Mac1+ cells). Additionally, an average of 26.0% of these cells co-expressed B220 and Mac1, compared to 2.1%, in controls. Surprisingly, when injected into secondary mice, the B220+/Mac1' cells showed the highest frequency of leukemia propagation (>1 in 36 cells) as compared to the Mac1$^+$/B220$^-$ bulk population population (1 in 19717) and the B220$^+$/Mac$^+$ cells (1 in 437). Moreover, at a single cell level, the leukemic IGH DJ rearranged B220$^+$/Mac1$^-$ cells could give rise to the B220$^+$/Mac1$^+$ as well as the Mac1$^+$/ B220$^-$ populations with clonal DJ rearrangements, and induce identical leukemias demonstrating its capacity to give rise to the myeloid leukemic bulk at the single cell level. Depletion of the B220$^+$ cells from the leukemia before secondary transplantation effectively prevented leukemia development in mice. This murine model closely recapitulated C/A$^+$ human AML; BM cells of a majority of patients (7 of 9) displayed clonal IGH DJ rearrangements and in 3 of 3 patients tested, a significant proportion of C/A$^+$ IGH rearranged CD45RA$^+$ (B220) cells could be detected. *Conclusions.* These results indicate that LSCs can have surface markers different from both normal stem cells and the tumor bulk, which might be useful for specific targeting of such LSCs while sparing the normal stem cell pool.

0033

IDENTIFICATION OF HEME OXYGENASE 1 (HO-1=HSP32) AS A CRITICAL SURVIVAL FACTOR AND NEW THERAPEUTIC TARGET IN AML

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Background. Heat Shock Protein 32 (Hsp32), also known as Heme Oxygenase 1 (HO-1), has recently been identified as a stress-related survival molecule that acts anti-apoptotic and cytoprotective in inflammatory reactions. Recent data suggest that Hsp32 is also expressed in neoplastic cells in various malignancies. Methods and *Results.* In the present study, we provide evidence that Hsp32 is constitutively expressed in primary leukemic cells in patients with acute myeloid leukemia (AML, n=17) and in various AML cell lines such as HL60, KG1, KG1a, and U937. Expression of Hsp32 mRNA was demonstrable by RT-PCR, and the Hsp32 protein by immunocytochemistry and Western blotting. In addition, we were able to demonstrate expression of Hsp32 mRNA and of Hsp32 protein in the CD34$^+$/CD38$^-$ progenitor/stem cell fraction in the leukemic clone in patients with AML. The Hsp32-inducing compound hemin (10 μM) was found to promote expression of Hsp32 in AML cells. Incubation with the Hsp32-targeting drugs pegylated zink protoporphyrin (PEG-ZnPP) or styrene maleic acid-conjugated ZnPP (SMA-ZnPP), resulted in a dose-dependent inhibition of growth of leukemic cells at pharmacologic concentrations (IC50: 5-30 μM for cell lines and primary AML cells). The SMA-ZnPP-induced growth-inhibition of AML cells were found to be associated with induction of apoptosis as evidenced by light microscopy, electron microscopy, and by a Tunel assay. In consecutive experiments, combination experiments were performed using SMA-ZnPP and AML cell lines. In these experiments, SMA-ZnPP was found to synergize with cytarabine in producing growth inhibition in all AML cell lines tested. *Conclusions.* Hsp32 is a novel survival factor and interesting target in AML. The clinical significance of this observation remains to be determined in forthcoming trials.

0034

ABSENCE OF JNK ACTIVATION IS A PREDICTOR OF ANTHRACYCLINE RESISTANCE IN AML

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Standard chemotherapy is often unsuccessful in AML. Besides other extensively studied causes of treatment failure, as MDR, defective apoptosis emerges as an important mechanism of drug resistance. Anthracyclins are reported to activate the JNK signaling pathway in drug-sensitive AML cell lines, including U937 cells. In U937 derivative drug-resistant cells (URD) we have shown absence of basal and anthracycline-induced activation of JNK. As we have observed that URD cells are remarkably less susceptible to anthracycline-induced apoptosis compared to parental U937 cells, the different pattern of JNK response may account for their resistant phenotype. *Aims.* To investigate the impact of JNK on the apoptosis induction and the resistance to standard chemotherapy in AML cell lines and in primary AML blasts. *Methods.* We performed siRNA inhibition of JNK in U937 cells and we evaluated apoptosis susceptibility after treatment with the anthracycline Daunorubicin(DNR), (1 μM). DNR-induced apoptosis was also examined in URD cells transfected with a constitutively active form of SEK1 (SEK-ED), an upstream activator of JNK (URD/SEKED cells). Bone marrow blasts from 30 AML patients at diagnosis and without any previous therapy were exposed *in vitro* to anthracycline treatment (1 μ DNR, 16hr) and the basal levels as well as the inducibility of JNK activity were correlated with the

drug-induced apoptotic response of blast cells. Experimental results were also associated with patient clinical data. *Results.* RNA silencing of JNK in U937 cells resulted in a 57,4% decrease in DNR-induced apoptosis compared with wild type and cells transfected with empty vector (annexin V+ve cells=26.46±2.02% vs 62.12±0.28%). Additionally, URD/SEKED cells, characterized by active JNK, became more susceptible to DNR and underwent drug-induced apoptosis (annexin V+ve cells=21.6±1.7% vs 6.42±2% in control cells). 44.2% of primary AML samples exhibited increased basal JNK activity levels, but they were not correlated with sensitivity to DNR. In 46% of AML samples a drug-induced upregulation of basal JNK activity preceded the induction of significant apoptosis, in relation to untreated controls. (p=0,00048<0,05). The pattern of JNK activation in these patients was similar to that detected in U937 drug-sensitive cells. On the contrary, in the remaining 54% of AML samples basal JNK activity remained immutable in response to drug treatment, and this was accompanied by a failure of DNR to induce significant apoptosis (p=0,4697>0,05). Moreover, the latter cohort of patients shared clinical features indicative of a poor response to treatment, such as older age (mean=78,6 years vs 57,16 years in the JNK activation group, p=0,0036<0.05) and antecedent hematological disorders, mainly myelodysplasia (61,53% vs 16,6% in the JNK-activation group). CR1 rates were significantly lower in these patients (28,57% vs 77,7% in the JNK-activation group). *Conclusions.* Our findings suggest that JNK is a critical proapoptotic mediator of Daunorubicin and impaired activation of JNK is associated with a failure of DNR to induce apoptosis in AML. In accordance with that point of view, restoration of JNK activity may increase chemotherapy effectiveness in AML. Moreover, the activation levels of JNK may serve as a marker reflecting differences in disease biology which affect treatment outcome in AML.

0035

DEFINED NICHE CONDITIONS AND A STEM CELL PHENOTYPE BOTH CONTRIBUTE TO THE *IN VITRO* RESISTANCE OF PRIMARY AML CELLS TO A FLT3 TYROSINE KINASE INHIBITOR

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Background. Relapse in acute myeloid leukaemia is considered to be mediated by the survival of leukaemic stem cells and by protection of blasts in the bone marrow niche following remission induction chemotherapy. *Aims.* The aim of this study was to study the differential response of both bulk cells and CD34+CD38-CD123+ leukaemic stem and progenitor cells (LSPC) to *in vitro* FLT3 inhibition under different culture conditions. *Methods*. We devised a flow cytometric chemosensitivity assay to allow 48 hour culture of leukaemic blasts with and without defined niche conditions (fibronectin, IL-3, SCF, IL-6, Ang-1) followed by enumeration of the viable (7-amino-actinomycin D negative, forward scatter high) LSPC. The FLT3 inhibitor AG1296 was applied to 11 AML samples, of which 9 had internal tandem duplications of the FLT3 gene (FLT3 ITDs). *Results.* 3/11 samples were very sensitive (>50% kill) and 5 were quite sensitive (10-50% kill) to AG1296 in bulk suspension culture without niche support. However when niche conditions were used and the LSPC compartment was analysed, the concentration of LSPC was enhanced rather than inhibited by AG1296 treatment. *Conclusions.* We conclude that LSPC from samples with FLT3 ITDs are resistant to AG1296 under defined niche conditions. Whilst pharmacological FLT3 inhibitors may be more effective than AG1296, we suggest that it would be useful to assay the effects of such agents on LSPC under niche conditions, since the current study does not provide a rationale for the clinical use of single agent FLT3 inhibitors to target residual LSPC post chemotherapy.

0036

ANTITUMOR ACTIVITY OF BORTEZOMIB ALONE AND IN COMBINATION WITH TRAIL IN HUMAN ACUTE MYELOID LEUKEMIA

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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. *Aims.* 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). *Methods.* cell viability was determined using the CellTiter 96 AQueous One Solution Cell Proliferation Assay. Immunohistochemistry or immunofluorescence were performed using a monoclonal mouse anti-human p65 (Rel A) to evaluate the cellular localization of NF-κB. *Results.* in each sample Bortezomib was able to induce cell death of AML blasts. The cytotoxic effect was dose and time-dependent (concentration of Bortezomib ranging from 0.001 to 10 μM for 24 and 48 hours) and was associated with downregulation of Bcl-xL and Mcl-1, with an upregulation of TRAIL-R1, TRAIL-R2 and p21 and the activation of executioner caspases. NF-κB was in the nucleus of AML blasts and do not translocate after Bortezomib exposure. Moreover, low doses of Bortezomib were able to prime TRAIL-resistant AML cells for enhanced TRAIL-mediated killing. *Conclusions.* the combination of proteasome inhibitors and TRAIL is effective for treatment of AML patients even though refractory to conventional chemotherapy.

0037

ALLELIC IMBALANCES AND MUTATIONS OF AML1, MLL, AND FLT3 GENES ANALYSIS IN RADIATION-ASSOCIATED ACUTE MYELOID LEUKEMIA

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Background. Ionizing radiation is established as a cause of leukemia in humans. However, little is known about the molecular mechanisms by which radiation induces the leukemia. *Aims.* The aim of this study was the identification of molecular leukemia markers that are associated with exposure to ionizing radiation. *Methods.* The cohort of patients consisted of 154 unselected adult acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients, diagnosed between 1988 and 2006 in Ukraine. Of these patients, 84 had experienced radiation exposure due to the Chernobyl accident (radiation-associated cases), and 70 developed spontaneous AML and served as controls. Fifty-one and 59 AML samples were analyzed for the presence of AML1 and MLL abnormalities respectively using fluorescent in-situ hybridization and/or reverse transcription polymerase chain reaction (PCR). AML1 mutations were screened in 6 radiation-associated cases of MDS or AML following MDS by direct sequencing of genomic DNA. Using Affymetrix high-density single nucleotide polymorphism 10K mapping arrays, we performed a whole-genome loss of heterozygosity (LOH) and DNA copy number changes analysis of 19 radiation-associated AML cases. The conventional comparative genomic hybridization (CGH) was done on 25 radiation-associated and 12 spontaneous AML samples. One hundred and twenty four patients (71 radiation-associated and 53 spontaneous AML cases) were examined for the presence of FLT3 internal tandem duplications (ITD) by genomic PCR. *Results.* Translocations involving AML1 fused to ETO but no other translocation partner was found to be significantly less frequent in radiation-associated AML (1/24) than in spontaneous cases (9/29, p=0.02). No difference in MLL translocations frequency was found between radiation-associated and spontaneous AML cases (0/27 and 1/32 respectively). An AML1 point mutation was detected in 1 out of 6 MDS/AML following MDS patients. The hexanucleotide duplication of CGGCAT in exon 8, inserted after base position 1502 was found in a patient who developed MDS following an acute radiation syndrome. Most significant was a high frequency of LOH at 5q and/or 7p, and 7q detected in 8 cases (42%) of radiation-associated AML and their combination with DNA loss at 5q and/or 7q and 7p (10/26 in radiation-associated vs. 1/12 in spontaneous cases, p=0.06). There was no significant difference in the prevalence of FLT3 ITD between patients with (8/71) and without history of radiation exposure (9/53, p=0.4). Six out of 17 patients with ITD were found to harbor more than one FLT3 mutant alleles. Multiple duplications were found in 5 of the 8 Flt3 mutated radiation-associated AML, but in 1 of the 9 spontaneous cases (p=0.0498). *Conclusions.* Chromosomal translocations of the AML1 and MLL genes are not common among the AML patients exposed to ionizing radiation. Radiation may contribute to the development of leukemia through

AML1 gene point mutation. We hypothesize that LOH/DNA loss at 5q and/or 7 constitutes an important genetic mechanism involved in leukemogenesis following accidental radiation exposure. The higher prevalence of multiple ITD alleles in radiation-associated AML cases with FLT3 mutations may reflect an underlying radiation-induced genetic instability.

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0038

INHIBITION OF SDF-1/CXCR4 AXIS AFFECTS SURVIVAL AND DIFFERENTIATION OF HUMAN AML CELLS

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A number of recent studies showed that the chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 participate in the retention of human acute myeloid leukemic (AML) cells within the bone marrow (BM) microenvironment, and their release into the circulation. We have also showed that AML cells constitutively express and secrete SDF-1 dependent cell surface elastase, which regulates their migration and proliferation. To elucidate the molecular events and genes regulated by SDF-1/CXCR4 axis and elastase in AML cells, we examined gene expression of U937 AML cell line treated with neutralizing anti-CXCR4 or elastase inhibitor (EI) compared to untreated cells, using DNA microarray technology. Unsupervised hierarchical clustering analysis showed very similar gene expression profiles of anti CXCR4 mAb and EI treated cells, both differ significantly from the untreated control. 230 of the 8400 genes investigated were repressed, and 164 were induced after culturing AML cells in the presence of anti CXCR4 mAb or EI compared to untreated cells. Inhibition of CXCR4 or elastase was accompanied by down regulation of the transcripts of primary granule proteins. Functional classification of SDF-1/CXCR4 axis or elastase regulated genes revealed down-regulation of HOXA9, HOXA10, ETS2, as well as other transcription factors that are over expressed in AML, and are important for the development of leukemia. In contrast, transcriptional factors and regulators known to be induced during myeloid differentiation like C/EBPα, ID1, RUNX3 and HHEX were up-regulated in the treated cells. In addition, interleukin receptors, growth factors (G-CSFR and GM-CSF), complement component (C1QR1) were up-regulated in the treated cells. These data were confirmed by real time PCR for selected marker genes of myeloid cells differentiation. Interestingly, many of the differentially expressed genes were common to the transcriptional program of normal terminal granulocytic differentiation (Theilgaard- Monch & Borregarrd 2005. Blood 105:1785), suggesting that inhibition of SDF-1/CXCR4 axis may induce myeloid differentiation in AML cells. We further analyzed the effect of CXCR4 pathway inhibition on AML cell differentiation and growth. Treatment of AML cell lines with the SDF-1 antagonist AMD3100 triggered a proliferative arrest and mimicked myeloid differentiation, including morphologic changes and increased expression of myeloid differentiation antigens. In summary, our study showed that inhibition of SDF-1/CXCR4 axis or elastase in AML cells affects similar pathways related to differentiation and malignant transformation, implying a critical role for those molecules in regulating leukemic development. Repression of SDF-1/CXCR4 decreases survival and induces differentiation of AML cells, suggesting a potential new therapeutic approach for AML.

0039

RETROVIRAL INSERTIONAL MUTAGENESIS SCREEN IN A C/EBPALPHA PROLIFERATIVE GENETIC BACKGROUND

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The CCAAT enhancer binding protein α (C/EBPα) transcription factor plays a key role in the regulation of growth and differentiation of the granulocytic lineage in the hematopoietic system and CEBPA (encoding C/EBPα) is often mutated or deregulated in AML patients. Consistently, mice lacking C/EBPα have no mature neutrophils and die within a few hours. However, homozygous knockin mice in which wild type Cebpa has been replaced with a mutant allele (BRM2), which abolish the growth-repressing ability of C/EBPα, are viable, but at 8 weeks of age they display myeloid dysplasia with absence of neutrophil granulocytes. Strikingly, in older CebpaBRM2/ BRM2 knockin mice the myeloid dysplastic phenotype progress into other myeloid malignancies such as myeloid proliferative syndrome and acute myeloid leukemia-like malignancy. This strongly suggests that secondary mutations in other loci must occur when developing leukemia in the CebpaBRM2/BRM2. In order to identify genes that cooperate with Cebpa mutations in the development of leukemia in CebpaBRM2/BRM2 mice a so-called retroviral insertion mutagenesis screen was performed. Inbred newborn CebpaBRM2/BRM2 and wildtype mice were injected with SRS19-6 retrovirus, which incorporated into the genome and resulted in activation of oncogenes and leukemic progression. When leukemia was evident the mice were euthanized and analyzed. As expected the CebpaBRM2/BRM2 mice had a shorter latency than wildtype mice (186 vs. 276 days). The mice had enlarged spleen, thymus, and/or lymph nodes and were thoroughly investigated by histology, flow cytometry and southern in order to determine the leukemic phenotypes. Most of the CebpaBRM2/BRM2 mice developed an AML-like phenotype, whereas T cell leukemias were most prominent in wildtype mice, showing that mutating Cebpa specifically directs leukemic progression towards a myeloid direction. Finally, the retroviral insertion loci were identified through a splinkerette-aided PCR strategy. This led to the identification of several novel putative and previously unexplored oncogenes, which might collaborate with mutated Cebpa in the development of AML.

0040

CONSTITUTIVE PI3 KINASE ACTIVATION REPRESENTS A FAVORABLE PROGNOSTIC FACTOR IN DE NOVO AML PATIENTS UNDER 60 YEARS

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Background. Abnormal activation of kinases may promote leukemogenesis by conferring cell proliferation and survival advantage in acute myeloid leukemia (AML). New targeted therapies are currently developed to disrupt such activation, to improve the long term prognosis of AML patients. However, the prognosis impact of kinase activation on AML patients was only rarely evaluated. The most important studies related to FLT3 activating mutations, which implied an adverse outcome. Recent reports suggest that PI3 kinase (PI3K) activation may confer a poor survival to AML patients. *Methods.* Primary bone marrow blast cell samples from 92 patients registered for the LAM2001 trial of the GOELAMS were analyzed at diagnosis and PI3K constitutive activation was correlated with outcome. As previously reported (V Bardet, Haematologica, 2006, 91, 757-764), PI3K activation was established with the status of AKT phosphorylation on Ser 473, determined using either Western Blot analysis (in samples >90% blasts) or flow cytometry in the CD45Low positive population of blast cells, after 4 hours of cytokine and serum starvation. In such conditions, two groups of patients were defined, one with constitutive activation of PI3K ($PI3K^+$), contrary to the second ($PI3K^-$). *Results.* Median age at diagnosis was 46 (17,3-64,5) years. 42 patients were males and 50 were females. Leukocytosis was 12000/∝L (range 400-251800). Patients were classified in low, standard and high-risk cytogenetic subgroups in 16%, 61% and 23%, respectively. 17/79 (21,5%) had FLT3-ITD mutation, 9/60 (15%) had Ras (N-Ras or K-Ras) mutations and 18/72 (25%) had NPM mutations. 46 of 92 (50%) patients had constitutive PI3K activation ($PI3K^+$ group). In univariate analysis, no difference was observed between the two groups for all previously described parameters, except concerning FLT3 mutational status: 5/40 (12,5%) $PI3K^+$ patients had FLT3-ITD versus 12/39 (30%) in the PI3K- (p=0,048). All patients received one course of induction chemotherapy (3+7 regimen), and 31 (34%) a second induction course at day 15. Complete remission was obtained in 74 patients (80%) with no difference between the two groups (p=0,60). Among responders, 30 (41%) had autologous stem cell transplantation, 24 (32%) had allogenic bone marrow transplantation and 16 (22%) had consolidation chemotherapy. With a median follow-up of 26 months (range 3 days-37 months), 48 patients died. Overall survival was 56% in the $PI3K^+$ group and 33% in the $PI3K^-$ group (p=0.007). Among responders, 30 patients relapsed. Relapse free survival was 72% in the $PI3K^+$ group and

41% in the PI3K- group (p=0.001). In multivariate analysis, the PI3K activation plays a significant prognostic role on overall and relapse free survival, after adjustment for FLT3 status (p<0.01 and p=0.0073 respectively) and cytogenetics (p=0.033 and p=0.0075 respectively). *Conclusions.* Thus constitutive PI3K activity detected in leukemic cells at diagnosis confers a better prognosis in AML. As we observed significantly more relapses in PI3K- patients, we can make the assumption that PI3K activity may confer a particular sensitivity to chemotherapy in AML. Furthermore, targeting PI3K signalling in AML may be harmful and a better understanding of downstream effectors is needed.

0041

LS104, A NOVEL KINASE INHIBITOR, INDUCES APOPTOSIS, SYNERGIZES WITH CYTOSTATIC DRUGS AND IS TARGETING THE RECEPTOR TYROSINE KINASE FLT3

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Background. Fms-like tyrosine kinase 3 (FLT3), a member of the class III tyrosine kinase receptor family, is expressed in up to 90% of adult acute myeloid leukemia (AML). Internal tandem duplications (ITD) of the juxtamembrane domain are found in approximately 35% of AML-cases, are associated with poor clinical outcome and are considered to represent an attractive target for molecular therapy. LS104 is a novel small molecule substrate inhibitor. *Aims.* To characterize biologic activity of the novel kinase inhibitor LS104 in FLT3 ITD-positive leukemic cells. *Methods.* As a cellular model to investigate FLT3-ITD specific activity we employed 32D myeloid cells stably transfected with FLT3-ITD or wt-FLT3. In addition, we used the human myeloid-leukemia derived MV4;11 cell line harbouring FLT3-ITD and the human lymphoid-leukemia derived RS4;11 cell line expressing wt-FLT3. *Results.* In MTT assays, pronounced inhibition of cell growth was seen at nanomolar concentration (IC_{50}=50 nM) which could be partially rescued by addition of IL3. LS104 at a concentration ranging from 3-10 µM readily induced apoptosis as evaluated by cell cycle analysis, annexin-V assays and PARP cleavage. Similar results were observed using primary AML-blasts. Throughout the experiments, FLT3-ITD expressing cells showed significantly higher sensitivity towards LS104 than cells expressing wt-FLT3. Interestingly, efficacy of LS104 to induce apoptosis was significantly reduced in 32D cells transfected with a FLT3-ITD isoform (N676K) previously reported to be associated with clinical resistance of FLT3-ITD to the kinase inhibitor PKC-412 (Heidel-F *et al.,* Blood 2006). These results strongly suggest that the mechanism of action of LS104 is inhibition of FLT3 kinase activity rather than inhibition of other kinases targeted by LS104. Immunoblot and phosphoprotein-FACS analysis demonstrated inhibiton of tyrosine phosphorylation of FLT3-ITD and of its downstream target S6 ribosomal protein in transfected 32D cells and primary AML blasts. To estimate non-specific toxicity we tested the effects of LS104 on differentation and proliferation of normal bone marrow cells in colony assays. Upon incubation of up to 10 ∝M LS104, bone marrow mononuclear cells showed normal differentiation to CFU-GMs with only slight reduction in colony numbers. Using the intrinsic fluorescence activity of the novel compound, a rapid and dose dependent cellular uptake lasting up to 24h could be demonstrated by FACS analysis and fluorescence microscopy. Combination of LS104 with the cytostatic drugs cytosine arabinosid (Ara-C) and daunorubicin (DNR) showed synergistic and additive effects in induction of apoptosis in cell-lines and primary AML blasts, respectively. *Conclusions.* Our data show that LS104 inhibits signalling of FLT3-ITD kinase activity and induces apoptosis in cell lines and primary AML blasts harbouring FLT3-ITD. CFU-GM growth of bone marrow mononuclear cells from normal controls was only slightly inhibited. Together, this indicates a therapeutic window which provides a preclinical framework for clinical testing of LS104 in FLT3-ITD-positive AML. A phase I trial for patients with refractory hematologic malignancies is currently in preparation.

0042

CHROMOSOME 21-ENCODED MIR-125B AND ITS ROLE IN THE LEUKEMOGENESIS OF MYELOID LEUKEMIAS IN CHILDREN WITH TRISOMY 21

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Background. Children with Down Syndrome (DS) have a 20 to 40 fold increased risk of developing acute myeloid leukemia (ML-DS), primarily the megakaryoblastic subtype (AMKL). Approximately 10% of newborns with DS show a transient myeloproliferative disorder (TMD) and more than 20% of the neonates with TMD suffer ML-DS within the first years of their life. Recently, acquired mutations of the transcription factor GATA1 have been identified in leukemic blasts from virtually all DS patients with ML or TMD. The factors on chromosome 21 that predispose to TMD or ML-DS are still unknown. So far four miRNAs (miR-99a, miR-125b, miR-155 and let-7c) which are localized on chromosome 21 are known. *Aims.* We hypothesized that there might be a role of miRNAs in the development of TMD and ML-DS. *Patients and Methods.* The expression levels of miRNAs were analyzed in sorted cells from patient samples [ML-DS (n=4), TMD (n=4), AMKL (n=4)], and megakaryocytes from healthy donors (n=3) by qRT-PCR. CMK, a TMD derived cell line, served as standard reference. For normalization, ribosomal 5S was used as housekeeping gene. Myeloid progenitor cells (MPC, CD34pos, CD33pos) were retrovirally transduced with MIGR1 containing miR-99a, miR-125b-2, miR-155 or let-7c, respectively (MPCmiR125b MPCmiR155 MPCmiR99a/let7c / MPCMIGR1). Green fluorescent protein (GFP) gene was used for selection of successfully transduced cells. MegaCult®-C (StemCell Technologies) and CollagenCultTM (Stem Cell Technologies) were used for the evaluation of proliferation and differentiation capacity. A cocktail of cytokines (FLT3 ligand, stem cell factor, thrombopoietin and interleukin 6) was added to stimulate proliferation. Expression of putative target genes of the analyzed miRNAs has been obtained by Gene Set Enrichment Analysis (GSEA) of recently reported gene expression profiles (Bourquin *et al.,* PNAS 2006), using the databases for the prediction of miRNA targets (Targetscan/ Pictar). Specific target genes of miRNA 125b such as CD61, CD34, and NCAM, were quantified by immunophenotyping. *Results.* miR-125b was clearly overexpressed in ML-DS (10.0 fold), TMD (5.6 fold) and AMKL (3.2 fold) compared to normal megakaryocytes (p patients vs. control <0.003). Comparable results were obtained with miR-155 (DS-ML 3.4fold; TMD 2.2fold; AMKL 2.3fold; p patients vs. control <0.02). By contrast, let-7c and miR-99a expressions did not differ from controls. In the colony assays the number of colonies of MPCmiR125b was significantly higher than from MPCMIGR1 (1.8 to 7 fold, χ^2=0.0004). Furthermore, MPCmiR125b were less differentiated than MPCMIGR1, analyzed by morphology (numbers of CFU-GM 0 vs. 9) and immunophenotyping (percentage CD34/CD33 26% vs 9%). GSEA revealed a significant decrease of the expression of miR-125b target genes. By contrast, MPCmir155 and MPCmiR99a/let7c revealed a strong inhibition of proliferation. *Conclusions.* MPC transduced by miR-125b revealed a high proliferation and reduced differentiation, which supports the hypothesis, that at least miR-125b is involved in the leukemogenesis of megakaryoblastic leukemias.

0043

FUNCTION AND CLINICAL APPLICATION OF EMBRYONIC GENE NANOG IN CANCER

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Background and Aims. Each year about 9,000 people die from acute myeloid leukemia (AML), a form of malignancy affecting the hematopoietic stem cells in the bone marrow. The average age of a patient with AML is 65 years old. Overall, the survival rate in adults under 65 is about 33%, but it is 4% for people over 65. The division of normal cells is a regulated process, but when that regulation breaks down, cells become cancerous and divide out of control. Only a few cancer cells have the ability to replicate indefinitely. They are called cancer stem cells, and they are responsible for cancer's long-term growth and possibly metastasis. The embryonic gene Nanog is responsible for maintaining the embryonic stem cells (ESCs) in an immature state to maintain their self-renewal capability and allow long-term proliferation. Our aim was to determine if decreasing Nanog expression could decrease proliferation in HL60 cancer cells as it does in embryonic stem cells. *Methods and Results.* Polymerase Chain Reaction (PCR) shows that Nanog is expressed in an AML cell line HL60, SA726 AML cells primary human AML cells, and circulating mononuclear cells from a patient diagnosed with glioblastoma, the deadliest and most common type of brain cancer. Nanog expression did not decrease when the SA726 AML cells were subjected to all-trans retinoic acid differentiation factor for a week. Trypan blue staining shows that cell proliferation decreased when HL60 was subjected to 25 micromolars of sodium orthovanadate. Real-time PCR suggests a decrease in Nanog expression after HL60 AML cells were subjected to 25 micromolars of sodium orthovanadate for 72 hours. Nanog and other embryonic genes may be highly specific cancer molecular biomarkers for new diagnosis and therapeutic targets. Possible cancer treatments

in the future may target Nanog and its signaling pathways using small molecule inhibitors or gene-based therapy by delivering shRNAi- or Zinc Finger Nuclease-plasmids to target cancer stem cells. Also, two suspended blastocyst-like clusters (Figure 1) were found on day 7 of SA726 AML cell culture on Mouse Embryonic Fibroblast feeder cells in Minimum Essential Medium 10% Fetal Calf Serum. These are indicative of embryonic behavior of the cancer cells.

Figure 1. Suspended blastocyst-like SA726 AML cell clusters.

Conclusions. These preliminary results suggest that a decrease in the cancer cell population happens concurrently with a decrease in Nanog expression. This may justify a future study to investigate a possible causal relationship.

Suspended blastocyst-like SA726 AML cell clusters

0044

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

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Background. Mutations in NPM1 exon 12 and the resulting shift of NPM1 into the cytoplasm are the most specific and frequent events in acute myeloid leukaemia 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(NPM1M). 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 IKB proteins and activated in the nucleous after degradation of IKB. The activation of NF-κB is responsible for chemoresistance to different drugs including anthracyclines. *Aims.* The aim of the study was to analyze the possible cytoplasmatic interaction between the mutated form of NPM1 and NF-κB. METHODS 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 and Western blot using NPM1 and p65 antibodies were performed to identify the amount and localization of both proteins. Co-immunoprecipitation assay was perform to study the interaction of the two proteins. To study the *in vitro* chemosensitivity, etoposide incubations were performed in both NPM1WT and NPM1M cells and apoptosis was evaluated. Downstream genes transcriptionally activated by NF-κB have been evaluated by RT-PCR and RQ-PCR. *Results.* NPM1M 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 NPM1M cells when compared to the NPM1WT cells (p=0,001). Immunofluorescence analysis confirmed the cytoplasmatic localization of NPM1M protein and the prevalent nuclear localization of NPM1WT. In addition, immunofluorescence analysis shows different pattern of NF-κB localization in NPM1M cells when compared to NPM1WT cells. In particular, in NPM1M cells NF-κB is mainly localized in the cytoplasm in the inactive form and in NPM1WT cells is mainly nuclear localized. These data were confirmed by Western blot. The cytosolic interaction of NPM1M 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 NPM1M cells. Finally, TNFα, a potent stimulus for NF-κB activation was unable to activate NF-κB in NPM1M cells. *Conclusions.* 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 NPM1M patients. These data may provide a possible explanation for the chemosensitivity observed in NPM1M 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.

0045

THE HOMEOBOX A9 GENE (HOXA9) IS REQUIRED FOR SURVIVAL OF HUMAN MLL-REARRANGED ACUTE LEUKEMIAS

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Background. Leukemias that harbor translocations involving the mixed lineage leukemia gene (MLL) possess unique biological characteristics and often have an unfavorable prognosis. Gene expression analyses demonstrated a unique profile for MLL-rearranged leukemias with consistent high-level expression of select Homeobox (HOX) genes including HOXA9, implicating an important role for HOX genes in MLL-translocation associated leukemias. *Aims.* In this study we aimed to elucidate the role of aberrant HOXA9 expression in human MLL-rearranged and non-rearranged leukemia's utilizing RNAi mediated knockdown. *Methods.* To establish efficient knockdown, 6 different shRNA constructs targeting human HOXA9 were synthesized applying the BROAD Institute (Cambridge, USA) selection algorithm to minimize off-target effects. The shRNA constructs were then stably introduced into a panel of 17 AML/ALL cell lines (7 MLL-rearranged, 10 non rearranged) and primary AML patient cells utilizing a pLKO.1 lentiviral vector system. The shRNA constructs which showed highest knockdown efficiency (75-80% knockdown) as measured by quantitative PCR and Western Blot analysis and no induction of interferon response genes were used for further experiments. *Results.* Initial analysis of phenotypic effects after HOXA9 knockdown in (9;11) MOLM-14 cells revealed sustained inhibition of cell proliferation 48h after transduction as determined by MTT assay followed by a progressive induction of apoptosis starting at 72h. This effect was almost completely rescued by introducing an exogenous HOXA9 expression vector. To investigate if the HOXA9 knockdown related effects are specific for MLL-rearranged cells, cell growth and viability were next analyzed in the 17 AML/ALL cell line panel. Interestingly, impaired cell proliferation and induction of apoptosis was significantly higher in the MLL-rearranged cell lines than in the non-rearranged cells (mean viability at 72h: 51% vs. 91%: p=0.007) and significantly correlated with the baseline HOXA9 mRNA expression before knockdown, with the greatest effect in high-level HOXA9 expressing cells (R=0.8, p=0.00017). Analysis of MLL-rearranged and non-rearranged primary human AML cells demonstrated a similar induction of apoptosis after HOXA9 knockdown with a higher effect on viability in association with the presence of an MLL translocation (p=0.004) and high-level HOXA9 expression. Expression analysis after HOXA9 knockdown revealed concomitant downregulation in a gene set previously found to be aberrantly expressed in human MLL-AML and also associated with *self-renewal* in a recently defined murine MllAf9 leukemia stem cell model, including HOXA5, HOXA10, MEIS1, RUNX2 and MEF2C. To assess the *in vivo* effects of HOXA9 knockdown, luciferase-expressing SEMK2 (t4;11) cells were transduced with HOXA9 shRNA or GFP control shRNA and transplanted into SCID-beige mice. Engraftment and leukemia burden was monitored by subsequent *in vivo* bioluminescent imaging. There was a significantly lower leukemia burden in the HOXA9 group when compared to controls with a peak difference at day 15, shortly before the control group animals succumbed to overt leukemia. At this point, all mice in the HOXA9 group were still clinically healthy. *Conclusions.* Our data implicates an important role for aberrant HOXA9 expression in the survival of human MLL-rearranged leukemias and suggests targeting HOXA9 or downstream programs may be a novel therapeutic approach which warrants further exploration.

Acute myeloid leukemia - Clinical I

0046

PROGNOSIS OF ACUTE MYELOID LEUKEMIA IN FIRST RELAPSE: IMPORTANCE OF THE HOVON/SAKK INDEX

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Background. The outcome of adults with acute myeloid leukemia (AML) in first relapse is highly unsatisfactory, as very few patients achieve durable second complete remissions (CR) with intensive reinduction chemotherapy (CT). Breems et al recently proposed a Prognostic Index (PI) to be applied to patients ≤ 60 years in first relapse, which may prove to be a major tool in therapeutic decisions. Aim. To evaluate the reproducibility of this PI in a cohort of uniformely treated patients at a single center. Methods. Of 151 consecutive new patients (age 17-60 years) with de novo or secondary AML (excluding promyelocytic leukemia) treated from May 98 to April 06, 107 achieved CR1, among whom 55 had relapsed as of June 06. The 50 patients who received intensive reinduction CT are the object of this analysis. Patients were stratified into the 3 risk groups of the PI according to the following parameters: length of CR1, cytogenetics at diagnosis, age at relapse, and whether previous stem cell transplantation had been performed. Overall survival (OS) from relapse was compared between the 3 groups. *Results.* Forty two percent of the patients achieved CR2. The median OS was 5 months; the overall 1-year survival rate was 25%. The PI defined 3 groups with significantly different 1-year OS: 100% for the favorable group (comprising 8% of the patients) 40% for the intermediate-risk group (42% of the patients) and 4% for the poor-risk group (50% of the patients) [$p<0.05$]. *Conclusions.* The distribution of our patients among the 3 prognostic groups is remarkably similar to the original HOVON/SAKK cohort. This index is simple, reproducible, and segregates subsets of patients with very different outcomes at relapse. It should be used to identify candidates to conventional salvage CT vs investigational strategies or palliative support.

0047

IMPAIRED NUTRITIONAL STATUS AMONG RUSSIAN AND NORWEGIAN PATIENTS RECEIVING INTENSIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA

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Background. The 5-year survival rarely exceeds 50% for patients with acute myeloid leukemia (AML) treated with intensive chemotherapy. During treatment repeated and prolonged hospitalisations, often in isolation wards, are necessary. Our clinical experience is that body weight is reduced and the patients experience nausea, loss of appetite and fatigue, indicating that during treatment the nutritional status of the patients deteriorates. However, these alterations have not been quantified or systematically studied. Such documentation is necessary in order to give recommendations concerning nutritional supplements. *Aim.* In this prospective study we investigated the nutritional status in AML patients receiving intensive chemotherapy treatment. *Methods.* From 2004-2006 we recruited newly diagnosed adult AML patients consecutively admitted to either Pavlov University (n=26, mean age 60 years) or to Ullevaal University Hospital (n=14, mean age 46 years). Written, informed consent was obtained from all patients, and the protocol was approved by ethics committees in both countries. The patients were treated with induction chemotherapy consisting of cytarabin and daunorubicin followed by 3-4 courses of consolidation therapy with cytarabin every 3-4 weeks. Nutritional status was monitored at diagnosis, at start of each chemotherapy course, and finally 9 months after diagnosis, or until they died or underwent allogeneic stem cell transplantation. Measurements included body mass index (BMI), skinfold thickness (a marker of fat tissue), hand grip strength (a marker of muscle mass) and blood samples. *Results.* Among the Russian patients 20 (64%) did not survive beyond the induction therapy, and 6 (19%) completed the study. The corresponding numbers among the Norwegian patients were 14 (100%) and 8 (57%). The Table 1 shows significant and similar reductions in both country-groups of markers of anthropometry as well as decreased levels of albumin and transferrin, sex-hormones and fat-soluble vitamins. Intriguingly, the gonadotrophines and vitamin A remained low nine months after diagnosis. Neither BMI, thyreoid status nor the plasma levels of water soluble vitamins (B12, B6 and C) changed in either country-group during follow-up. *Summary.* For a successful outcome of AML treatment, repeated intensive chemotherapy courses are necessary. Such treatment apparently leads to a catabolic condition characterized by decreased fat and muscle mass, lowered fat soluble vitamin levels, and hypofunction of the pituitary-gonadal axis. Whether nutritional support is warranted during this treatment requires intervention studies aimed at improving the nutritional status among AML patients.

Table 1.

0048

FRACTIONATED DOSES OF GEMTUZUMAB OZOGAMICIN (3,3,3 REGIMEN) IN ADDITION TO ESCALATED DOSES OF DAUNORUBICIN AND CYTARABINE FOR THE REINDUCTION TREATMENT OF RELAPSED AML: A PHASE 1/2 STUDY OF THE ALFA GROUP

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Gemtuzumab Ozogamicin (GO, Mylotarg®) used as a single agent at a dose of 9 mg/m^2 for 2 doses given 14 days apart was shown to induce a 26% response rate (RR) in patients (pts) with relapsed AML. However grade 3-4 liver toxicity was observed in 20% of patients with a risk of VOD. To avoid excessive toxicity lower dosage of GO are proposed in trials combining GO in addition to conventional induction chemotherapy for untreated AML pts . The MRC AML15 trial used a dose of GO of 3 mg/m^2 on day 1 of induction course with DA, DAE or FLAG-IDA with promising results in terms of DFS. The ongoing SWOG study is using a dose of GO of 6 mg/m^2 on day 1 with DNR: 45 mg/m^2×3 days (d) and Ara-C 100 mg/m^2 × 7d. We adressed the question of the possibility of using a higher cumulative dosage of GO in addition with a 3+7 regimen. In a previous phase 2 study in 57 relapsed AML patients we demonstrated that fractionated doses of GO: 3 mg/m^2 on days 1,4 and 7 (3,3,3 GO regimen) had a good efficacy/safety profile without excessive hematological and liver toxicities (no grade 3-4 liver toxicity).[1] Thus, we designed a phase 1/2 trial to assess the safety and activity of escalated doses of DNR and AraC in addition to the 3,3,3 GO regimen. Three dose levels of a 3+7 chemotherapy regimen were tested: level 1: DNR: 45 mg/m^2 + AraC: 100 mg/m^2; level 2: DNR: 60 mg/m^2 + AraC: 100 mg/m^2; level 3: 60 mg/m^2 + AraC: 200 mg/m^2. Inclusion criteria were relapsed AML patients over 50 years. To date, 11 patients have been treated: 5 in the first dose level, 4 in the second, 2 in the third. Median age was 65 years (range: 55-75). During the induction period, grade 3-4 adverse events included: sepsis in 3 patients (dose level 1, 1 pt; dose level 2, 2 pts), bleeding in 1 patient (dose level 1), diarrhea in 2 patients (1 pt in first and second dose level), pneumonia in 2 pts (1 pt in first and second dose level). No grade 3-4 elevated liver function tests nor hyper-

bilirubinelia were observed and no pts developped VOD. 3 pts died during the induction period. Causes of death were disease progression in 2 pts (dose level 2) and sepsis in 1 pt (dose level 2). 5 pts achieved complete remission (dose level 1, 2 pts, dose level 2, 1 pt and dose level 3, 2 pts). Median duration of neutropenia <0.5 10^9/L was 25 days and median duration of thrombocytopenia <50.10^9/L was 28 days for responding patients. We conclude that combining a 3,3,3 GO regimen with conventional doses of DNR and AraC regimen is feasible. Inclusions in level 3 cohort are ongoing.

Reference

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0049

ADDING LOW DOSE GEMTUZUMAB OZOGAMICIN TO FLUDARABINE ARA-C AND IDARUBICIN MAY IMPROVE DFS AND OS IN ELDERLY PATIENTS WITH NON M3 AML: RESULTS OF A PROSPECTIVE, PILOT, TRIAL AND COMPARISON WITH A HISTORICAL COHORT OF PATIENTS

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Background. Elderly AML patients and patients with AML evolved from MDS or therapy related display a very poor prognosis. In the last decade the association of fludarabine, Ara-C and anthracycline proved to be an effective and well tolerated induction regimen for this group of patients and, more recently, the introduction of gemtuzumab ozogamicin (GO) has opened new perspectives in the treatment of AML. *Methods*. We report here final results of a prospective multicentre trial testing the combination of chemotherapy (Fludarabine 25 mg /sqm, Ara-C 1 g /sqm, idarubicin 5 mg / sqm all for 3 days) followed by gemtuzumab ozogamicin (3 mg/sqm at day 4), for induction treatment of patients with CD33+ acute myeloid leukaemia (AML). Responding patients received the same regimen as consolidation therapy. *Patients*. Forty-six consecutive patients were treated between May 2004 and September 2006. The median age was 66 years (60-80), the karyotype was unfavourable in 12 patients (26%), intermediate in 33 (71%) and favourable in 1 (3%). *Results.* Neutrophil (N>0.5×10^9/L) and platelet (Plt > 25×10^9/L) recovery required a median of 17 days (range 10-26) and 16 days (range 7-30) from the end of therapy, respectively. There was one early death during induction. Eleven major infectious complications were recorded (sepsis in 6 patients, pneumonia in 1, pleuritis in 1, aspergillosis in 3). Non-haematological toxicity was very mild. In particular, none of the patients experienced grade 3 or 4 hepatic toxicity, but 4 patients had transient elevation in liver function tests (specifically bilirubin). No evidence of VOD or sinusoidal obstructive syndrome was documented. Of the 45 evaluable patients 24 achieved a complete remission (52%), 66% and 33% in good-intermediate /poor karyotype patients, respectively. Median duration of CR is 8 months (3-26). The cumulative incidence of relapse is 37% with an actuarial 3 year survival of 54%. These results were compared with 47 patients matched for age and karyotype who received the same treatment (FLAI), without GO. The proportion of patients achieving CR was comparable. However, patients with de novo AML receiving GO (n=26) had a significantly lower risk of relapse at 2 years when compared to patients not receiving GO (n=35) (40% vs 80%, p=0.01) and significantly better overall 2 years survival (40% vs 14% p=0.02). Patients with secondary AML had comparable outcome whether or not they were given GO. *Conclusions.* this GO based induction chemotherapy can be given to elderly patients with AML with a good toxicity profile. In keeping with a recent prospective randomised trial, the addition of GO seems to reduce the risk of relapse and therefore to prolong disease free survival.

0050

AN ANTHRACYCLINE FREE REGIMEN BASED ON CONTINUOUS SEQUENTIAL INFUSION OF FLUDARABINE AND CYTARABINE FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA. RESULTS FROM A PHASE II STUDY ON 100 PATIENTS

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New approaches are needed for elderly patients with acute myeloid leukemia (AML). The combination of fludarabine (F) with cytarabine (ARA-C) ± G-CSF has been proven to be effective in refractory or relapsed AML. However, little is known as concerns continuous sequential infusion of the two drugs in newly diagnosed patients. In a phase II study, we investigated the efficacy and toxicity of a regimen including F + ARA-C administered as sequential continuous infusion (CI-FLA) in a series of untreated non-M3-AML patients aged more than 60 years. F at loading dose of 10 mg/sqm over 15 min at day 0, and after three hours and half ARA-C at a loading dose of 390 mg/sqm over 3 hours were given; at the end, F at 20 mg/sqm/ci/24 hours for a total of 72 hours and ARA-C at 1440 mg/sqm/ci/24 hours for a total of 96 hours were started. G-CSF was added at day +15 at a dose of 5 microg/kg. Patients achieving CR were programmed to receive an additional course of CI-FLA. After consolidation, G-CSF at 10 microg/kg was given from day 15 in order to mobilize $CD34^+$ cells. Between June 2001 and June 2006, 100 patients received the treatment. Median age was 68 years (range 61-81). In 42 patients (42%) an antecedent myelodysplastic syndrome preceded overt AML. Cytogenetic analysis showed normal karyotype in 50 patients, complex karyotype or other unfavourable chromosomal abnormalities in 38 cases, no mitoses in 12 cases. Finally, 65 patients were affected by one or more concomitant diseases requiring specific treatment. Overall, 63 (63%) patients achieved CR, all but one following one course of CI-FLA. There were 18 induction deaths (18%), while 19 patients (19%) were refractory to induction treatment. The median number of days to neutrophil > 0.5×10^9/L and platelet > 20×10^9/L was 19 (7-34) and 19 (9-38), respectively. Patients needed a median of 3 platelet units (0-19) and 7 blood units (1-38), respectively. Most patients required broad spectrum empiric antibiotic therapy, while 19/100 cases (19%) needed intravenous antifungal treatment. Documented infections occurred in 15 cases (15%). Forty-four patients out of 63 (70%) were eligible for the programmed consolidation course. Five of the first 20 consolidated patients died from infectious complications during subsequent pancytopenia; therefore, the remaining last 24 patients received reduced consolidation with F+ARA-C at 48 and 72 hours, respectively. Forty-four patients were monitorized for the mobilization of $CD34^+$ cells, collection being successful in 33 (75%). Median number of $CD34^+$ cells/kg collected was 6.8×10^6 (2-60.3), median number of apheresis being 2 (1-2). Overall, 27 (27%) patients received autologous stem cell transplantation (ASCT). Disease free survival and overall survival are 11 and 13 months, respectively. Survival at 5 years is projected to 25%. In conclusion, this study demonstrates that CI-FLA is an effective and well-tolerated regimen for elderly patients with AML. Therapeutic results are extremely encouraging as to CR achievement, $CD34^+$ cell collection and ASCT feasibility and compare favorably with conventional anthracycline/ARA-C based therapy.

0051

FACTORS INFLUENCING THE COLLECTION OF PERIPHERAL BLOOD STEM CELLS IN ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

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Several studies have investigated factors influencing peripheral blood stem cell (PBSC) mobilization in patients with non myeloid malignancies; conversely, there are very few reports on low number of patients concerning the efficiency of PBSC mobilization in patients with acute myeloid leukemia (AML). We analyzed the effects of different potentially influential variables on successful mobilization of CD34 positive ($CD34^+$) from a series of 160 consecutive patients with AML in first complete remission (CR1). Data were collected from a cohort of 160 consecutive patients with AML in CR1. The median age was 52 years (14-78). There were 134 patients with de novo AML (84%) and 26 patients (16%) with secondary AML (s-AML). According to MRC criteria, cytogenetic findings at diagnosis were classified as favourable in 11%, intermediate in 69% and adverse in 20% of patients. Patients up

to 60 years (n=116, or 72%) received anthracycline + cytarabine + etoposide as induction, followed by consolidation including intermediate dose cytarabine + mitoxantrone, while those aged more than 60 (n=44, 28%) were treated in induction/consolidation with fludarabine/cytarabine given as continuous sequential infusion. Consolidation therapy followed by G-CSF at 10 microg/sqm given from day 15 to the last apheresis was used as mobilization regimen. The following variables were analyzed: age > vs 60 years, de novo vs s-AML, anthracycline vs fludarabine based induction treatment, cytogenetics at diagnosis, presence of FLT3 mutations (either ITD or D835 mutation), WBC at diagnosis (more or less than 50×10^9/L), number of courses (i.e. 1 or 2) needed for achievement of CR. The above parameters were correlated to successful mobilization (defined as collection of $>2\times10^6$/L). Overall, 137 patients (86%) had a successful mobilization of stem cells, while 23 (14%) failed to mobilize. The median number of $CD34^+$ cells collected was 6.9×10^6/L (range 2.1-25). Either univariate or multivariate analysis (see Table 1) failed to demonstrate significant influence on successful mobilization for any of parameter which were considered into the study. More in detail, no effect was observed as median number of $CD34^+$ cell collection, median number of apheresis, peak of SC in PB and median number of $CD34^+$ cells collected per single apheresis were concerned. In addition, none of the above parameters was significantly related to hematopoietic reconstitution after autologous stem cell transplantation in terms of WBC and platelet recovery, while hematopoietic recovery was significantly related to the number of $CD34^+$ infused cells. We conclude that successful mobilization in AML is unpredictable by using the variables analyzed in our study.

Table 1. Multivariate analysis of different paramenters on mobilization.

0052

THE DETERMINATION OF LEUKEMIC PHENOTYPE AND MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA BY USING FLOW CYTOMETRY

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The hematological remission in acute leukemia is defined as the achievement of complete hematological recovery in peripheral blood and the a decrease of leukemic blasts below 5%. The persistency of malignant cells below this threshold level (<5%) is called as minimal residual disease (MRD). A number of publications have reported that the presence of MRD after the treatment(s) might predict relapse of the underlying disease. One of the covinient methods used for the detection of MRD is immunophenotyping with flow cytometry (FCM). In this study we aimed to evaluate both the leukemic cell immunophenotype at the diagnosis and the amount of MRD at the follow-up in AML patients (using FCM events). 166 patients diagnosed as AML between January 2004 and June 2006 in our center were analyzed retrospectively. Median age was 49 years. Male/Female was 104/62. Method: Immunophenotyping at the period of diagnosis was performed by using the monoclonal antibodies specific for CD45, HLA-DR, CD34, CD33, MPO, CD15(CD16), CD13, CD24, CD11b, CD117, CD64, CD10, CD2, CD19, TdT and (CD4)(CD7). The data were collected in FC500 (Beckman Coulter, France) flow cytometer and were analyzed using the RX-P software program. We usually analyzed approximately 10.000 cells at the diagnostic samples and at least 500.000 cells at the follow-up samples for the detection of MRD. The classification of MRD was evaluated in four groups according to Kern et al recommendations (Crit Reviews Oncol/Hematol 2005). *Results.* The diagnostic samples in AML patients for FCM were obtained from bone marrow (n=133) or peripheral blood (n=33). The median value of immature cell ratio was 63% (21-98%). We detected leukemia associated immunophenotype (LAIP) in one and/or more than one categories at the diagnosis (Table 1).

Table 1.

Clssification	*LAIP*	*The detected parameter*	*%*
Cross-lineage (n=55 patients)	CD2+,CD19+, CD4+, CD7+ etc	61	7.05
Asynchronous (n=148 patients)	CD117/34/33±13 CD11b+/CD117+/CD34+ CD34+/CD15+/CD33+/±CD13+ etc	289	33.4
Lack of expression (n=patients)	CD15+/CD33+/CD13- HLA-DR+/CD34-/CD13+ OR CD33+ CD11b+/CD24-/CD34+ OR CD117+ etc	298	34.5
Overexpression (n=111 patients)	CD11b-/CD117+/CD34+(+) CD15+/CD13++/CD33++ HLA-DR++/CD33++/CD34++ ect	217	25.1
Total		865	100

In summary The 55 of the diagnostic samples, were positive for cross-lineage expression of lymphoid antigen, 148 show asynchronous expression of antigens, 155 present a lack of myeloid antigen expression and 111 have had myeloid antigen overexpression. The most frequent LAIP in the diagnosis was the persistency of immature myeloid antigen during the maturation (33.4%) or the lack of expression of myeloid antigen (34.5%). Only 73 patients were able to be analysed for MRD on day of 14th to 54th following remission induction therapy. We could not evaluate 17 patients (23%) for MRD at the follow-up in which hematological remission was not achieved after a remission-induction therapy. Eighty-nine percent of 56 patients in the remission had any LEIP permitting for the evaluation of MRD. The amount of MRD was 0.1% to 10% in different follow-up periods. We observed relapse within six months in 36% of 55 patients having MRD. *Conclusions.* We found less myeloid-antigen associated abnormal immunphenotype (7.65%) in the diagnostic samples compared with the previous published studies (10-24%). However, the ratio of leukemic immunophenotype was detected as 92.6%. Evaluation of MRD with regular follow-up of FCM events may allow us to predict the probability of relapse.

0053

WILMS' TUMOUR 1 MUTATION IN NORMAL KARYOTYPE AML

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Background. Our recent studies demonstrated that the Wilms' tumour 1 (WT1) gene represents an important mutational target in normal karyotype (NK) Acute Myeloid Leukemias (AML) (Fitzgibbon *et al.,* 2005; Summers *et al.,* 2007). In this study, we have set out to determine the potential clinical relevance of these mutations. *Methods.* Diagnostic NK AML patient DNA samples (n=285: de novo 272, secondary 13) were obtained from previously conducted Medical Research Council AML 10 and 12 trials and AML cell lines (n=67) from the DSMZ-German Collection of Microorganisms and Cell Cultures. Mutation analysis for WT1 was performed using standard PCR-based direct sequencing and high-resolution Capillary Electrophoresis (CE) of exons 7 and 9. FLT3 and NPM mutation status were obtained using standard approaches. Statistical analysis was performed using the Mantel-Haenszel test for initial response, and log-rank test for long term outcomes. *Results.* Mutation of WT1 was observed in 32/285 (11%) NK AMLs. These occurred predominantly in exon 7 (26/32) with the vast majority being frameshift mutations (30/32 cases) arising as a result of insertions or deletions (range 1-28 bps). Two non-synonymous substitutions, D396N and H397Y of

unknown significance were identified in single cases. Mutations could be separated into 3 distinct patterns based on CE mutant quantification: low (6 cases < 30% mutant), intermediate (15 cases 30-60% mutant) and high (9 cases > 70% mutant) mutation load. There was no significant difference in the frequency of FLT3-ITD (47% Vs 34%) and NPM mutation (50% vs 62%) between WT1(M) and WT1(WT) cases. Patients with mutations in WT1 had an inferior initial response to therapy (Complete Remission [CR] WT1(M) 75% Vs WT1(WT) 90%, Odds Ratio [OR] 4.04, Confidence Intervals [CI] 1.30-12.5; p=0.02) and a higher rate of Resistant Disease [RD] (RD WT1(M) 16% Vs WT1(WT) 5%, OR 5.85, CI 1.29-26.4; p=0.02). Although Overall Survival (OS 34% vs 47%) and Disease Free Survival (DFS 33% vs 47%) were lower in the WT1(M) Vs WT1(WT) cases, this did not reach statistical significance. CE mutation analysis in 67 AML cell lines yielded mutation in the UF-1 (PML-RARA) and CTS (MLL-AF6) cell lines suggesting that WT1 mutations are likely to occur in other cytogenetic risk groups. *Conclusions.* WT1 mutations occur in 11% of NK AML at diagnosis. Mutations result in insertion or deletions in the DNA binding domain of the protein and have the potential to interfere with WT1 derived minimal residual disease detection. The locations of the common epitopes used for immunotherapy locate outside of the mutated region. WT1 mutations are associated with a lower CR rate and a higher rate of RD. Although OS and DFS are lower in the mutated group, this did not reach significance. Additional cases are being investigated to determine whether WT1 mutation status warrants inclusion as a poor risk marker in AML.

0054

ANALYSIS OF NPM1 MUTATIONS USING A SIMPLIFIED DNA-BASED PCR APPROACH DEMONSTRATES THAT ONE THIRD OF YOUNGER ADULT PATIENTS WITH NORMAL KARYOTYPE CARRY A MUTATION

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Background. There is a need for guidelines to improve risk stratification of patients with AML who have a normal karyotype. In 1999, it was discovered that rearrangements of the FLT3 gene confer a poor prognosis in AML. These were found in around 25% of AML, mostly in patients with a normal karyotype. In 2005, it was discovered that mutations in the NPM1 gene conferred a favorable prognosis. Analysis of these two mutations greatly improves prognostic ability for normal karyotype AML. The analysis of NPM1 mutations has generally been performed using histochemistry, sequencing of DNA, or RNA-based methods (RT-PCR) however many laboratories do not have the facilities to perform these analyses. Furthermore, as FLT3 analysis is performed on DNA, it is advantageous to have a DNA-based method to study these two aberrations in parallel. *Aims.* To establish and test a DNA-based method of NPM1 analysis. *Methods.* We have developed a DNA-based method for NPM1 analysis using PCR and melting temperature which gives rapid clearcut results which demonstrate the presence of a mutation. Although this method does not detect the specific type of mutation, this does not influence prognosis, and it is not crucial to determine it. We have used this method to analyze 154 patients with AML (primary and secondary) for both NPM1 and FLT3 mutations. 60 of these had a normal karyotype. FLT3 length mutations were analyzed by PCR and acrylamide gel electrphoresis. *Results.* Figure 1 shows a typical result of such an analysis. The tracing with a single peak represents normal DNA. The tracing with two peaks represents an AML patient sample which carries an NPM1 mutation.

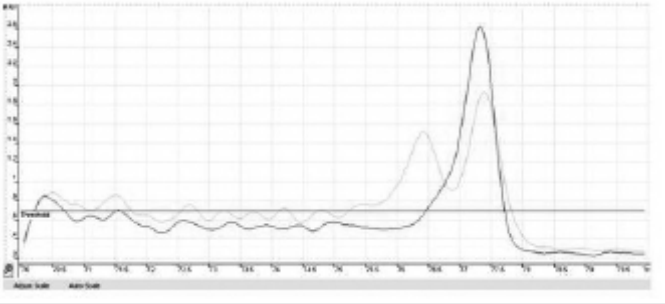

Figure 1. Melting curve analysis for NPM1 mutations.

In total, we found that 21 patients had NPM1 mutations, of whom 20 had a normal karyotype, representing 33% of normal karyotype AML patients. Of these, 10 patients also had FLT3 rearrangements, whereas 10 had only NPM1mutations without FLT3 rearrangements. Although our sample is small, our findings were in accordance with the literature: patients with NPM1 mutations had a longer survival than those without such mutations, but only if FLT3 rearrangements were not present. Two thirds of the NPM1 positive patients were female. Our percent of normal karyotype/NPM1 positive patients (33%) is somewhat lower than that reported in the literature [45.7% (Theide, 2006) to 50-60% (Falini, 2006))]. The slightly lower percent could be due to a number of factors, such as the small sample size of our group. Alternatively, it could be due to the younger age of our patient population. The mean age of our patients positive for NPM1 mutations was 46 years (range 17-79) as compared to 48 (17-87) without NPM1 mutations. Falini (2005) reported that the NPM1 positive patients were significantly older (51.8 years) than NPM1 negative patients (41.9 years) (Falini, 2005). Thiede (2006) also reported a slightly older age for NPM1 positive compared to negative patients, in a population whose median age was 60 years. Summary and *Conclusions.* We conclude that melting temperature DNA-based NPM1 analysis is useful in rapid determination of NPM1 status. Patient age may influence the development of NPM1 mutations.

0055

ARE COMORBIDITY SCORES USEFUL DECISION MAKING TOOLS FOR INTENSIVE CHEMOTHERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA?

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Aim. We have recently reported results of standard intensive chemotherapy in 416 patients with AML aged 65 years or more (median, 72 years) treated in the ALFA-9803 trial. We report here on the impact of pretreatment comorbidities on mortality before day 100 (d100 mortality, 22% in the whole patient population) and survival in this large cohort of patients. *Methods.* For each patient, we calculated the original Charlson Comorbidity Index (CCI) (Charlson, J Chron Dis 1987) and the refined Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) (Sorror, Blood 2005). *Results.* As expected, both scores correlated pretty well (CCI 0/1/2=353/57/6; HCTCI 0/1/2/3+ = 268/85/42/21; p<0.001). Due to usual inclusion criteria which represent a first important selection step including notably the absence of heart or renal failure, only 5 comorbidities were observed in at least 5% of patients : coronary artery disease (10%), arythmia (8%), infection (8%), diabetes (7%), and chronic pulmonary disease (5%). Neither CCI or HCTCI correlated with advanced age in this patient population. Performance Status (PS) correlated strikingly with initial WBC but not with HCTCI, making it more a characteristic of AML impact on host status than purely host-related. In multivariate analysis, the HCTCI influenced d100 mortality (p=0.01) and overall survival (p=0.008) more significantly than the original CCI. Other independent factors were age, PS, and cytogenetics. Such an additive weighted score is, however, of relatively limited interest for decision making at the individual patient level. An HCTCI>1 demarcated only 15% of high-risk patients with a d100 mortality rate of 35% and higher HCTCI levels only 5% of patients or less. In addition, the predicted d100 mortality was 21% in a patient with HCTCI>1 due to cardiac abnormality and/or diabetes and/or obesity versus 47% in a patient with HCTCI=1 due to infection only, indicating that some HCTCI items did not impact on outcome. Further analysis of the prognostic impact of each comorbidity or combination led us to propose only four independent criteria (pre-treatment documented infection, chronic pulmonary disease, PS>1 if age>74y, and high-risk cytogenetics) to consider a patient as unfit for intensive chemotherapy, even if he/she fulfilled all usual inclusion criteria. This 4-criteria non-additive tool was associated with a higher decisional value allowing to demarcate 28% of patients with a d100 mortality rate of 43% and a specificity of 86%. This new definition of unfit patients was validated in a set of patients from one independent French institution.

0056

PROGNOSTIC SIGNIFICANCE OF FLT3/ITD MUTATION IN PATIENTS WITH AML1/ETO ASSOCIATED ACUTE MYELOID LEUKEMIA

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Background. AML1/ETO fusion gene in acute myeloid leukemia (AML) usually predicts a good response to chemotherapy with a high remission rate and a relatively long median survival. An internal tandem duplication of the FLT3 gene (FLT3/ITDs) is known to be associated with poor outcome in AML patients. In these days, animal study showed that the dual presentation of AML1/ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. *Aims.* To determine the prognostic role of FLT3/ITD mutation in patients with AML1/ETO positive AML. *Methods.* FLT3/ITD mutation status was evaluated by performing DNA polymerase chain reaction assays on 38 bone marrow samples obtained at initial diagnosis from the patients with AML1/ETO fusion gene positive AML. GeneScan analysis was performed to confirm the FLT3/ITD mutation and to measure mutant levels. *Results.* Of total 38 patients, 9 patients (23.7%) demonstrated the aberrant FLT3/ITD mutations. The median age of patients was 37 years (range, 18-75 years). There were 23 males (60.5%) and 15 females (39.5%). There was no statistically significant difference in age, gender, leukocyte count, hemoglobin level, platelet count and percentage of peripheral or bone marrow blasts between the patients with FLT3/ITD and the patients without FLT3/ITD. To analyze the response to or outcome of therapy, we evaluated 33 patients who received intensive induction chemotherapy containing cytarabine. In univariate analysis, there was no significant difference in complete response rate (100% vs. 95.8%, p=0.53). However, the presence of FLT3/ITD was associated with higher relapse rate in these patients (77.8% vs. 30.4%, p<0.05). A trend for shorter leukemic-free survival (LFS) was observed in patients with FLT3/ITD compared with those without FLT3/ITD, but there was no statistical significance (8.7±2.7 ms vs. 18.9±7.9 ms, p=0.26). Also, there was no significant difference in median duration of overall survival (p=0.79). *Conclusions.* This study demonstrates that the presence of FLT3/ITD mutations is a significantly higher relapse rate in AML1/ETO positive patients. Therefore, a stratified treatment plan such as stem cell transplantation may be warranted for the AML1/ETO positive AML harvoring FLT3/ITD mutation.

0057

HAEMORRHAGE AND THROMBOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS: RELATION TO THROMBOTIC MARKERS

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Background. APL patients present with laboratory signs of disseminated intra-vascular coagulation. Clinical manifestations include both severe haemorrhage and thrombosis. Remission induction therapy with all-trans retinoic acid (ATRA) has produced high rates of complete remission and a rapid resolution of the APL-associated coagulopathy. *Aim.* In this study, in newly diagnosed APL patients, we prospectively evaluated: 1. The rate of haemorrhage and thrombosis; 2. The plasma levels of thrombotic markers before and after starting remission induction therapy; 3. The predictive value of these markers. *Methods.* Forty-four consecutive patients with APL (F/M = 20/24; age range = 8-84 years) were enrolled at our Centre from January 2000 to January 2007. All patients received induction therapy with Idarubicin + ATRA (GIMEMA AIDA 2000 protocol) and were prospectively monitored for thrombo-haemorrhagic episodes for 8 weeks. Blood samples were obtained from 18 of the 44 patients, at baseline (before ATRA = T0), and at days 7 (T7), 15 (T15), and 25 (T25) after starting ATRA. Control samples were from 25 healthy subjects. Plasma levels of clotting activation markers were measured by ELISA, including: D-dimer, thrombin-antithrombin complex (TAT), tissue factor pathway inhibitor (TFPI), and activated factor VII-antithrombin complex (FVIIa-AT). *Results.* At disease onset, among the 44 patients, 8 had haemorrhages (18%), including 3 (6.8%) fatal intracranial bleeding (1 before starting ATRA, and 2 after 1 and 3 days ATRA, respectively) and 5 non-fatal major bleedings (11.3%) at days 1-3 of therapy; furthermore 3 patients (6.8%) presented with thrombosis, including 1 fatal Budd-Chiari syndrome (2.2%), and 2 non-fatal events (4.5%). During induction therapy, 3 patients (9.0%) developed thrombosis at day 9, 15 and 40, respectively. The study of plasma markers in the subgroup of 18 patients showed that at T0, the levels of D-dimer, TAT, TFPI, and FVIIa-AT were all significantly (p<0.05) higher in APL patients than controls. During ATRA therapy the thrombotic marker levels declined. However, after a week (T7), D-dimer and TAT were significantly (p<0.05) reduced compared to T0, whereas TFPI and FVIIa/AT levels persisted elevated. Thereafter, TFPI level significantly decreased at T15 (vs T0, p<0.05), and last, FVIIa-AT showed a significant reduction at T25 (vs T0, p<0.05). Three of the 18 subjects of this sub-group had thrombosis, after 1, 9, and 15 days of ATRA, respectively, none had major haemorrhages. Among all markers, the pre-treatment TFPI levels > 74.9 showed a significant predicting value for thrombosis by the Fisher's exact test (RR= 5.14; CI = 85.34-60.81, p<0.05). *Summary and Conclusions.* These data confirm a significant rate of early deaths in APL due to the coagulopathy, in the ATRA era. 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, which suggests a role for these markers in the follow up of these patients. Finally, TFPI levels at diagnosis may be a promising marker of increased thrombotic risk, although this needs to be tested by large prospective clinical studies.

0058

FLOW-CYTOMETRIC ASSESSMENT OF MINIMAL RESIDUAL DISEASE DISCRIMINATES CATEGORIES OF RISK AMONG ADULT AML PATIENTS WITH INTERMEDIATE KARYOTYPE

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Background. According to the prognostic classifications of karyotypic abnormalities of AML (MRC and SWOG), the intermediate group includes patients either lacking good and poor karyotype or with normal karyotype. Therefore, it represents, by definition, a miscellaneous group for which the evaluation of the better treatment strategy is difficult due to its heterogeneity. Moreover, patients belonging to this intermediate group account for the large majority of AML cases enrolled into clinical trials. *Aims.* The aim of our study was to analyze the factors specifically affecting the outcome of patients bearing intermediate risk karyotypic abnormalities in a group of 121 AML cases entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61yrs) or AML13/AML15 (age >61 yrs), consisting in intensive induction and consolidation cycles. *Methods.* The clinico-biological variables evaluated in our model included age, FAB, WBC count, MDR1 phenotype, FLT3 mutations and level of post-consolidation bone marrow residual leukemic cells (BMRCL), assessed by multiparametric flow-cytometry (MPFC). By applying the maximally selected log-rank statistics, the threshold discriminating MRD negative from positive cases was set at 3.5×10^{-4} BMRLC, a level that allowed the identification of distinct subgroups of MRD^- and MRD^+ patients at post-Cons time-point.

Figure 1.

Results. Patients with $<3.5\times10^{-4}$ BMRLC at the end of consolidation therapy were considered MRD- and showed a better outcome, patients whose level of MRD were $>3.5\times10^{-4}$at the end of consolidation were considered MRD+ and showed a poor prognosis. Using the MRC classification, 17/121 patients (14%) had a good-risk cytogenetics, 97/121 (80%) an intermediate-risk and 7/121 (6%) a poor-risk. When we restricted the analysis to cases with intermediate-risk karyotype we found that: 1) patients in the MRD- and MRD+ group differed significantly in terms of relapse free survival (RFS), overall survival (OS) and relapse rate ($p<0.001$, 0.003 and <0.001, respectively); 2) Intermediate karyotype MRD' patients had an outcome comparable to those bearing good risk karyotype whereas intermediate karyotype MRD+ patients showed a dismal outcome comparable to adverse karyotype patients (Figure 1). *Conclusions.* These results suggest that the inclusion of MPFC assessment of MRD in patients with intermediate risk karyotype may be particularly useful in discriminating subgroups with different outcomes. This may allow clinicians to design risk-based therapeutic programs in a group of AML where karyotype does not represent a clear prognosticator likely due to the underlying molecular heterogeneity.

0059

PROGNOSTIC SCORE INCLUDING SERUM β-2 MICROGLOBULIN LEVELS TO STRATIFY PATIENTS WITH ACUTE MYELOID LEUKEMIA: ANALYSIS OF 1293 PATIENTS

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Background. Serum β2 microglobulin (β2M) levels reflect renal function and membrane turnover. *Aims.* The aim of this study was to evaluate the prognostic significance of β2M in acute myeloid leukemia (AML). *Methods.* Among 2,014 patients who presented to M. D. Anderson Cancer Center with newly diagnosed AML (> 20% myeloblasts) from 1990 to 2005, pretreatment β2M levels were determined in 1,293 patients using a radioimmunoassay (Pharmacia Diagnostic, Uppsala, Sweden)(normal values, 0.7-2.0 mg/L). Survival analyses were performed considering 38 covariates. Pretreatment parameters that remained independently significant were used to design a model to predict an individual patient s risk of death: the AML score. *Results.* The median age of patients was 61 years (range, 16-89 years). Cytogenetic risk was poor in 29%, intermediate in 60%, and favorable in 7%. Zubrod performance status (PS) was > 1 in 27%. The median follow-up of surviving patients was 27 months (range, 1-145 months). Overall, 96% received chemotherapy and 91% received ara-C-based induction therapy. In the multivariate analysis of the 2,014 patients, lack of β2M measurements was an adverse independent factor predicting survival. Since patients > 60 years are sometimes offered less intensive therapy and have shorter survival than younger patients, we performed separate multivariate analyses for these groups. β2M levels were among the top three independent prognostic factors for patients > 60 years, but not for younger patients. We then performed multivariate analyses in 1293 patients with β2M measurements. A training sample of 862 patients (2/3) was randomly selected.

Figure 1.

The following factors had independent adverse significance for survival: worse-risk cytogenetics, older age, poorer performance status, higher β2-microglobulin levels, lower hemoglobin levels, secondary (vs. *de novo*) AML, longer prothrombin time, and higher LDH levels. The prognostic factor model was then applied to a validation sample of the remaining one third of patients (415/1293), with complete data on the eight specified variables. The model was equally predictive in the validation sample. Based on the relative risks associated with each of the top four independent risk factors (cytogenetics, age, performance status, 'β2M level), we designed the AML score. Patients with poor-risk cytogenetics were given a score of 2, those with intermediate risk a score of 1, and those with favorable cytogenetics a score of 0. Each of the following factors was given a score of 1: age > 60 years, β2M > 3 mg/L, PS >1. The risk of death could be determined by summing the above scores present at diagnosis and ranged from 0 to 5. At 2 years, 74%, 51%, 36%, 19%, and 6% of patients with scores of 0, 1, 2, 3, 4-5, respectively, are expected to be alive (Figure 1). *Conclusions.* This large study strongly supports the utility of uncorrected serum β2M levels as an independent prognostic factor for survival in patients with newly diagnosed AML who are 60 years or older, regardless of cytogenetics and PS. The proposed score defines the key prognostic features in newly diagnosed AML and provides a refined stratification of risk assessment.

0060

MUTATION PROFILE AND PROGNOSIS OF ADULT ACUTE MYELOID LEUKEMIA WITH INVAGINATED *CUP-LIKE* NUCLEI

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Background : Acute myeloid leukemia with the most frequent recurrent cytogenetical abnormalities (t(15;17), t(8;21), inv(16)) display well recognized cytological features. In contrary, few morphological characteristics allow to predict the mutation profile (NPM1, FLT3, CEBPA) in AML. Recently, AML with prominent nuclear invaginations were successively shown to be associated with FLT3/ITD and NPM1 mutations (Kussick, Leukemia 2004; Chen, Blood, 2006). These AML also display a particular phenotype with a low expression of CD34 and HLA-DR. The aim of this study was to precise the mutation profile of these AML with invaginated cup-like nuclei. *Methods.* AML in 92 patients aged 15-50 years and treated in the ALFA-9802 protocol were investigated for the presence of cup-like nuclei, NPM1 mutation (NPM1m), FLT3 internal tandem duplication (FLT3/ITD), FLT3/D835 mutation and CEBPA mutation (CEBPAm). The criterion for this morphological feature was the presence of 10% ore more blasts with nuclear invagination embossing at least 25% of the nucleus. R*esults.* The incidence of NPM1m, FLT3/ITD, FLT3/D835 and CEBPAm were respectively 28%, 20%, 11% and 7%. Both NPM1m and FLT3/ITD were found in 12% AML. AML with cup-like nuclei (CL-AML) were found in 17/92 patients (18%) : 6 FAB-M1, 6 M2 and 5 M4. CL-AML were associated with a higher WBC (76 vs 25 G/L, $p= 0.07$) and a higher marrow blast rate (88% vs 70%, $p=0.02$). CL-AML mostly displayed a normal karyotype (81% vs 46%, $p=0.01$). All CP-AML presented with either a NPM and/or a FLT3 mutation. In this subgroup, NPM1m and FLT3/ITD were significantly more frequent (NPM1m : 76% vs 17%, FLT3/ITD : 71% vs 8%, $p<0.0001$) but no difference was observed concerning FLT3/D835 or CEBPAm ($p=0.99$). Among the 11 patients with both NPM1 and FLT3/ITD, 9 (82%) presented with CL-AML ($p<0.0001$). In our cohort, the sensitivity and the specificity of this morphological feature to predict the presence of concomitant mutations were respectively 82% and 89%. Complete remission rate was not different for CL-AML patients (89% vs 92%, $p=0.7$). However CL-AML was associated with a shorter survival in the whole population (3y-OS:30% vs 53%, $p=0.05$) or in the cytogenetical intermediate risk group (3y-OS:45% vs 67%, $p=0.09$). *Conclusions.* CL-AML consist in a relatively homogeneous group displaying a high frequency of both NPM1m and FLT/ITD rates and a poor outcome. Gene expression profiling may be useful to understand and to corroborate this observation.

Anemia and Bone marrow failure

0061

GRANULOCYTE TRANSFUSION IN THE MANAGEMENT OF PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND SEVERE INFECTION

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Background. Infection associated with chemotherapy induced neutropenia continues to be a major cause of morbidity and mortality in patients treated for hematological malignancies. The role of granulocyte transfusion (GT) in this clinical setting has not been established. *Aims and Methods.* We retrospectively analysed clinical characteristics and outcome in a cohort of patients with hematological malignancies receiving GT during neutropenia and severe infection. *Results.* A total of 30 patients with a median age of 46 years (range 3-82) who had received one or more GT, achieved from community donors after stimulation with G-CSF and corticosteroids, were included. Acute leukemia (80%) and non-Hodgkin lymphoma (17%) predominated as underlying hematological malignancy. All patients had severe (absolute neutrophil count $<0.1\times10^9$/L) and prolonged (median 16 days; range 6-63) neutropenia. In 28 patients (97%) neutropenia was induced by chemotherapy and 6 of those had received allogeneic stem cell transplantation. The etiology of febrile neutropenia was microbiologically verified in 20 patients (66%) and 19 of those had bacteremia. The major reasons for GT were persistent fever and clinical deterioration despite adequate antibiotic therapy in combination with progressive pneumonia (n=16), neutropenic enterocolitis (n=6), and soft tissue infections (n=3). GT were given daily for a median of 4 days (range 1-14). An increment in neutrophil counts after GT occurred in 62% of patients and median time to fever defervescence after GT was 5 days (range 1-33). In 11 patients resolution of fever and all signs of infection could directly be related to GT and 3 of those patients were long term survivors (>5 years). Mortalities at 30-days and 6-months post GT were 40% and 72%, respectively. GT was well tolerated and only 2 patients needed antihistamines/corticosteroids to avoid reactions. *Conclusions.* A substantial proportion of severely ill patients with complicated febrile neutropenia benefited from GT. Despite high mortality, the presence of long time survivors motivates further evaluation using well designed randomised prospective trials to evaluate the efficacy of this intervention in patients with hematological malignancies.

0062

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM MATCHED RELATED DONORS IN FANCONI ANEMIA PATIENTS: THE TUNISIAN EXPERIENCE WITH TWO CONDITIONING REGIMENS

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We transplanted twenty-four patients with Fanconi anemia (FA) using matched related donors (MRD) between January 1999 and June 2006. The patients were assigned to the following groups according to the conditioning regimen: group 1 (N=17), low-dose cyclophosphamide (CY; 40 mg/kg)-busulfan (BU; 6 mg/kg) conditioning regimen; group 2 (N=7), low-dose CY- Fludarabine (120 mg/m^2) conditioning regimen. Median age at transplantation was 10 years (range:3-22 years). Graft-versus-host disease (GVHD) prophylaxis included cyclosporine A and methotrexate (MTX; 5mg/m^2 at day 1, 3, 6). Twenty-three patients engrafted (for an absolute neutrophil count $>0.5\times10^9$/L) after a median time of 12 days (range,10-16 days). Eighteen patients (78%) had sustained grafts, whereas five others of the group 1 (29%) rejected grafts between day +39 and 22 months after transplantation. Four of them are still alive after successful second PBSC transplantation and one died. All of the group 2 patients had sustained grafts without major toxicity. Acute and chronic GVHD occurred, respectively, in 17% and 9% of only group1 patients. After a median follow-up of 53 months (range, 9-93 months), nineteen patients are alive. The Kaplan-Meyer survival is 79% at 7 years. We conclude that, in FA patients, fludarabine-based conditionning allowed engraftment with low toxicity.

0063

ALEMTUZUMAB PLUS CYCLOSPORIN A FOR TREATMENT OF PATIENTS WITH BONE MARROW FAILURE SYNDROME

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Patients with bone marrow failure syndrome are suffered from cytopenia and resulting requirement of transfusion. Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the standard therapy for severe aplastic anemia (AA) with suitable sibling donor. However, it is true in many cases that alloHSCT can not be performed even in very severe AA due to no suitable donor. In that regards, immunosuppressive therapy (IST) is another option for those patients. Alemtuzumab (ALM) targets not only B-cell but also Tcell, which is known to play an important role in pathogenesis of bone marrow failure syndrome (BMFS). Although there were several reports of ALM for immune cytopenia and pure red cell aplasia, no report were published regarding ALM for BMFS. We studied the feasibility of ALM and cyclosporine A (CsA) for patients with BMFS. Inclusion criteria were transfusion-dependent BMFS including AA, MDS, PNH. Prior IST or other drugs for BMFS except for ALM were not excluded. ALM was infused for 3 days (10 mg on day 1, 20 mg on day 2, and 30 mg on day 3) along with CsA for at least 6 months. Prophylactic ciprofloxacin, acyclovir, and bactrim were given for first 2 months. Without partial response (PR) or more within 6 months, subsequent alloHSCT or antithymoglobulin therapy was allowed. Total 12 patients were enrolled. Female were 9 (75%). Median age was 43 (16-74) years. Disease were 9 severe AA, 2 moderate AA and 1 hypoplastic MDS. All patients were transfusion-dependent. Median amount of transfused red cell, platelet concentrate and platelet apheresis prior to ALM-CsA were 2, 2 and 7 units, respectively. Prior therapy were none in 9 (75%), anti-thymoglobulin in 2 (16.7%), and danazol in 1 (8.3%). Only 1 patient could not receive full scheduled CsA because the patient underwent alloHSCT. Median follow-up was 326 (140-466) days. Response to ALM-CsA were none response in 7 (58.3%), PR in 4 (33.3%), and CR in 1 (8.3%). Therefore, overall response rate was 4/12 (41.7%). Median time to response was 47 (24-71) days (Figure 1). PR was converted to CR in 1 patient at 12 months after treatment. Neither fever nor skin rash during treatment did not affect the possibility of response to ALM-CsA (p=0.523, p=0.523, respectively). Toxicity during ALM was fever in 3 (25%), skin rash in 3 (25%), G1 ALT elevation in 7, G1 AST elevation in 4, and hyperbilirubinemia in 3 (G1 in 2, G3 in 1). There were no anaphylaxis, serum sickness or CMV reactivation. In spite of small number, AML-CsA showed comparable response rate within 3 months after treatment. Therefore in conclusion, ALM-CsA is feasible and tolerable therapy for BMFS.

Figure 1. Time to response.

0064

INCREASED EXPRESSION OF TOLL-LIKE RECEPTOR-4 AND -9 IN THE BONE MARROW OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA MAY CONTRIBUTE TO THE INFLAMMATORY MARROW MICROENVIRONMENT

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Background. Chronic idiopathic neutropenia (CIN) is an acquired bone marrow (BM) failure syndrome characterized by Fas-mediated apoptosis of the BM $CD34^+/CD33^+$ granulocytic progenitor cells. An inflammatory BM microenvironment consisting of activated myelosuppressive T-lymphocytes, pro-inflammatory cytokines such as Tumor Necrosis Factor-α (TNFα), Interferon-γ (IFNγ) and Interleukin-6 (IL-6) and pro-apoptotic mediators such as Fas-Ligand (FasL) are mainly implicated in the pathogenesis of the disease. Recent evidence suggests that activation of Toll-like-Receptors (TLRs) on antigen presenting cells (APCs) by a variety of microbial and/or host-derived ligands, may induce the production of pro-inflammatory cytokines by APCs. *Aims.* To evaluate the expression of TLRs in the BM of CIN patients looking for possible relationships between TLR induction and pro-inflammatory cytokine production in CIN BM. *Methods.* BM was obtained from 16 patients with CIN and 14 healthy controls. All patients fulfilled the diagnostic criteria for CIN as we have previously published (Blood 2003; 101: 2591-2600). Surface TLR-1, TLR-2, TLR-4 and intracellular TLR-3 and TLR-9 expression was evaluated by flow-cytometry in the $CD14^+$ BM cell fraction. TLR flow-cytometric analysis was also performed in the $CD45^+$ and $CD45^-$ cell fraction of trypsinised adherent cell layer of confluent (week-3) long-term BM cultures (LTBMCs), representing an approximation of BM microenvironment cells, in patients and controls. *Results.* Increased proportion of TLR-4+ and TLR-9+ cells was found in the $CD14^+$ BM cell fraction of CIN patients (6.48±5.29 and 40.36±26.31, respectively) compared to healthy controls (1.87±1.27 and 21.10±14.17, respectively; $p<0.05$ and $p<0.05$, respectively). A trend towards increased expression of TLR-1, TLR-2 and TLR-3 was also observed in patients (7.58±7.41, 56.14±15.68, 14.42±15.70, respectively) compared to controls (4.01±3.89, 43.36±24.57, 5.73±4.82, respectively); however the obtained differences were not statistically significant. Increased proportion of TLR-4+ and TLR-9+ cells was also observed in the CD45- fraction of patient LTBMC adherent layers (18.42±12.41 and 42.27±20.14, respectively) compared to controls (4.43±0.81 and 3.93±3.35, respectively; $p<0.05$ and $p<0.05$, respectively). *Summary and Conclusions.* CIN patients display increased proportion of $TLR\text{-}4^+$ and $TLR\text{-}9^+$ cells in the BM $CD14^+$ cell compartment and in marrow microenvironment. A degree of TLR up-modulation might be due to the presence of pro-inflammatory cytokines in patients BM. Alternatively, that specific up-regulation of TLR-4 by intrinsic stress proteins and TLR-9 by incorporated DNA from the apoptotic granulocytic progenitor cells may represent a contributory pathogenetic mechanism in CIN by further increasing the pro-inflammatory cytokine production in patients' BM.

0065

BLOCKADE OF INTRAVASCULAR HEMOLYSIS IN PNH WITH THE TERMINAL COMPLEMENT INHIBITOR ECULIZUMAB UNMASKS LOW-LEVEL HEMOLYSIS POTENTIALLY OCCURRING THROUGH C3 OPSONIZATION

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia characterized by intravascular hemolysis, often resulting in the need for red blood cell (RBC) transfusions. PNH RBCs lack two complement regulatory molecules - CD59, a terminal complement inhibitor, and CD55, a C3 convertase inhibitor. Eculizumab, a humanized monoclonal antibody that inhibits terminal complement by binding to C5, effectively controls intravascular hemolysis as determined by a dramatic reduction in lactate dehydrogenase (LDH) to levels in or just above the normal range. Control of intravascular hemolysis in these patients led to a reduction in, or cessation of, RBC transfusions. During eculizumab treatment, a majority of patients demonstrate evidence of residual, low-level hemolysis; LDH levels remain slightly elevated, haptoglobin levels are low or undetectable, and bilirubin levels are above normal. We hypothesized that this low-level residual hemolysis may be due to clearance of PNH RBCs through a C3b-mediated mechanism. *Aims.* To investigate C3 deposition on RBC in PNH patients before and on eculizumab. *Methods.* C3 deposition was investigated using a direct antiglobulin test (DAT) using monoclonal anti-C3d and two-color flow cytometric analysis with anti-CD59 and anti-C3. *Results.* The DAT was positive in 29 out of 39 PNH patients on eculizumab. Of these 29 DAT-positive patients, who were all receiving transfusions, 25 had DAT testing prior to eculizumab therapy and only one of these was positive. DAT was negative in all of 8 normal volunteers. By two-color flow cytometric analysis, the majority of patients on eculizumab demonstrated three distinct RBC populations: (i) $CD59^+/C3^-$ (normal RBCs); (ii) CD59-/C3- (PNH RBCs without C3 coating); and (iii) $CD59^-/C3^+$ (PNH RBCs coated by C3). No $CD59^+/C3^+$ RBCs were observed. Of 21 DAT positive eculizumab treated patients tested, the median proportion of total RBCs that were C3b positive was 17.6%. 18 of 29 [62%] eculizumab patients with a positive DAT received at least one transfusion during eculizumab therapy compared with 1 of 10 [10%] for DAT negative patients ($p=0.01$), although even patients who did not become transfusion independent during eculizumab treatment showed a marked reduction in transfusion requirement. The median hemoglobin value for the 29 DAT positive eculizumab patients was 9.8 g/dL compared with 11.3 g/dL in the 10 DAT negative eculizumab patients ($p=0.08$). No apparent relationship between LDH and DAT positivity was observed. *Summary.* It is proposed that resolution of intravascular hemolysis in PNH patients on eculizumab results in deposition of C3b on the surface of PNH RBCs which may explain, at least in part, the residual low level hemolysis occurring in some patients. This appears to be a previously undescribed mechanism of RBC clearance in PNH, most likely obscured by the rapidity of intravascular hemolysis in the absence of eculizumab therapy. Despite the low-level residual hemolysis, patients continue to receive significant benefit from eculizumab treatment.

0066

HIGH DEFINITION CONTRAST-ENHANCED MR IMAGING IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA SUGGESTS A HIGH FREQUENCY OF SUBCLINICAL THROMBOSIS

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder characterized by intravascular hemolysis and venous thrombosis. Thrombosis is the most feared complication in PNH and is reported to occur in more than 40% of patients. Proposed mechanisms of thrombosis include depletion of the coagulation regulator nitric oxide (NO) by intravascular hemolysis and increased sensitivity of PNH platelets to activation due to deficiency in complement regulatory molecules, particularly CD59. The occurrence of subclinical thrombosis in PNH patients has not been previously studied using modern imaging techniques. B-type natriuretic peptide (BNP) has been shown to increase in proportion to right ventricular dysfunction in pulmonary hypertension. *Aims.* To evaluate the incidence of subclinical thrombosis in PNH. *Methods.* PNH patients were evaluated with a comprehensive state-of-the-art MRI protocol (which included the use of both blood pool and conventional Gadolinium based contrast agents) for the detection of subclinical thromboses and its sequelae. The detailed protocol consisted of: (a) lung perfusion and pulmonary MRA, (b) cardiac MR - including quantitative studies of both ventricles, right heart flow dynamics and delayed enhancement for the detection of left ventricular damage, and (c) abdominal MR for the assessment of hepatic and portal venous systems and kidneys. Plasma BNP levels were measured directly by immunoassay. *Results.* 10 PNH patients (median age 31.5 yrs) with large PNH clones but without previous clinical evidence of venous or arterial thrombosis underwent imaging. Five (50%) of the patients were on primary anticoagulant prophylaxis with warfarin. There was evidence of significant renal hemosiderosis, which was distributed throughout the cortices, in 9/10 patients. Two patients had small myocardial scars suggestive of previous unsuspected ischemic damage. Six patients had subsegmental perfusion defects mainly distributed in the peripheries of the lung fields indicative of previous small pulmonary emboli. No such subclinical thromboses would be anticipated in an age-matched control population. 8 patients had mildly reduced right ventricular ejection fractions (mean 42.2±1.8%; normal range 48-63%). The plasma BNP level was high in all 10 patients (median 29.4 pmol/l; range 18.7-373.90; normal subjects 4.89±1.00 pmol/l). No intra-abdominal defects were iden-

tified with the current protocol. *Summary.* we identified abnormalities suggestive of previous subclinical thromboses in 6 of 10 hemolytic PNH patients by high-resolution MR imaging, including in patients on primary prophylaxis with warfarin. Effective prevention of thrombosis is an important aspect of the therapy in PNH.

0067

THE INCIDENCE AND PREVALENCE OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) AND SURVIVAL OF PATIENTS IN YORKSHIRE

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal stem cell disorder characterized by the expansion of a population of blood cells deficient in glycosylphosphatidylinositol (GPI) linked proteins. This results in the classical clinical features of intravascular hemolysis and thrombosis. PNH is known to be a rare disorder, but its incidence and prevalence have so far been poorly defined with very few studies. *Aims.* To better define the incidence and prevalence of PNH. *Methods.* Survival data was collected on all patients diagnosed with PNH in the strategic health authorities of North and East Yorkshire, Northern Lincolnshire and West Yorkshire between January 1991 and July 2006. All patients were diagnosed by flow cytometry for GPI-linked antigens on red cells and neutrophils at a single reference laboratory (HMDS). *Results.* The population of the study region is 3,742,835 (based on the 2001 census of Britain). 76 PNH patients were diagnosed during this time period giving an incidence of 0.13/100,000/year. Based on incidence and survival rates, the estimated 15 year prevalence of PNH is 1.59 per 100,000 resulting in a predicted prevalence of 59 patients in the study region. We have previously demonstrated that a neutrophil clone size >50% is a predictor of increased thrombotic risk; the current study predicts that 25% of patients will have >50% PNH neutrophil clone size, 43% with >10%, and 82% with >1%. Platelet count $>100\times10^9$/L has been used as a criteria to consider primary prophylactic anticoagulation in PNH patients with substantial hemolysis if the neutrophil clone size is >50%. In the current study, the platelet count is $>100\times10^9$/L in 32% of patients and <30 x 109/L in 27%. The primary clinical manifestation of PNH is intravascular hemolysis and although levels of hemolysis vary considerably between patients even those with relatively small PNH clones will have some degree of hemolysis. Levels of LDH (a sensitive marker of hemolysis) were elevated above the upper limit of the normal range in 82.5% of patients. Of the 59 patients in the study region, 33% reported hemoglobinuria. Overall survival was 78% with a median follow-up of 6.25 years (range 0 to 15 years). Survival was compared between patients with a) hemoglobinuria (89%) vs. those without (76%); b) neutrophil clone size 50% (81%) vs. <50% (80%) and c) Platelet counts $<30\times10^9$/L (61%) vs. 30×10^9/L (86%) by univariate analysis. Hazard ratios and 95% confidence intervals were estimated using Cox s proportional hazards model adjusted for age and sex. Worse survival was only significantly predicted by a platelet count $<30\times10^9$/L (log rank test, p=0.008). *Conclusions.* With a population of 57,105,375 (2001 census of Britain), Great Britain should have an estimated 75 new cases of PNH per year and a predicted prevalence of 908 patients. The USA will therefore have 4713 cases of PNH based on its July 1, 2005 census bureau population estimate of 296,410,404. This study is the first to accurately report the incidence and prevalence of PNH in a given population in a well-defined geographical area.

0068

THIOL-DEPENDENT FORMATION OF LECTIN-INDUCED NEUTROPHIL AGGREGATES

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Background. Recruitment of circulating leukocytes to vascular endothelium requires multistep adhesive and signaling events including selectin-mediated attachment and rolling, leukocyte aggregation and activation and others. *Aims.* To evaluate the significance of thiol-dependent signaling for stable aggregate formation, the aggregation/dissociation of neutrophils initiated by two crosslinking plant lectins, namely Viscum album agglutinin (VAA) and by potato lectin Solanum tuberosum (STA) in the presence of sulfhydryl agent N-ethylmaleimide (NEM) were comparatively studied. *Methods.* Cell aggregation/disaggregation was measured with the methods of light transmission and optical microscopy. For analysis of mechanisms of cell aggregate formation by lectins we used the method of haptenic-sugar-resistant (HSR) intercellular contacts identification.[1] *Results.* It was found that despite intimate cell contacts in the neutrophil aggregates by N-acetyl-D-glucosamine-specific lectin STA and galactose-specific lectin VAA only the later led to establishment of HSR complexes. NEM concentration-dependently inhibites VAA-induced HSR-contacts in cell aggregates. The treatment of neutrophils by NEM increases in a dose-dependent manner rate and degree of STA-induced aggregation and induces the formation of HSR-contacts in neutrophil aggregates by STA. *Conclusions.* The ability of NEM to modify lectin-dependent formation of neutrophil aggregates supports the importance of thiol-dependent signaling for this process. The obtained differences in the mechanisms of action of NEM are determined apparently by type of glycoreceptor involved in the adhesion interactions.

Reference

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0069

HLA-DRB AND DQB1 GENES IN PREDISPOSITION TO APLASTIC ANEMIA

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Background. Aplastic anemia (AA) is considered to be an autoimmune disease with active destruction of blood-forming cells by lymphocytes. Most cases of AA can be pathophysiologically characterized as T-cell-mediated, organ-specific destruction of bone marrow hematopoietic cells. Immunogenetic studies have reported about a strong association between AA and antigen HLA-DR2 (DRB1*15) in different populations, although the exact role of HLA-DR2 in the immune mechanisms of AA remains unclear. Genomic organization of HLA-DR region includes a few group haplotypes (DR51, DR52, DR53, DR1, DR8), which differ not only by encoded products but also in a number of expressed genes (one or two). The aims of present work have been to define the role of different HLA-DRB specificities and the expression of second DRB gene (DRB3, DRB4 or DRB5) in predisposition to AA. *Methods.* 38 AA patients were HLA-A, -B, -C, -DRB1 (3, 4, 5) and -DQB1 typed (2n=76). 328 unrelated donors of blood components were taken as controls (2n=656). HLA class II genes were typed by PCR-SSP. Statistical analysis was performed using exact Fisher's test. The strength of association between HLA specificities and disease was estimated by evaluation of relative risk (RR). *Results.* In AA patients we have found insignificantly increased frequency of HLA-DRB1*15 (0.25 vs 0.128 in controls, RR=2.27) and significantly increased frequency of HLA-DQB1*06 (0.382 vs 0.213 in controls, p<0.05, RR=2.28). The frequencies of HLA-DRB1*01 and HLA-DQB1*0501 were decreased (0.013 vs 0.131 in controls, p<0.01, RR=0.09 and 0.013 vs 0.137, p<0.001, RR=0.08, accordingly). The presence of second expressed DRB gene (DRB1+DRB3 or DRB4, or DRB 5), i.e. group haplotypes DR51+DR52+DR53, was revealed in AA patients more often than in controls (0.973 vs 0.793, p<0.001), and the absence of second expressed DRB gene (in DR1 and DR8 group haplotypes) took place in AA patients rarely than in controls (0.026 vs 0.155, p<0.01). In case of two expressed DRB genes in one DR group haplotype RR of AA development was increased to 9.4 and in case of absence of second expressed DRB gene RR was diminished to 0.14. *Conclusions.* Although DRB1*15 and DQB1*06 contribute to the development of AA, the increased number of expressed DRB genes also can play an important role in predisposition to AA development.

0070

EXPRESSION OF CD203C ON NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS: COMPARISON TO NORMAL MAST CELLS AND UPREGULATION BY CROSS-LINKING OF THE FCγRI

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Background. In patients with systemic mastocytosis (SM), several activation-linked cell surface antigens such as CD2 or CD25, are *overexpressed* on neoplastic mast cells (MC) compared to normal MC, and thus can be employed as diagnostic parameters. The ectoenzyme E-NPP3 (CD203c) is a novel activation-linked basophil antigen that is expressed at low levels on normal MC. *Methods.* Using multicolor flow cytometry,

we examined the expression of CD203c on bone marrow MC in 85 patients with SM, in normal bone marrow (n=38), and in primary lung MC (n=3). Lung MC or neoplastic MC were incubated with an anti-IgE antibody or stem cell factor before being examined for CD203c expression. In patients with ASM or MCL, we examined expression of CD203c-mRNA in highly purified FACS sorted MC by RT-PCR. *Results.* In patients with SM, MC expressed significantly higher levels of CD203c compared to normal bone marrow. There were no significant differences in expression of CD203c among the SM variants analyzed, although slightly lower amounts of CD203c were detected in those with SM-AHNMD. As assessed by RT-PCR, purified neoplastic MC as well as HMC-1 cells were found to express CD203c mRNA. Incubation of lung MC or neoplastic MC with anti-IgE resulted in upregulation of expression of CD203c. However, in patients with SM with anaphylaxis, MC did not exhibit higher baseline levels of CD203c compared to SM patients without anaphylaxis. *Conclusions.* CD203c is a novel activation-linked MC antigen, that is upregulated in response to IgE receptor cross-linking and is overexpressed on neoplastic MC in SM.

0071

RESULTS OF IMMUNOSUPRESSIVE THERAPY IN CHILDREN WITH SEVERE APLASTIC ANAEMIA. REPORT POLISH PAEDIATRIC LEUKAEMIA AND LYMPHOMA STUDY GROUP

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Introduction. Bone marrow transplantation from HLA identical family donors is the treatment chosen for children with severe aplastic anaemia (SAA). When no donor is available, combined immunosuppressive therapy is given. *Aim.* evaluation of results immunosupressive therapy in children with severe aplastic anaemia. *Material and Methods.* SAA was recognised in 85 children (31 girls, 54 boys) aged 2-17,5 years in the eleven centre Polish Paediatric Leukaemia and Lymphoma Study Group (PPLLSG) in Poland between 1993-2003 years. All patients received protocol according Severe Aplastic Anaemia Working Party of the Europe Bone Marrow Transplant (EBMT) protocol: antilymphocyte globulin or antithymocyte globulin, cyclosporin A, prednisolon and granulocyto- or granulocytomacrophagic-cell stimulation factor was additionally administered during deep neutropenia. Haematological response was evaluated on 84, 112 or 180 day of the therapy. *Results.* complete remission occurred in 43 patients (50,5%), partial remission in 22 (25,4%), no response was obtained in 20 children (23.7%) in 180 day of the therapy. Period of observation was from 12 months to 10.5 years. During this time relapse occurred in 6 patients (7%). We observed 16 deaths: 7 early during the first 3 months of immunosupressive therapy (IS) and 9 after the first 3 months of IS. *Conclusions.* The actuarial survival at 10-years after immunosupressive therapy is 81,2% in our group. Transformation to leukaemia or myelodysplastic syndrome (MDS) was not observed in none of our patients. We notice one case with paroxysmal nocturnal hemoglobinuria (PNH).

0072

PHASE II STUDY OF THYMOGLOBULIN, CYCLOSPORINE, AND G-CSF FOR THE INITIAL TREATMENT OF APLASTIC ANEMIA AND LOW RISK MYELODYSPLASTIC SYNDROME

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Background. Treatment of aplastic anemia (AA) often involves the use of equine anti-thymocyte globulin (ATG) in combination with cyclosporine. Hematological responses occur in 40% to 50% of patients treated with equine ATG alone. With the addition of cyclosporine, response rates of up to 65%, and 5-year survival rates of over 80% have been reported. Rabbit ATG (Thymoglobulin) is commonly used to treat refractory or relapsing AA patients because of its demonstrated efficacy and a desire to minimize the risk of potentially life-threatening allergic reactions associated with re-administration of equine preparations. It has been previously demonstrated that most patients who received Thymoglobulin after unsuccessful treatment with equine ATG achieved response (Di bonna E, Br J Haematol, 1999). High response rates achieved with Thymoglobulin have also been reported in patients with relapsed AA (Scheinberg P, Br J Haematol, 2006). The efficacy of Thymoglobulin in AA may also be extended to myelodysplastic syndrome (MDS), as several lines of evidence suggest that the underlying autoimmune mechanisms leading to the development of AA may overlap with the disease process in at least a subset of patients with MDS. *Aims and Methods.*We investigated the efficacy and tolerability of the combination of Thymoglobulin, cyclosporine, and G-CSF as a first-line therapy for patients with AA or low-risk MDS. Thymoglobulin 3.5 mg/kg (or 2.5 mg/kg if patients were 55 years or older) was initially administered daily for 4 days. After treatment of the first 10 patients resulted in an acceptable safety profile, the study was amended to allow administration of Thymoglobulin 3.5 mg/kg for 5 days in all AA patients and MDS patients younger than 55. Methyl prednisone (1 mg/kg intravenously) was administered daily for 5 days and was followed by a tapering dose of oral prednisone. Cyclosporine (5 mg/kg), and G-CSF (5 μg/kg) were also administered daily and were continued for up to 3 to 6 months, at the discretion of the treating physician. All patients received prophylactic broad-spectrum antibiotics. *Results.* To date, we have enrolled 17 patients, and 15 (8 with AA and 7 with MDS) have received treatment. The median age was 61 years (range 45-73 years) for AA patients, and 60 years (range, 41-71 years) for MDS patients. The median absolute neutrophil count (ANC) for AA patients was 0.74×10^9/L (range, 0.1-1.0 $\times10^9$/L) and 0.5×10^9/L (range, 0.2-2.5$\times10^9$/L) for MDS patients. The median Hgb for AA patients was 6.9 g/dL (range, 3.9-7.9 g/dL) and for MDS patients was 8.1 g/dL (range 6.4-10.7 g/dL). The median platelet count for AA patients was 10×10^9/L (range, 0-153$\times10^9$/L) and for MDS patients was 10×10^9/L (range, 6-16$\times10^9$/L). Flow cytometry for a PNH clone was negative in all patients. Seven patients (4 with AA and 3 with MDS) were positive for HLA-DR15. All AA patients had diploid cytogenetics; 5 MDS patients were diploid and 2 had cytogenetic abnormalities (-18 and add{18p}). Seven of 8 (87.5%) patients with AA have responded including 3 CRs, 3 PRs and 1 HI. One patient with MDS had a CR. Cytopenias and neutropenic fever were the only grade 3 or 4 side-effects observed. Other side-effects (grade 1 and 2) included infusion-related reactions, rash, myalgias, hypomagnesemia, elevation of serum creatinine, and hyperbilirubinemia. *Conclusions.* The combination of Thymoglobulin, cyclosporine and G-CSF is safe and effective in the first-line treatment of AA and has activity in low-risk MDS.

0073

LEAD-INDUCED ANEMIA CAUSED BY THE SAME AYURVEDIC FORMULATION IN BELGIAN PATIENTS

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Background. An almost forgotten, but still existing cause of normochromic normocytic anemia is intoxication by lead. Heavy metals, in particular lead, have been a regular constituent of traditional formulations like Indian Ayurvedic medications. Since the renewed public interest in complementary and alternative medicines, cases of lead poisoning are emerging around the world. To our knowledge, these are the first reported cases in Belgium. *Case report.* Two Belgian women, both about 40-years old, presented at the emergency department of their hospital with abdominal discomfort. Laboratory investigation showed a normochromic normocytic anemia with hemoglobin levels of 10.0 and 8.1 g/dL respectively. No explanation for the anemia and abdominal pain was found after extensive clinical, endoscopic and radiological investigation. Suspicion of a lead intoxication was based on the prominent basophilic stippling of erythrocytes, both observed in the peripheral blood smear and the bone marrow aspirate. Both patients' blood lead levels were dangerously high, 80 and 75 μg/dL respectively. Despite the risk to develop an encephalopathy and nephropathy at these levels, no clinical abnormalities could be found. Upon further questioning both patients admitted to take some Ayurvedic medications. Toxicological analysis revealed that, in both, the same orange-red pills contained a remarkable high amount of lead, namely 31 mg per tablet. *Discussions.* Traditional remedies have become more and more popular in industrialised countries. One of the patients recently travelled to India where Ayurvedic medications are widely available. However, in both cases, the lead intoxication was caused by the same Ayurvedic formulation, prescribed in Belgium by an Indian *healer*. The Belgian Federal Agency for the Safety of the Food Chain has been notified. Subsequently, three

new cases of lead poisoning due to the same medication also were reported. *Conclusions.* Health care workers should be aware of the potential health risks posed by some traditional or folk remedies. Although in general, these products are believed to be safe, they may contain hazardously toxic amounts of heavy metals, particularly lead. Also patients have to know that not all substances found in nature are harmless. Under-reporting of consumption of these *herbal* medicine products may be substantial indicating how important it is for physicians to include questions about traditional remedies. Furthermore, governments should take measures to protect the public from potentially harmful products like Ayurvedic formulations.

0074

STROMAL CELLS ALTERED FUNCTION IN PATIENTS WITH APLASTIC ANEMIA

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Background. The cause of hematopoiesis dysfunction during aplastic anemia (AA) is not fully known. A few assumptions were made to explain the alterations occurred. Ineffective hematopoiesis could be caused both by disordered interactions between hematopoietic stem cells (HSC) and stromal cells and alterations in HSC or stromal microenvironment itself. *Aims.* The goal of this investigation was to characterize stromal precursors (CFU-F) and mesenchymal stem cells (MSC) of AA patients. *Methods.* CFU-F frequency was calculated using standard assay. Mononuclear bone marrow cells from donors and AA patients were plated into the 25 cm^2-cultivation flasks (106, 5×10^5 and $2{,}5\times10^5$ cells per flask) in α-MEM with 20% of fetal calf serum. Two weeks later the colonies were stained with crystal violet dye. MSC were isolated by cultivating $2{,}5\times10^6$ mononuclear cells from the bone marrow in the 25 cm^2-cultivation flasks in α-MEM with 10% of fetal calf serum Cells were passaged leaving one third on average once in 10 days. To induce adipogenic differentiation MSC were cultivated with isobutyl-1-methylxanthine, dexametasone, indomethacin and insulin. *Results.* There were no CFU-F detected in the bone marrow of 3 out of 15 tested AA patients; 2 out of 15 contained regular-sized colonies, equal to the ones of the donors and in 10 cases the formed colonies were much bigger than the donors' ones judging by size (28,7±1,3 mm^2 versus 9,1±0,5 mm^2 , $p<0.001$). CFU-F frequency in the bone marrow of AA patients was twice as large as in the donor bone marrow (63,5±18.3 and 29,7±5,7 correspondingly), however the difference is insignificant. Total cell production was also more intensive in MSC cultures derived from the bone marrow of AA patients than in the donors' ones. First 4 passages in donor cultures resulted in total $50{,}8\pm1{,}27\times10^5$ cells from one cultivation flask and took 30-60 days, while in AA patients cultures - $60{,}98\pm23{,}9\times10^5$ cells in 30-40 days. After the adipogenic induction MSC from AA patient unlike the donor's one doesn't show changed morphology and an increased number of lipophylic granules. The latter has even decreased in some cases. Obviously there are some alterations of MSC response depending on the type of inductor. *Conclusions.* The data obtained display apparent changes in stromal cells function from the bone marrow of AA patients. The increase in CFU-F frequency and size of the colonies could be a consequence of alterations in the expression level of such genes as FGF, BMP4, HGF etc, and needs further investigation. The depression of hematopoiesis often leads to negative changes in the stromal microenvironment. Observed improvement in MSC growth in culture together with its differentiation potential distortion might be an evidence of both the initial damage of stromal cells and it's secondary alteration due to profound bone marrow aplasia. In any case when analyzing the pathogenesis of aplastic anemia one should necessarily take into the consideration the changes occurred in stromal microenvironment of the patients.

0075

ALTERATIONS IN OSTEOBLASTIC AND VASCULAR NICHES REVEALED IN ADHERENT CELL LAYERS OF LONG-TERM BONE MARROW CULTURES FROM PATIENTS WITH APLASTIC ANEMIA

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Background. Up to date the exact pathogeneic mechanism of acquired aplastic anemia (AA) is still unknown. Beside immune-mediated destruction of marrow hematopoietic progenitor/stem cells (HSC) recent scientific data suggest that disturbed interaction between bone marrow (BM) stromal microenvironment and HSC may cause this disorder. In our previous studies we have shown that expression of genes regulating BM niches is changed in adherent cell layers (ACLs) of AA patients as compared with donors. Expression of Ang-1 - one of genes regulating self-renewal and quiescence of HSC in osteoblastic niche in BM was decreased in ACLs of AA patients. Expression of genes regulating vascular niche was also altered. Expression of VCAM-1 was decreased whereas expression of VEGF was increased in cultures of AA patients. Moreover futher cultivation led to normalization of expression of genes described here in ACLs of AA patients. *Aims*. The aim of this study was to find out whether normalization of gene expression in ACLs of AA patients during long-term cultivation was due to the absence in cultures of inhibiting factors existing in AA patient's organism. *Methods*. Long-term bone marrow cultures (LT-BMC) were established from 5 donors. Blood serum was obtained from 13 AA patients. Serum from healthy donor was used as a control. Serum from different AA patients was added for 3 weeks of cultivation in LT-BMC of donors in amount of 10% from the total media volume once a week. Gene expression analysis was made after 3 and 6 weeks of cultivation of donor cultures. To characterize alterations in the expression of several genes in ACLs after serum administration semi-quantitative analysis of RT-PCR products was performed using PhosphoImager Cyclone, Packard Bell (USA) after Southern blot hybridization with appropriate sequences. The expression level of β-actin was used as a normalization factor. *Results.* 7 of 12 samples of blood serums derived from AA patients significantly decreased the expression of Ang-1 in ACL's of donors administered with these serums separately. Expression of VCAM-1 also decreased in donor cultures treated for 3 weeks with 9 of 12 serum samples. Moreover expression of VEGF increased in ACL's of donors administered with 12 of 13 serum samples separately. Tendency in changes of gene expression in this experiment coincided with our previous studies of gene expression in ACL's of AA patients. *Summary/Conclusions*. Serum of AA patients contains some factors that change functioning of BM stroma *in vivo* and correspondingly gene expression in ACL of donors administered with AA patient's serum *in vitro*.

0076

FURTHER CHARACTERIZATION OF THE CYTOKINE PROFILE IN ACQUIRED APLASTIC ANEMIA

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Background. Myelosuppressive cytokines TNF-α and IFN-γ act as final effectors of apoptosis in haematopoietic cells in Acquired Aplastic Anemia (AAA). Combined Immunosuppression (IS) with ATG and CyA improves haematopoiesis in most patients but has a relapse rate of about 30%. *Aims.* To characterize the role of TNF-α, IFN-γ and IL-4 in the clinical course of AAA treated with combined IS. *Methods.* A first group of 59 subjects, 42 in Active Disease (AD) (onset, relapse, never responding to IS) and 31 in Non Active Disease (NAD) (responding to IS), median age 15 years (range 2-56 years) was tested for TNF-α, IFN-γ and IL-4 expression in $CD3^+$ and in $CD14^+$ marrow cells. Twentynine subjects (18 AD and 19 NAD) were also tested for BFU-e growth after block of TNF-α and/or IFN-γ in culture system. A second group including 21 patients, median age 10 years (range 2-32) was prospectively tested for TNF-α, IFN-γ and IL-4 expression in $CD3^+$ marrow cells at diagnosis and at response evaluation time (RET) after IS. *Results.* First group= IFN-γ and IL-4 were not differently expressed in $CD3^+$ and in $CD14^+$ marrow cells of NAD vs AD patients. TNF-α was more expressed in CD3+ and in $CD14^+$ cells of AD than of NAD patients without significant difference. After blocking TNF-α and IFN-γ in culture systems BFU-e incremented in AD patients and, significantly ($p<0.05$), in NAD patients, while not incrementing in normal controls, thus suggesting that a residual TNF-α and IFN-γ activity was still present particularly in the marrow of patients responding to IS. This finding led us to investigate prospectively the kinetics of these cytokines in a second group of AAA patients. *Second group.* The expression of TNF-α and IFN-γ at diagnosis was not different in patients who at RET were Responders vs patients who were Non Responders to IS. The expression of TNF-α and IFN-γ (median absolute number of $CD3^+$ marrow cells expressing cytokine in the cytoplasm) was significantly lower at RET (443 for TNF-α and 590 for IFN-γ) vs Diagnosis (1368 for TNF-α, 1028 for IFN-γ) in Responders (p 0.02). No

significant difference was found at RET vs Diagnosis in Non Responders. IL4 was significantly lower at RET (1324) vs Diagnosis (4748) (p 0.003) in Non Responders but not in Responders. *Conclusions.* 1.TNF-α and IFN-γ were confirmed to be over-expressed in marrow of both AD and NAD AAA patients. 2. TNF-α and IFN-γ decline more in patients Responding vs Non responding to IS. 3. Myelosuppressive cytokines remain in the marrow also of patients who respond to IS. This smouldering activity may contribute to relapse in case of immune attack reactivation. 4. The reduced expression of IL-4 in may contribute to the disease by not counterbalancing the Th1 polarization in Non Responding patients. Overall these data indicate TNF-α and IFN-γ as crucial effectors of marrow damage in AAA and suggest to strengthen IS with targeted anti cytokine treatments that may reduce the risk of relapse after IS.

0077

HIGH PREVALENCE OF ANAEMIA AMONG FRENCH HOSPITALIZED CANCER PATIENTS: A ONE-DAY CROSS-SECTIONAL SURVEY

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Objective. To evaluate the prevalence of anaemia (haemoglobin [Hb] <12.0 g/dL) among hospitalized cancer patients in France. *Material and Methods.* A written invitation to participate in a one day survey of anaemia among hospitalized French cancer patients on November 14, 2006, was sent to all practicing hospital-based French oncologists, haematologists and pulmonary specialists (n=4165). 255 physicians provided data. Age, gender, cancer type and treatment, Hb value, type of anaemia and treatment for anaemia were recorded. Unless otherwise specified, data are presented as mean±standard deviation. *Results.* 4,161 patients (mean age, 62.2±14.6 years; males, 53.2%) from 255 hospitals were described. The patients were admitted for chemotherapy (CT) (57.6%), initial staging and follow-up (19.6%), radiotherapy (5.2%), supportive care (22.6%), and/or other (20.6%). 57.8% of patients had a hospital stay >24 hours. 76.9% of patients received cancer treatment (recurrent disease: 44.2%); 42.7% of patients had a performance status of ≥2. 23.8% of patients had haematological malignancies (including lymphoma, 8.9%; and acute leukaemia, 6.3%) and 76.2% had solid tumours (including lung cancer 15.9%; colorectal cancer 14.4%; and breast cancer 12.8%). 57.6% of solid tumours were metastatic. Hb levels ranged from a minimum of 4.2 to a maximum of 18.9 g/dL (11.2±1.9). 63.8% of patients had anaemia (77.3% among patients with haematological tumours; 59.5% in patients with solid tumours). 57.8% of anaemia cases were considered to be related to cancer treatment. 59.1% of patients receiving CT were anaemic. 47.4% of anaemic patients were not being treated for their anaemia. Among patients receiving 1 or more treatment for anaemia, the treatments included transfusion (28.5%), erythropoietin stimulating protein (27.0%), and nutritional supplements (iron and/or vitamins) (19.1%). Further analysis of patients not treated for anaemia is underway. *Conclusions.* These results confirm the findings of ECAS, a large European survey of anaemia in cancer patients. Better identification of the causes of anaemia in cancer patients may optimize patient care. Detailed data on haematological patients will be presented.

0078

INTRAVENOUS IRON IMPROVES RESPONSE RATES TO RECOMBINANT HUMAN ERYTHROPOIETIN IN ANAEMIC PATIENTS WITH HAEMATOLOGICAL MALIGNANCY ON CHEMOTHERAPY WHICH IRON PARAMETERS TO USE?

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Background. Recombinant human erythropoietin (rhEpo) may be used to avoid transfusion and improve quality of life in the anaemia of cancer. Poor response rates to rhEpo are a factor contributing towards its poor uptake in clinical practice. Supplementation with intravenous iron improves response rates to rhEpo. Biochemical markers of iron status may guide rational use of iron supplementation, but they are often unreliable, being altered by the same factors that disrupt iron metabolism and contribute to the anaemia of malignancy. Functional iron deficiency (FID) is a status whereby iron stores are adequate by biochemical parameters but rendered inaccessible to erythropoiesis, perhaps through cytokine dysregulation. *Aims.* ASH/ASCO-based guidelines and a protocol-based approach to the use of intravenous iron and rhEpo in the treatment of non-myeloid haematological malignancy have been used in our centre for the last 4 years. Monitoring of several biochemical iron status markers was integral to the protocol. The aim was to determine which iron parameters most consistently predicted FID and hence the need for intravenous iron. *Methods.* A prospective audit was carried out of patients with non-myeloid haematological malignancy on chemotherapy and who were anaemic and receiving rhEpo. Intravenous iron supplementation was based on the following iron parameters: haemoglobin, mean corpuscular haemoglobin, serum ferritin, transferrin saturation, zinc protoporphyrin (ZPP), reticulocyte count and reticulocyte haemoglobin concentration (CHr). Measurements were made at baseline, weeks 4 and 8, then as indicated by a suboptimal haemoglobin (Hb) response. The data were examined to determine which measures most frequently triggered the addition of intravenous iron at baseline (indicating FID) and which measures most frequently indicated the development of FID during rhEpo therapy, leading to later addition of intravenous iron. *Results*. The iron status indices of 54 patients receiving rhEpo were analysed. 68% had myeloma, 17% CLL, 6% Waldenstrom's macroglobulinemia and 9% other non-Hodgkin's lymphoma. Their mean baseline haemoglobin was 9.2 g/dL. 50% of patients received intravenous iron. Abnormal baseline ZPP was most frequently the iron-deficiency trigger for commencing intravenous iron supplementation on first instigating rhEpo therapy. In patients started on rhEpo alone, the development of functional iron-deficiency during rhEpo-driven erythropoiesis was best monitored by changes in transferrin saturation and serum ferritin, leading to commencement of intravenous iron. However, in some patients, intravenous iron was instituted in spite of biochemical parameters not themselves indicating iron deficiency, because of downward trends in transferrin saturation and serum ferritin. Intravenous iron commencement enabled rhEpo maintenance doses to be reduced and even discontinued. With this approach, the overall response rate was 90% (81% > 2g/dL Hb increase) and mean Hb increment was 3.25 g/dL. *Summary and Conclusions*. Rational use of intravenous iron to support rhEpo therapy in haematological malignancy is possible with readily available, inexpensive biochemical parameters of iron status. ZPP most frequently reflects initial iron depletion, while transferrin saturation and serum ferritin trends best reflect development of functional iron deficiency during rhEpo therapy.

0079

SEQUENTIAL MONITORING OF ERYTHROCYTE HEMOGLOBINIZATION AND IRON MARKERS DURING R-HUEPO THERAPY IN ANEMIC PATIENTS WITH MULTIPLE MYELOMA AND LYMPHOMA

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Background. Recombinant human erythropoietin (r-HuEPO) stimulates erythropoiesis and increases iron demands in the erythroid marrow leading to functional iron deficiency (FID). The development of FID is an important cause of r-huEPO unresponsiveness in anemic patients with multiple myeloma (MM) and lymphoma. Close monitoring of sensitive markers of erythrocyte hemoglobinization and iron status are useful in recognizing FID early in the course of r-huEPO treatment. *Aims.* The aim of this study was to monitor the sequential alterations in erythrocyte hemoglobinization (EH) and iron markers in order to recognize the development of FID early in the course of r-huEPO treatment, in anemic patients with MM and lymphoma. *Methods.* Fourty-one patients with a median age of 71 years (range 18-84) with symptomatic MM or lymphoma and disease related anemia, were enrolled. All patients received epoietin β at a dose of 30.000IU/wk for 6 consecutive weeks. The evaluated parameters of iron status and EH, were: serum ferritin, transferrin saturation (TSAT%), soluble transferrin receptor (sTfR), the ratio of sTfR to the logarithm of ferritin (sTfR-F index), the percentage of hypochromic erythrocytes (HYPO%) and reticulocyte haemoglobin content (CHr). These parameters were evaluated in serial measurements at baseline and on weeks 1, 2 and 6. The gradual development of FID during sequential weeks was indicated by an increase in HYPO% and sTfR-F index and a reduction in CHr and TSAT%. Statistical analysis was performed with paired samples Wilcoxon test and correlations with the Spearman's test. *Results.* HYPO% and sTfR-F index gradually increased during all sequential measurements showing statistical signif-

icance, from baseline to week 6 (p<0.001). CHr was significantly decreased between baseline and weeks 2 and 6, as well as between week 1 and 6. In contrast, TSAT% was significantly decreased from baseline to week 6 and from weeks 1 and 2 to week 6 (p<0.001). HYPO% and sTfR-F index changes between baseline and week 6 were positively correlated (p<0.01) whereas CHr was negatively correlated with HYPO% and sTfR-F index (p<0.01). *Conclusions.* These results indicate that FID is gradually observed early in the course of r-HuEPO treatment in anemic patients with MM and lymphoma. Sequential monitoring of sensitive erythrocyte hemoglobinization (CHr and HYPO%) and iron status markers (sTfR-F index and TSAT%) is very useful for the early recognition of FID during r-huEPO therapy. This could help to optimise r-huEPO treatment by using intravenous iron in a rational manner, avoiding the risks of inappropriate iron co-administration.

0080

RIBOSOMAL PROTEIN S24 GENE ANALYSIS IN DIAMOND BLACKFAN ANEMIA IN ITALY

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Background. Diamond Blackfan anemia (DBA; MIM 105650) is a rare, pure red cell aplasia of childhood due to an intrinsic defect in erythropoietic progenitors. Somatic malformations are present in about 40% of patients. Although the vast majority of cases are apparently sporadic, 10-20% show an autosomal dominant pattern of inheritance. DBA is clinically heterogeneous, and within the affected families anemia may be mild or absent; some patients may show only increased mean corpuscular volume (MCV) and/or elevated erythrocyte adenosine deaminase activity. Approximately 25% of patients carry heterozygous mutations in the RPS19 gene which encodes for a structural ribosomal protein. Recently, Gazda et al. identified mutations in ribosomal protein S24 (RPS24) in 2% of patients. These data strongly suggest that DBA is a ribosomal disease. *Aims.* Our aim was to analyze the RPS24 gene in Italian DBA patients. *Methods.* We have collected DNA of 110 Italian unrelated patients with DBA; in 25 of them we documented a RPS19 mutation. So far we have sequenced exons and intron-exon boundaries of RPS24 in the 85 patients without a mutation in RPS19. Enzymatic digestion was performed to search for the mutation in all the available family members of one mutated patient and in 50 healthy subjects. *Results.* We found 2 new changes in the RPS24 gene. A missense mutation (371nt A>G) in exon 4 causes a non-conservative amino acid substitution that results in replacement of an asparagine with a serine at codon 124. The patient shows lip and palate cleft, short stature and is steroid-dependent. The mutation was inherited from her healthy father, and it was also found in a healthy sister. Incomplete penetrance has been reported also for RPS19 mutations. The second mutation is a deletion of codon 22 in exon 2 (64-66delCAA). The patient does not show somatic malformations or short stature; he is now transfusion dependent. No other members of his family have been analyzed so far. The mutations were not identified in 100 normal chromosomes. *Conclusions.* We have identified 2 new mutations in RPS 24 in Italian DBA patients. The prevalence of RPS 24 mutations in the population matches that found in US patients by Gazda *et al.* 2006. Although these mutations are not a common polymorphisms further data are needed to demonstrate their role in the pathogenesis of DBA.

0081

APLASTIC ANAEMIA: MANAGEMENT CONSTRAINS IN ZARIA, NIGERIA

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Background. Aplastic Anaemia (AA) is one of the bone marrow failure syndromes characterized by pancytopenia with a hypocellular marrow, often *empty bone* marrow in the absence of foreign cells. It can be inherited or acquired and definitive treatment consists of allogeneic hemopoietic stem cell transplantation (HSCT) or immunosuppression. *Aim.* To assess some diagnostic and treatment constrains in a resource limited setting with a view to raising awareness towards the formulation of an AA policy and consequent establishment of stem cell transplantation facility in Nigeria. *Methods.* Over a 4 year period (March 2002 to December 2006), a total of 309 patients had bone marrow studies out of which only 14 had Clinical and bone marrow histological features consistent with AA. Prospective clinic based assessment of patients was carried out. Blood cell count was carried out by standard manual methods (no automated haematology analyzer). All the patients were seronegative for transfusion transmissible viruses (HIV, Hepatitis B and Hepatitis C). Bone marrow studies include both aspiration cytology and trephine biopsy, normal marrow cytogenetics and immunophenotying could not be determined. Bone marrow microvascular density could not be done due lack of expertise on such diagnostic tool. Ferrokinetic studies were also not done due to lack of radiolabelled isotopes. The modality of specific treatment was the use of steroids and cyclosporin, other immunosuppressive agents such as Antilymphocyte Globulin, Androgens, Erythropoietin and haemopoietic growth factors were not available. Achieving cure with HSCT is still far from our reach. *Results.* Adult male patients predominate, with a male to female ratio of 1.6:1. Patient's ages range from 18 to 67 years. One childhood AA was reported in a 5 year old boy. Transfusion dependence was the commonest mode of presentation in 85.7% of the patients while the remaining 14.3% presented with epistaxis. The mean haematological parameters at presentation were packed cell volume of 0.14L/L, white cell count of 2.2×10^9/L, platelet count of 78×10^9/L and a corrected reticulocyte count of 0.02%. Management consists largely of supportive care with blood transfusions; packed red cells for anaemia and fresh whole blood when platelets are required in bleeding patients as there are no cell separators to produce platelet concentrates. Specific therapy was with prednisolone in 3 patients, high dose methylprenisolone (HDMP) in 7 patients, 1 patient had a combination of HDMP with cyclosporin and non in the remaining 3 patients. One patient achieved bone marrow recovery and still alive as at the time of this report, 1 patient had an unstable aplasia, after 1 year she developed a low grade bone marrow lymphoma and died in the following year, 5 patients were lost to follow up and 6 patients had a progressive aplasia with death ensuring within 4 months of diagnosis. Management is hampered by ignorance, poverty, lack of some diagnostic tools and inability to afford the recommended choice of therapeutic agents in a country with a Gross Domestic Product (GDP) per capita of $ 330-348 where 70.2% of the population subsists on less than $ 1 per day. *Conclusions.* Improved outcome might be achieved by health education, provision of basic diagnostic tools, availability/reduction in the cost of standard therapeutic agents and HSCT centre as is obtained in other countries.

Bleeding disorders

0082

ACQUIRED HEMOPHILIA IN URUGUAY (1977–2007) IN MEMORIAM PROF. ROBERTO DE BELLIS

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Background. acquired factor VIII inhibitor in a non-hemophiliac patient (acquired hemophilia A), is a rare life-threatening bleeding disorder. Treatment is an emergency and must control hemorrhagic manifestations and ultimate suppression of antibody production. Current management is challenging with the availability of novel agents, but immunosupression could lead the patient to fatal infectious complications. The experience of thirty years in the diagnosis and treatment of this patients in Uruguay is presented. *Methods.* 21 patients were studied from 1977 to 2007 in three medical institutions in Uruguay. Gender: 12 women and 9 men. Age: from 18 to 87 years old (median age 78). To dosage of factor VIII inhibitors we employed in the first years Carol Kasper's tecnique and in the last three years Nijmejen & Kasper technique. *Results.* the underlying condition in these cases were: idiopathic 9, lymphoproliferative disorders (non Hodgkin's lymphoma, chronic lymphoid leukemia, multiple myeloma) 4, PAR 2, puerperal conditions 2, hepatic transplantation 1, lupus erithematosus 2 and metastatic cáncer 1 patient. All of them presented with severe spontaneous haemorrhage. The main areas of bleeding were: central nervous system, calf haematoma, gastrointestinal, cellular subcutaneous, hematuria and oropharinx. Prolonged APTT, and reduced factor VIII:C level was detected. The factor VIII inhibitor titre ranged from 4,5 to 160 Bethesda Units (BU). All received high dose steroids and factor VIII concentrates (cryoprecipitate or recombinant human factor VIII). 7 patients received cyclofosfamide intravenous, and 14 of them intravenous immunoglobulin. One patient received rituximab 375 mg/m^2 during 4 weeks with excelent outcome. Other patient received factor rVII in two oportunities, with very good outcome too. 9 patients died of bleeding complications and 1 ot them of septic shock with normal values of factor VIII: C, in remission. *Conclusions.* Our experience confirms that significant clinical bleeding is the major presenting symptom of acquired hemophilia A. On this serie the most frecquent condition was idiopathic, affecting mainly elderly patients and associated with high mortality due to bleeding or immunosupression. Factor rVII and rituximab were two new options for succesfull treatment but have a very high cost. Treatment must be individualiced in order to obtain the fastest resolution of bleeding but avoiding the consequences of immunosupression that can lead the patient to fatal infections.

0083

EXCLUSION OF HAEMOPHILIA A BY DIRECT MUTATION DETECTION IN TRIGEMINI PREGNANCY

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Haemophilia A (HA) and haemophilia B (HB) are common X-linked bleeding disorders resulting from the inherited deficiency of coagulation factors VIII (fVIII) and IX (fIX), respectively. Female relatives of patients with haemophilia may be carriers and many of them request carrier status determination and prenatal diagnosis. In the framework of the molecular diagnostics service for haemophiliacs as a tertiary national referral center, since 1998, prenatal diagnostics was required in 42 cases out of 153 HA and 2 cases out of 31 HB affected families (28 male and 16 female fetuses). In cases of male fetuses, cycle sequencing or large gene inversion detection as direct mutation analysis, (in 2 and 12 cases respectively) and linkage analysis using intragenic polymorphisms in 14 cases was used as a method for prenatal diagnosis. Overall, 19 healthy and 9 haemophilia affected male fetuses were diagnosed. Furthermore, we hereby report an unusual case, a 33-year-old obligate HA-carrier woman (fVIII gene, intron 22 inversion carrier) who conceived three embryos during an *in vitro* fertilisation procedure and requested prenatal diagnosis. Transabdominal chorion-villus sampling was performed on the 9th week of pregnancy with successful sample acquisition form all three embryos. Long-distance PCR for intron 22 inversion detection indicated two healthy boys and one carrier girl. Knowing that all of the fetuses are free of HA-manifestation, the couple desisted from reduction. On the 35th week of pregnancy a caesarean section was performed on the bases of comlex indications and a 1950 g and a 2200 g boy and a 2350g premature girl were born. Our results further confirm, that molecular genetic investigations play an important role in haemophilia prenatal diagnostics.

0084

HAEMOSTATIC THERAPY DURING SURGICAL OPERATIONS TO HAEMOPHILIA PATIENTS WITH INHIBITOR

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Introduction. We present our experience surgical treatments of haemophilia patients with inhibitor. Haemostatic therapy is one of the leading problems in treatment of haemophilia patients with inhibitor. *Patients and Methods.* In total in our department there were 67 surgical operations to patients with inhibitor. All patients were with sever haemophilia. 68% of them were patients with high responding inhibitors, usually greater than 10 BU. All of them were HCV+. The majority of surgical operations were a high degree of risk, there were: total knee and hip replacement (6), extirpation of pseudotumour (10), osteosynthesis (6), synovectomy (16), ectomy of iliopsoas hematomas with intra-abdominal bleeding (3) and others. To all patients with a high titer before operation carried out plasmapheresis for decrease until inhibitor was not reduced up to 2 BU or lower. We used different hemostatic treatment. The last 3 years haemostatic therapy during the operation realized by preparation Novo Seven - from 120 mkg/kg each two hours per day of operation up to 90 mkg/kg each 4 hours for 9 day, then FEIBA 50 U/kg 2 times day till 14 days. *Results.* In 75% of cases there were good results (positive functional and anatomic results). 12% of cases have become complicated by bleedings. Most of them have been connected with insufficient hemostatic treatment and the expressed local inflammation. Fatal outcomes were not. The best results were when the hemostatic treatment was carried out by recombinant activated factor VII (rFVIIa-NovoSeven) during the operation and the first days after and then by activated protrombin complex concentrate (FEIBA). *Conclusions.* Modern hemostatic therapy in a complex with additional methods allows carrying out the haemophilia patients with inhibitor of surgical operations of any complexity. It is necessary to provide a good hemostasis both during operation, and in the first days after operation. At operative interventions of a high degree of risk hemostatic therapy is better for carrying out NovoSeven or FEIBA.

0085

MANAGEMENT OF HEMOPHILIA IN THREE PREMATURE NEONATES

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We describe the management and outcome of hemophilia in three premature infants. Only 3 other patients have been described in literature thus far. Two twins born at 33 weeks of gestation with moderate hemophilia A (factor VIII activity of 2%) were treated with prophylactic factor VIII replacement because of prematurity and the associated increased risk of intracranial hemorrhage. We preferred minimal blood sampling to avoid the insertion of a central venous catheter (CVC). The first born twin received a CVC for only two days for temporary difficult venous access during a clinical suspicious but non proven infection at day 9. He received prohylactic treatment for 17 days and no bleeding complications were observed. Recovery of factor VIII was 94%. No presence of factor VIII inhibitors were found for up the age of 18 months. The second twin developed respiratory distress and an infection with staphylococcus aureus. No bleeding episodes and no cranial hemorrhage were observed until day 7. On day 8 the infant became septic with bacteremia caused by enterobacter cloacae with rapid deteoriation. In the final phase of the septic shock a pulmonary bleeding occurred and a cranial ultrasound showed a intraventricular bleeding. He died on day 8. The third child was a premature infant born at 31 weeks of gestation with prolonged bleeding form a scalp pH-measurement. He was diagnosed with hemophilia B and started with prophylactic factor IX replacement 17 hours after birth. A grade II intraventricular bleeding was found on day 2 and he received intensive replacement therapy to achieve a through factor IX level of 70% for 17 days. A central venous catheter was inserted to avoid continuous access. The mean recovery of factor IX was 46% (range 28%-69%) and the half life time was 5,8 hours. A cerebral ultrasound on day 17 showed no signs of intracerebral hemorrhage and he was discharged at our hospital at day 28. No presence of factor VIII inhibitors were found for up the age of 16 months.

0086

THROMBELASTOGRAPHIC CHART RELIABLE REFLECTION OF HEMOSTATIC IMPACT OF ITP IN CHILDREN

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Background. Immune Thrombocytopenic Purpura (ITP) in children continues to be confronted with life threatening bleedings despite its generally accepted benign evolution. This risk is dependent primarily on the hemostatic efficiency of platelets, rather than the platelet count. *Aims.* Therefore, looking for a supplementary information about hemostatic consequence of thrombocytopenia and taking into account the multiple targets of platelets, we aimed a bedside evaluation of the whole dynamic process of hemostasis by means of thrombelastography (TEG) in ITP patients. *Method.* Our study included 34 patients: 35,29% with severe, 26,47% with moderate, and 14,70% with mild ITP. We analyzed TEG parameters: r (reaction time), k (clot kinetics to reach 20 mm amplitude), Angle α (clot strengthening), MA (maximum aplitude), G (clot strength) in correlation with platelet count and some hemostatic explorations: bleeding time (BT), clot retraction rate (CRR), prothrombin consumption ratio (PCR) and APTT. *Results.* In our study, we assessed the following hemostatic abnormalities ascribed to thrombocytopenia: PCR (p<0,01), CRR (p<0,01), BT (p<0,03); APTT wasn't changed in a significant manner (p=0,3). TEG was very sensitive, reproducible and specific revealing a defining reduction of MA (X=32,32 mm) (p<0,001) and G(X = 2,76) (p<0,001) and a slight prolongation of k (p<0,1). This simple point of care investigation succeded to draw the attention on risky situation even in moderate or mild ITP. *Conclusions.* Minimal tissue factor triggered whole blood TEG provided a valuable bed side tool for the follow-up of the kinetics of hemostasis in ITP patients.

0087

EHLERS-DANLOS SYNDROME A NOT SO UNCOMMON CAUSE OF BLEEDING

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Background. Ehlers-Danlos syndrome (EDS) is a group of connective tissue dysplasias which comes to the attention of a hematologist as an unexplained bleeding disorder. The hemorrhagic manifestations can be mild with easy bruising to severe fatal arterial ruptures. *Aims and Methods.* From the year 1986 to 2006, seventy patients with EDS from the haematology database at Bradford Centre for Haemophilia and Thrombosis were reviewed with the aim of analyzing their bleeding manifestations and to correlate the symptoms with any pathological abnormality in the tests for haemostasis. A retrospective analysis of the case notes and the results of the laboratory tests which included Bleeding Time (BT) by Ivy method, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Platelet Aggregation studies by standard methods and PFA100, VWD screen and coagulation factor assays when appropriate were done. *Results.* Sixty seven of these patients were referred for evaluation of bleeding tendencies and three were detected during screening. All the patients were of Caucasian origin. The age at presentation ranged from eleven to sixty five years (mean age 36.9 years). Majority of them belonged to EDS type I. Bleeding manifestations recorded included easy bruising (39), epistaxis(15), menorrhagia(14), bleeding post dental procedures(7), post trauma(5), post surgical procedures(3).There were 4 major bleeds requiring blood transfusions including post partum haemorrhages(3) and gastrointestinal bleeds(1). The laboratory investigations revealed prolonged PT in two cases and APTT prolongation in one patient. Five out of twenty-nine had a prolonged bleeding time. Platelet aggregometry was done in fifteen patients with reduced aggregation detected in six-one with arachidonic acid and five with collagen. PFA 100 analysis was abnormal with collagen epinephrine in two of the six patients which was not corrected with platelet transfusion .They were found to have normal nucleotides on further evaluation. Three patients had type l VWD. Management of these patients involved supportive treatment with Tranexamic acid. One patient needed factor VIIa for controlling a nasal bleed. The three mild VWD patients responded to DDAVP. *Conclusions.* EDS accounts for a substantial number of patients with bleeding diathesis. In Bradford, Type I EDS, especially the milder variants is the most common form seen. Interestingly, none of the patients in our series were Asians though they contribute to 15% of population. The bleeding tendency is usually mild with easy bruising being the most common. Major bleeds requiring transfusions were rare and there were no fatalities.84% of the patients with EDS had normal haemostasis test results and the few abnormalities found were nonspecific. Three patients had type 1 VWD.Treatment is supportive with Tranexamic acid and avoiding elective surgeries. DDAVP may be helpful and factor VIIa may be needed for severe bleeds.

0088

SUCCESSFUL MANAGEMENT OF AN INFANT WITH KASABACH-MERRITT SYNDROME USING ARTERIAL EMBOLIZATION AND SYSTEMIC DRUG THERAPY

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Kasabach-Merritt syndrome (KMS) is a rare life-threatening disorder in which significant thrombocytopenia and consumption coagulopathy are accompanied by rapidly expanding hemangiomas of trunk, extremities and/or abdominal viscera, sometimes associated with bleeding anemia. Many treatments have been tried for this difficult syndrome. These include systemic treatments, such as corticosteroids, interferons, antiplatelet agents, vincristine, as well as local ablation with surgery. We report an infant with severe KMS and successful treatment with combination therapy using steroid, vincristine, interferon (IFN)-α and arterial embolization. A 2-month-old boy presented with rapidly enlarging mass which his parents described as having developed from a 1-2 cm birthmark on her left cheek. Despite treatment with steroids, interferon alfa-2a, vincristine, fresh frozen plasma, platelets, and blood transfusion, the mass increased in size with worsening of the coagulopathy. Systemic drug therapies were continued. At the same time selective left external carotid artery embolization with microparticles was performed. A percutaneous biopsy with 18 G needle was also performed after the embolization. The needle tract was embolized with a liquid embolizing agent for a possible oozing. The diagnosis of Kaposiform hemangioendothelioma was made according to the findings. Kaposiform hemangioendothelioma (KHE) is an aggressive vascular proliferation that has been recognized as a separate entity from other vascular tumors. After ten days of embolization therapy, the coagulopathy was corrected. 1.5 months after the embolization, the mass size was considerably decreased. In conclusion, KMS may be successfully treated with combination therapies consisting of steroid, interferon, vincristine and embolization.

0089

OFF-LABEL USE OF RFVIIA AND ITS CLINICAL EFFECTIVENESS: RESULTS FROM A SCOTTISH NATIONAL AUDIT

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Background. Recombinant FactorVIIa (rFVIIa) is licensed to treat bleeding in haemophiliac patients with inhibitors. Several case series and reports and few randomised studies have highlighted its potential value in controlling bleeding in intracerebral haemorrhage (ICH) and massive haemorrhage secondary to different causes. *Aims.* To assess local practice and policy on the issue of rFVIIa for off-label indications in Scotland between January and June 2006. To determine quantity, indications and apparent clinical efficacy of rFVIIa used during the 6-months audit period. To assess possible associations of rFVIIa efficacy with platelet count and coagulation screen before its use. *Methods.* All haematology departments in Scotland were asked to complete a questionnaire. The first part of the questionnaire was directed to ascertain if rFVIIa stocks were available in each acute hospital and if there were clear release procedures for its off-label use; the second part was directed to collect all the clinical and laboratory data on individual issues of rFVIIa during the audit period. Data from a previous similar audit from 2003 were used for comparison. Chi-square testing was used for statistical analysis on categorical variables. *Results.* The questionnaire was returned by 96% of the hospitals (26/27). 88% of the hospitals keep a stock of rFVIIa on site, 46% having a written protocol for its release. In 88%, Consultant Haematologist authorisation is required. Off-label use has increased since 2003 (48 v 34 episodes/year) especially in non-Haemophilia Centre Hospitals. Other than increasing use for ICH, indications have changed little over time, mainly including trauma, gastrointestinal bleedings and cardiovascular surgery. Immediate response rate [RR; defined by either major reduction or cessation of haemorrhage according to subjective clinical assessment], 24-hour survival rate [24SR] and 28-day survival rate [28SR] have not

changed - combined data showing 68% RR, 79% 24SR and 52% 28SR. A high coagulopathy score [CS; scoring 1 point for each of platelet <50 ×10×9/L, fibrinogen <1.0 g/L, and INR or APTTr > 1.5] is associated with non-significant lower RR and 24SR. Combining data from both audits, comparing CS 2-3 v. CS 0-1 by Chi-squared test gives RR of 58% v 78% and 24SR of 62% v 84% (p<0.1 for both). Platelet count <50, alone, is associated with significantly worse RR and 24SR of 45% v 83% (p<0.01) and 60% v 85% (p<0.05) respectively. There were no adverse events related to rFVIIa use reported. *Conclusions.* The current audit shows that most acute hospitals in Scotland keep stocks of rFVIIa on site for emergency use. Off-label use of rFVIIa has increased compared to previous audit because of an increased use in District General Hospitals. This might reflect wider availability of rFVIIa. It has also started to be used for ICH. rFVIIa appears to be safe when used to treat massive blood loss or ICH. It is less effective in patients who are more coagulopathic, particularly if platelets are <50 at time of administration. Overall, despite a high subjective clinical RR of 68%, 28-day survival is poor at about 50%.

0090

DISAPPEARANCE OF HIGH TITRE INHIBITOR IN CONGENITAL HEMOPHILIA A USING RITUXIMAB INDUCTION AND MAINTENANCE

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The development of Factor VIII (FVIII) inhibitors is currently the most serious complication in the management of Hemophilia A. Rituximab is a chimeric monoclonal antibody targeting CD20 antigen on neoplastic and normal B cells, approved for use in B cell malignancies as well as rheumatoid arthritis. Its use in other autoimmune diseases remains investigational. Rituximab appears to be an alternative therapy to reduce or eliminate the F VIII inhibitor in selected cases. However, the doses and appropiate schedule, as well as long-term side effects need further investigations. We report the case of a boy with severe Hemophilia A who developed a FVIII inhibitor successfully treated with rituximab, after failure of 2 courses of immune tolerance therapy with recombinant and plasma-derived FVIII. The boy had been diagnosed with severe Hemophilia A (FVIII <1%) at the age of 9 months after he suffered spontaneous hematomas. Prophylactic therapy with recombinant FVIII (Recombinate®, Baxter) was started, but soon after he developed a FVIII inhibitor. The titer of inhibitor was 45 BU at diagnosis. Immune tolerance therapy was started with a daily administration of 150 IU/Kg of the same recombinant FVIII. Thirteen months after, the treatment was stopped due infection of intravenous device and lack of response, since inhibitor level fluctuated ranging from 45 to 350 BU. At the age of 6, a second course of immune tolerance was started with human plasma-derived FVIII (Fanhdi®, Grifols) containing Von Willebrand Factor (200 IU/Kg/day) and intravenous immunoglobulins 1g/Kg/day for 2 days, every 3 weeks. Inhibitor titre remained high, between 30-1120 BU and again, immune tolerance therapy was discontinued after 12 months because of the lack of response. From then until the age of 8 years, he suffered numerous bleeding episodes into target joints. In that moment, Rituximab was commenced in off-label use after parent's informed consent. Immediately prior to starting rituximab, inhibitor titre was 100 BU. Rituximab at 375 mg/m^2 was administered weekly for 4 doses, then monthly for 4 doses and finally every 8 weeks. Therapy with human plasma-derived FVIII containing Von Willebrand Factor (200 IU/Kg/day) was associated from the second dose of rituximab. Inhibitor titre dropped to 2 BU in the first 4 weeks and turned undetectable after the 7th dose; FVIII recovery was 41%. No bleeding episodes have occurred since the start of rituximab therapy, and tolerance has been good, with only one episode of urinary tract infection. In our experience, rituximab has a significant place as rescue therapy of patients with Hemophilia A complicated with inhibitors. Extended or maintenance therapy could be used in cases of failure of induction therapy.

0091

TYPE 2B 'MALMO OR NEW YORK' IS NOT SO RARE AMONG ITALIAN PATIENTS WITH VON WILLEBRAND DISEASE: THE ROLE OF P1266L/Q MUTATIONS OF THE VON WILLEBRAND FACTOR GENE IN SEVEN CASES

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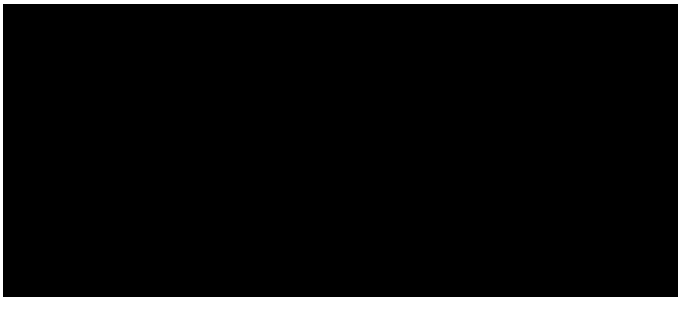
Background. Type 2B von Willebrand disease (VWD) is due to a unique gain-of-function variant of von Willebrand factor (VWF) that spontaneously interacts with circulating platelets resulting in loss of VWF high molecular weight multimers (HMWM) in plasma and, in some cases, reduced platelet counts. Diagnosis of 2B is based on increased ristocetin-induced platelet aggregation (RIPA) in patient's platelet rich plasma (PRP). Type 2B 'Malmo or New York' is caused by the mutation P1266L that is associated with increased RIPA, but with normal multimeric distribution in plasma and no thrombocytopenia. *Aims.* To determine the incidence and the molecular defects of this rare VWF variant among a large population of VWD patients regularly followed at two Northern Italian Hemophilia Centers. *Methods.* Criteria for VWD type 2B were those recommended by the ISTH-SSC-SC on VWF. RIPA and multimeric analysis were performed according to standardized methods. Bleeding severity score (BSS) was calculated in all patients enrolled in this study. Sequence analysis of the portion of exon 28 encoding for the A1 domain was performed to identify nucleotide substitutions in all cases where RIPA was <0.8 mg/ml. *Results.* 64 patients from 16 different families satisfied the criteria for VWD type 2B. Seven patients from 3 unrelated families clearly showed normal multimeric distribution in plasma and no thrombocytopenia also after stimuli. Clinical and laboratory parameters are summarized in Table 1. Patients from family A showed five distinct substitutions (3686T>G, 3692A>C, 3735G>A, 3789G>A, 3797C>T), patient B1 two (3789G>A, 3797C>T) and patient from family C three (3692A>C, 3789G>A, 3797C>A) distinct substitutions. Two of these were silent, whereas the other caused the following amino acid changes: three in family A (V1229G, N1231T, P1266L), one in B1 (P1266L) and two in family C (N1231T, P1266Q). Mutations V1229G and N1231T have already been reported, whereas mutation P1266Q is novel. The substitutions found in families A and B correspond to the pseudogene sequence, sustaining the possibility of a gene conversion between the VWF gene and pseudogene. In family C, the nucleotide substitution causing P1266Q mutation does not match the pseudogene sequence and the patients do not show the substitution 3735G>A that is present in the pseudogene. *Conclusions.* We confirm that the substitution of proline 1266 with leucine appears to be the main cause of type 2B *Malmo New York* phenotype while its replacements with a glutamine apparently results in lower VWF activities in family C. Based on these data, gene conversion appears to be more frequent than expected and capable to generate different set of mutations.

Table 1.



0092

EFFICACY AND SAFETY OF HIGHLY PURIFIED VWF/FVIII CONCENTRATES IN THE TREATMENT OF INHERITED VON WILLEBRAND DISEASE: RESULTS FROM THE RETROSPECTIVE ITALIAN STUDY ON 103 CASES

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Background. Efficacy of highly purified VWF/FVIII concentrates with standardized VWF:RCo content has been proven in prospective clinical

trials. The aim of this retrospective study is to verify the efficacy and safety of highly purified VWF/FVIII concentrates in treatment and prevention of bleeding in inherited Von Willebrand disease (VWD). *Methods.* 103 patients were treated with study concentrates (Fanhdi and Alphanate) from 2001 to 2006 at 15 Hemophilia Centers according to Italian guidelines for VWD management. 47 males and 56 females, median age 50 years (range 6-83 years), had types 3 (10), 2A (14), 2B (20), 2M (7) and DDAVP-unresponsive 1 VWD (52) with a median bleeding severity score (BSS) of 8 (range 0-27). *Results.* Study drugs were given to treat 113 bleeding episodes in 51 cases and to prevent excess bleeding during 111 surgical procedures in 72 cases with a good/excellent clinical response in 97% (bleedings) and 99% (surgeries). To prevent the recurrence of GI bleeding (8) CNS hemorrhage (1) hemarthroses (1), menorrhagia/urogenital bleeding (6), secondary prophylaxis was also carried out in 16 cases with types 3 (3), 2A (2), 2B (2), 1 VWD (9) all characterized by BSS >6. A regimen of 40 U VWF:RCo/kg every other day or twice a week, given over a median period of 505 days (range 24-1484) totally prevented bleeding in 13 cases, strongly reducing the incidence in the other ones. No adverse reactions occurred in 4.909 infusions for a total of 10.505.000 IU FVIII. *Conclusions.* These results confirm the efficacy and safety of study concentrates not only in the management of bleedings and surgeries but also in secondary prophylaxis in severe inherited VWD.

0093

PHARMACOKINETICS OF THE REFORMULATED B-DOMAIN DELETED RECOMBINANT FACTOR VIII CONCENTRATE USING CHROMOGENIC AND ONE-STAGE ASSAYS WITH POOLED NORMAL PLASMA AND REFACTO LABORATORY STANDARD

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Background. The use of RLS in one stage clotting assay has been proposed to reduce the underestimation of FVIII plasma concentration measurements after infusion of BDD-rFVIII in hemophilia A patients. Both BBD-rFVIII and RLS have been recently reformulated. *Aims.* Aim of this study was to evaluate the performance of reformulated RLS in the measurement of FVIII plasma concentration after infusion of the reformulated BDD-rFVIII. *Methods.* In 13 hemophilia A patients, FVIII <1%, inhibitor <0.6 UB/mL, age >12 years, not on anti-retroviral therapy for HIV, 25 UI/kg of BDD-rFVIII were injected intravenously. Venous blood samples were collected at 0.25, 0.5, 1, 3, 6, 9, 24, 28 and 32 h after the end of the infusion. One-stage clotting assay was performed using a FVI-II immune depleted plasma. The Electrachrome Factor VIII Chromogenic Assay was performed according to the manufacturer's instructions. The reference calibration curves were built using PNP or RLS from Wyeth Pharma gmbh. Pharmacokinetic analysis was performed using a model-independent method fitted with WinNonlin (Pharsight, Inc). Comparisons among assays and standards were performed using an ANOVA model for repeated measures. *Results.* Table 1.

Table 1.

	Chromogenic Assay				One stage assay				
	Refacto ST		Plasma ST		Refacto ST		Plasma ST		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
r2	0.98		0.99		0.97		0.99		
Cmax (U/mL)	0.63	0.14	0.63	0.09	0.60	0.13	0.58	0.13	ns
Tmax minutes)	17	5	18	6	15	0	17	5	ns
Half_life (hours)	9.4	3.8	7.7	4.3	10.4	2.8	13.0	4.7	<.05
AUCinf	431.0	148.0	469.0	166.0	468.0	161.0	575.0	147.0	<.05

Conclusions. We confirm previous results of a better sensitivity of the one stage method for the lowest concentrations of FVIII, with a more accurate evaluation of the terminal half life. Measured Cmax is slightly superior to the 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.

Chronic lymphocytic leukemia and related disorders - Biology I

0094

GENE EXPRESSION PROFILING OF PERIPHERAL BLOOD T CELLS IN PATIENTS WITH INDOLENT B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background. The impact of nonmalignant cells on the growth and sustenance of the malignant cells in many different cancers is evident. There is substantial evidence that T cell functions were dysregulated in B-cell chronic lymphocytic leukemia (B-CLL) and T cells may contribute to the survival and growth of the leukemic clone. In addition, the absolute number of T cells, largely devoid of antileukemic activity, is elevated in CLL patients. *Aim.* The aim of this study was to delineate genes in T cells of indolent B-CLL patients that may support the growth of the leukemic clone. We have also attempted to identify genes whose dysregulation may be the underlying cause of the observed T cell expansion and aberrant functions. *Methods.* We performed Affymetrix gene array analysis on highly purified peripheral blood $CD3^+$ T cells of treatment-naïve, indolent B-CLL patients (n=5) and compared with multiple myeloma (MM) patients (n=5) and healthy donors (n=5). Microarray results were confirmed with T cells of 14 CLL patients, 6 MM patients, and 10 healthy donors using quantitative real time PCR (QRT-PCR). T-cells were purified using selection with immunomagnetic beads. For the QRT-PCR assay, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were analyzed separately. The contamination of monocytes, B cells, and NK cells was <1%. *Results.* The results demonstrate that a large number of genes (356) involved in different cellular pathways and activities including signaling, proliferation control, apoptosis, metabolism, immune response, and cytoskeleton formation are dysregulated in CLL in comparison to MM patients and healthy donors. Three genes that showed the highest upregulation were the chemokines XCL1, XCL2, and the cytokine IFN-γ. CCL4 and CCL5 are two other important chemokines that also were found to be specifically upregulated in T cells of B-CLL patients as well as KLF6 and TRAF1. *Conclusions.* The four highly upregulated chemokines, as well as the other molecules that we have identified, may have an effect on the survival of the neoplastic cells in B-CLL. The results of the present study may be of significance for a better understanding of B-CLL pathobiology and development of therapeutic strategies.

0095

SECONDARY GENETIC EVENTS IN CLONAL CD8+/TCRAB+ LARGE GRANULAR LYMPHOCYTE PROLIFERATIONS

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Background. Expansion of large granular lymphocytes (LGL) is a type of lymphoproliferative disorder that ranges from reactive expanded activator T-cells to T-cell leukemia and shows variable presentation and clinical course. The vast majority concern $CD8^+$/$TCRab^+$ T-LGL lymphoproliferations. The development of T-LGL leukemia is likely to be a multistep process. In T-LGL leukemia the lymphocytes are clonal in nature as evidenced by clonally rearranged TCR genes. Due to various immunogenetic factors an initial polyclonal response results in clonal outgrowth, representing a pre-leukemic state. Secondary genetic events are likely to be essential for transformation of this exaggerated immune response into clinically malignant leukemia. However, well-defined clonal chromosomal aberrations have rarely been reported, whereas little is known about the secondary genetic events leading to clinically malignant $CD8^+$/$TCRab^+$ T-LGL leukemia. *Aims.* To determine genetic aberrations involved in the transformation of persistent clonal $CD8^+$/$TCRab^+$ T-LGL populations into clinically malignant T-LGL leukemia and to obtain more insight into the molecular pathogenesis of $CD8^+$/$TCRab^+$ T-LGL leukemia. *Methods.* In the present study we included 13 symptomatic

patients with CD8+/TCRab+ T-LGL proliferations. The diagnosis of T-LGL leukemia was established by clinical and laboratory parameters and all patients had a tumorload of at least 80%. Extensive immunophenotyping and BIOMED-2 PCR-based GeneScan analysis of TCRB gene rearrangements were performed in all peripheral blood (PB) samples. Genomewide analysis of DNA copy number changes in PB samples from all patients was performed using high-resolution array-based comparative genomic hybridization (array-CGH) containing about 3500 bacterial artificial chromosomes (BACs). *Results.* All patients presented with anemia and/or (severe) neutropenia with an increased T-LGL proliferation. The aberrant T-LGL population showed membrane expression of CD3, CD8, CD57 and TCRab and carried monoclonal TCR gene rearrangements. In three cases clear genetic aberrations could be detected. One case had a complete loss of the X chromosome and chromosomal gain at 10q11. In the second case loss at 4p16 and gain at 8q24 were found. This kind of gain loss pattern could be derived from an imbalanced translocation between chromosome 4 and 8. The final case had chromosomal gain at 6p22 and 14q13 with single copy loss at 6q16. We are currently looking further into candidate genes located in these regions which might be involved in the etiopathogenesis of T-LGL leukemia. *Conclusions.* Genetic instability in LGL leukemias seems to be less common and more subtle than in other mature T-/NK-cell malignancies. Although no recurrent genetic aberrations were found, we did identify some chromosomal regions that might harbour genes that are possibly relevant for the pathogenesis of T-LGL leukemia.

0096

THE MUTATION STATUS OF THE RESIDUAL ATM ALLELE IS AN IMPORTANT DETERMINANT OF TUMOUR PROGRESSION AND PATIENT SURVIVAL IN CLL CASES CONTAINING AN 11Q DELETION

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Background. Chromosome 11q deletions occur in up to 20% of CLL tumours and are associated with an adverse outcome. The minimal region of deletion includes the Ataxia Telangiectasia Mutated (ATM) gene, and mutations in ATM also occur in CLL patients and exert a detrimental effect on patient survival. ATM is a DNA damage response protein and defects in its function lead to impairment in p53-dependent apoptosis. Interestingly, the relationship between chromosome 11q deletions, ATM mutations and the integrity of the DNA damage response has not been specifically addressed in CLL. *Aims.* Our aim was to determine if CLL patients with a chromosome 11q deletion could be divided into two subgroups based on the functional status of the remaining ATM allele and whether these patient subgroups have differential progression or survival.

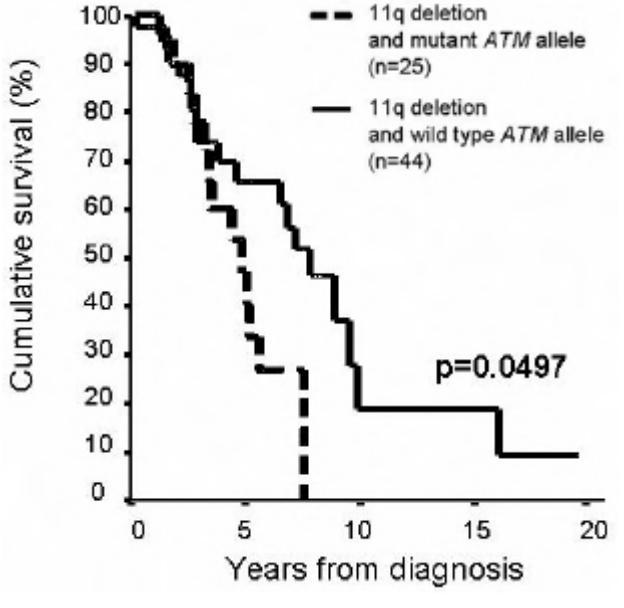

Figure 1. ATM mutations affect survival in 11q deleted CLLs

Methods. We established a cohort of 72 CLL cases that all had a chromosome 11q deletion and determined the sequence of the 62 coding exons of the residual ATM allele. We subsequently related ATM mutation status to clinical outcome, the size of the 11q-deleted tumour subclone, and the cellular responses to irradiation and cytotoxic drug exposure *in vitro*. Informed patient consent and ethical approval was obtained. *Results.* We found that the residual ATM allele is mutated in 35% of CLL tumours with an 11q deletion, thus distinguishing two genetic subgroups of these tumours; those with a second wild type and those with a second mutant ATM allele. Interestingly, the presence of a mutation in the residual ATM allele was associated with a further reduction in patient survival beyond that which is already dictated by the chromosome 11q deletion (p=0.0497). This subgroup of tumours also demonstrated impairment in the cellular response to irradiation and cytotoxic drug exposure *in vitro*, which contrasted from the preserved responses in the 11q-deleted tumour subgroup with a residual wild type ATM allele. Finally, we also found evidence for acquirement of ATM mutations during clonal evolution and observed that there was a highly significant association between the presence of ATM mutations and the expansion of the 11q-deleted subclone (p=0.002). *Conclusions.* CLL tumours with 11q deletion can be divided into two subgroups based on the integrity of the residual ATM allele. Complete loss of ATM function is associated with an impaired response to cytotoxic chemotherapeutics *in vitro*. This provides a mechanism to explain the poorer clinical outcome of patients with bi-allelic ATM abnormalities by comparison to patients with an isolated 11q deletion. Sub-clones that have acquired bi-allelic ATM defects can develop and expand during CLL clonal evolution, and we believe the use of DNA-damaging cytotoxic agents may have the potential to select these sub-clones, which in turn leads to the acceleration of disease progression.

0097

HIGH RESOLUTION SNP ARRAYS AND DIFFERENTIAL MIRNA EXPRESSION IDENTIFY NOVEL GENOMIC ABERRATIONS IN CLL

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Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with an extremely variable clinical course. Recurrent genetic aberrations and VH mutation status are important independent predictors of disease progression and survival, allowing a risk assessment of individual patients in the course of the disease. FISH detects genomic aberrations (13q-, +12, 11q-, 17p-) in over 80% of cases, the most frequent being 13q14 deletions, associated with a favorable prognosis. In order to characterize this subgroup we defined the minimal region deleted using 11 FISH probes, and we used the high-resolution 50k Affymetrix SNP arrays (SNP-A) to study the entire genome of 8 B-CLL cases from a previously well genetically characterized series of 54 patients, all harboring 13q14 deletion. This tool allows for precise detection of simultaneous analysis of LOH and gene copy number. There was a good correlation between the FISH and SNP-A *Results.* SNP-A allowed the identification of 8 novel aberrations, 2 of them recurrent. Two cases with unmutated IgVH genes presented gains on 2p14-2pter recently described by Pfeifer *et al.* (Blood 2006), spanning the REL and BCL11A oncogenes; and 2 cases with mutated IgVH genes presented gains on 19q13.33-19qter. Besides, we detected 9 non-recurrent LOH regions with normal copy number. LOH by UPD usually occurs in fragile sites in the genome, a common location for miRNA. Recently, there has been major progress in the identification of microRNA expression profiles that could distinguish normal from malignant B cells, and identify a unique microRNA signature associated with prognostic factors. To study the relationship between LOH regions and miRNAs expression we analyzed the expression of 157 miRNAs in these patients and in PB from normal donors by real-time PCR. Consistent microRNA down-regulation was seen in miR154, the miR181a and miR181b cluster, miR199a, miR199-s, miR224, miR-299, miR-326, miR-370 and miR-let7e; whereas miRNA upregulation was seen in the miR-96 and miR-183 cluster, miR-155 and miR-210. The miR181 has been reported to be differentially expressed in CLL, and overexpression of miR-155 has been related to aggressive forms of CLL. Interestingly, 3 of the downregulated miRNAs (30%) were localized in the 14q32 region, where the IgVH genes are located. The MDR in 13q in our samples contained the miR-15a and miR-16-1 genes, located at 13q14.3. These microRNAs are frequently deleted and/or downregulated in patients with B-cell CLL and have an impor-

tant function in this disease, negatively regulating Bcl2 at a post-transcriptional level. We found both downregulated in our samples with 13q-, although if comparing with normal PB the difference was not statistically significant, probably due to normal cell contamination. We did not find a correlation between genomic deletions and miRNAs located in these regions downexpressed, suggesting that regulation of miRNA expression must be due to a more complex mechanism. In conclusion, the transforming events that lead to B-CLL are unknown, and although genetic abnormalities appear to promote disease progression, none of these is seen in every patient. Therefore, combining SNP-A technology and miRNA expression profile could be an interesting approach to understand the genetic changes that can occur in B-CLL, and to provide new biological insights into different CLL subgroups.

0098

RITUXIMAB SENSITIZES SOME B-CLL SAMPLES TO FLUDARABINE AND CHLORAMBUCIL *IN VITRO*, REGARDLESS OF P53/ATM STATUS

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Background. Aberrations of two co-operating genes, the p53 and ATM, significantly deteriorate prognosis and treatment options for B-CLL patients. Monoclonal antibody rituximab (anti-CD20) is preferably used in combination regimens in B-CLL, often in those containing fludarabine. Very few *in vitro* data exist, however, showing an effect of such common treatment on B-CLL cells with aberrant p53 and/or ATM. In this respect, the data are also missing for a potential application of rituximab with chlorambucil. *Aims.* The aims were to assess the *in vitro* effect of the above mentioned combinations of drugs on B-CLL cells with various p53/ATM status. *Methods.* An interphase FISH was used for a determination of p53 and ATM deletions. Functional FASAY analysis coupled to sequencing was employed to supplement the screening also for p53 mutations. A metabolic WST-1 assay monitored the drugs effect on cell viability. An *in vitro* system lacking active human plasma was used, thus omitting the CDC pathway. After rituximab pre-treatment (10 μg/mL, 72h) the chemotherapeutics were applied in four concentrations for additional 48h (F: 25-0.4 μg/mL; CLB: 50-6.25 μM). Two-way analysis of variance (ANOVA) was used for determination of rituximab pre-treatment significance. *Results.* For the rituximab/fludarabine combination we tested forty samples having a median 85% of B-CLL lymphocytes, with the following characteristics: 13 were wild-type, 10 harbored ATM deletion (median 83% of deleted cells) and 17 exhibited p53 defects of various complexity - both alleles inactivation (del/mut and mut/mut) as well as the separate (one allele) aberrations (del or mut). The sensitivity to fludarabine was determined for the concentration 1.6 μg/mL, which provided significant differences among the samples. The sensitivity was assessed as follows: resistant - viability ≥60%; medium - viability <60% and ≥40%; sensitive - viability < 40%. The p53-affected samples were mostly resistant (71%) and none were sensitive. Among ATM deleted samples, on the contrary, 40% were sensitive, what was more than in wild-type subgroup (23%). Rituximab alone slightly increased a metabolic activity in most of samples, usually to 110-130% compared to untreated controls, while rarely a decrease was also noted (up to 80%). When the viability of fludarabine-treated and rituximab/fludarabine-treated samples was assessed in relation to fully untreated control, the positive sensitization effect of rituximab pre-treatment (p<0,05) was noted as follows: within the p53-affected as well as ATM-deleted subgroups in 30% of samples and within the wild-type subgroup in 62% of samples. For the rituximab/chlorambucil testing, which was performed as a pilot study in the same manner in eight samples, the positive effect of antibody pre-treatment was also noted in some samples of all the three subgroups. *Summary and Conclusions.* Our results indicate that the p53/ATM status is critical for the sensitivity of B-CLL cells to fludarabine. Regardless of the p53 and ATM aberrations, some samples are available for the rituximab-mediated sensitization to this agent. Our pilot data also support a warranty of testing a combined regimen containing rituximab and chlorambucil.

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0099

TP53 MUTATION AND 17P DELETION ARE ASSOCIATED WITH POOR SURVIVAL IN PATIENTS WITH CLL IRRESPECTIVE OF THE INACTIVATION OF THE OTHER ALLELE BY DELETION OR TP53 MUTATION: RESULTS FROM A SINGLE CENTER ANALYSIS

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Background. CLL patients with 17p deletion have a dismal prognosis. The exact prognostic role of TP53 mutations in the absence of 17p deletion and any differential impact of the mutation in cases with 17p deletion (vs. sole deletion) is currently unclear. *Aim.* To assess the incidence and prognostic impact of TP53 mutations as assessed by direct sequencing and DHPLC in a well characterized patient population. *Methods.* We studied 126 CLL patients from a single institution. The population is well characterized clinically and detailed genetic characteristics are available (FISH, VH-Status, CD38 expression). We used direct sequencing (n=126) and DHPLC (n=80) to detect TP53 mutations in the coding exons (2-11). The mutation analysis was performed on samples taken a median of 0.6 months after diagnosis. *Results.* We found an overall incidence of TP53 mutations of 13.5% (17/126 patients). The mutations were mainly located in the DNA binding domain (Exons 4-9). All mutations detected by direct sequencing were also detected by DHPLC. DHPLC was also informative in determining the allele status in the presence of heterozygous polymorphisms. Twelve of 15 patients with 17p deletions also had a TP53 mutation (80%). We also found TP53 mutations in the absence of 17p deletions in 4.5% (5/111). In the subgroup of patients with trisomy 12q we did not find TP53 mutations as opposed to 17/ 103 (16.5%) in all other subgroups combined (p=0.04). To study the impact on overall survival we confined the analysis to patients who were studied within 24 months of diagnosis to avoid bias. In this subgroup (n=108) we found a significant impact of the presence of the TP53 mutation (median survival 65 months vs. 15 months, p<0.0001). When separating the groups according to presence or absence of TP53 mutation and/or 17p deletion we found a highly significant influence of the presence of the TP53 mutation and/or 17p deletion on survival from the date of study (Figure 1) (p<0.0001).

Figure 1. Survival stratified by 17p and p53 Status.

Overall survival (from the time of study) was similar in the groups with deletion 17p and mutation (7.9 months), sole TP53 mutation (5.1 months) and 17p deletion without TP53 mutation (4.4 months) (vs. 66.7 months) (Figure 1). In 8/17 patients the mutation was found within 1 year of diagnosis. 4 of 6 patients with TP53 mutations and availability of serial samples, showed acquisition of mutations over time. *Conclusions.* TP53 mutations are associated with poor survival in CLL. TP53 mutations occur at a low incidence without 17p deletion and appear to be associated with poor survival once they occur. While the majority of cases with 17p deletion also have TP53 mutations, the prognosis appears to be equally poor for patients 17p deletion in the absence of TP53 mutations. These findings need to be confirmed in prospective trials. The mechanisms underlying the poor prognosis of patients with 17p deletion without TP53 mutation are under investigation.

0100

ASSESSMENT OF ZAP-70 EXPRESSION BY FLOW CYTOMETRY USING TWO DIFFERENT ANTIBODIES AND ITS CORRELATION WITH IGVH MUTATION STATUS AND CYTOGENETICS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Prognosis of patients with chronic lymphocytic leukemia (CLL) is largely heterogeneous and may be estimated based on clinical and laboratory findings the most important ones of which include chromosomal aberrations, the IgVH mutation status, and the expression of ZAP-70. Various efforts have been made to define the optimum procedure of ZAP-70 assessment including selection of antibody clone and the method of analysis. The validation of ZAP-70 has been performed using the course of the disease as well as using the IgVH mutation status. *Aims.* To further clarify the role of ZAP-70 expression in CLL we prospectively analyzed ZAP-70 expression, IgVH mutation status, and chromosome aberrations in peripheral blood and bone marrow samples of patients with CLL. *Methods.* For the flow cytometric assessment of ZAP-70 expression the antibody clones 1E7.2 (Caltag, n=523) and SBZAP (Immunotech, n=166) have been used. IgVH mutation status has been analyzed in 407 cases and chromosome aberrations in 416 cases applying FISH with probes for del(6q), del(11q), +12, del(13q), del(17p), and t(11;14). ZAP-70 expression has been calculated as percentage of positive cells using normal T-lymphocytes as positive controls as well as using the ratio of mean fluorescence intensity (MFI) for ZAP-70 between T-lymphocytes and leukemic B-lymphocytes. *Results.* Significant correlations were found between the percentages of ZAP-70 positive cells and IgVH homology (1E7.2, $p<0.001$, r=0.239; SBZAP, $p<0.001$, r=0.437). Accordingly, patients with mutated and unmutated IgVH status (98% homology used as cut-off) significantly differed in the percentages of ZAP-70 positive cells (1E7.2, mean 32% vs. 46%, $p<0.001$; SBZAP, mean 11% vs. 35%, $p<0.001$). Even stronger correlations have been found, however, comparing the MFI ratios for ZAP-70 (normal T-lymphocytes to CLL cells) with the IgVH mutation status: 1E7.2, $p<0.001$, r=-0.283; SBZAP, $p<0.001$, r=-0.569. The respective differences between cases with mutated and unmutated IgVH status were: 1E7.2, mean MFI ratio 3.45 vs. 2.67, $p=0.001$; SBZAP, mean MFI ratio 6.33 vs. 3.14, $p<0.001$. The ratio of MFI between leukemic and normal B-lymphocytes correlated less strong with the IgVH mutation status. No significant associations of ZAP-70 expression with chromosomal aberrations have been found. *Conclusions.* The results of the present study 1) confirm the strong correlation between ZAP-70 expression and IgVH mutation status, 2) confirm the independence of ZAP-70 expression from chromosome aberrations, 3) argue in favor of analyzing ZAP-70 expression as MFI ratio between T-lymphocytes and leukemic B-lymphocytes, and 4) suggest that the SBZAP clone has a significant potential in the diagnostic work-up of CLL. Further studies are warranted to validate the role of the MFI ratio approach and of the SBZAP antibody in the clinical setting.

0101

IGVH1-69 GENE USAGE IN B-CLL - THE SIGNIFICANCE OF SOMATIC HYPERMUTATION AND CRD3 STRUCTURAL FEATURES

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Background. B-CLL is a heterogenous disorder with a highly variable clinical course. Although certain acquired cytogenetic aberrations, unmutated IgVH gene status, ZAP-70 positivity and an atypical phenotype have been linked with more aggressive disease and a poorer outcome, the search for further prognostic markers is ongoing. IgVH 3-48, 3-53 and 3-21 gene usage have previously been associated with more aggressive disease and shorter survival times. In addition, IgVH 1-69 is expressed at a high frequency in B-CLL supporting the concept of antigenic selection in disease pathogenesis. It has been suggested that comparison to germline immunoglobulin sequence using the NCBI/IgBLAST database only may result in overestimation of mutated IgVH frequency associated with IgVH 1-69 rearrangements in B-CLL. Thus the clinical significance of IgVH 1-69 gene usage remains to be clarified. *Aims.* The aims of this study were to determine the frequency of VH1-69 gene usage in B-CLL, to assess the structural features of the CDR3 region, mutational status of the VH1-69 gene using the up-dated IgBLAST and V-QUEST databases and to determine if patients with VH1-69 rearrangements differ in clinical course to those patients with alternative IgVH gene usage. *Methods.* Two hundred and seventy patients were recruited for this study. IgVH gene usage was determined using multiplex BIOMED-2 primers (InVivoScribe Technologies) and protocol. IgVH mutational status was determined by sequence analysis using BigDye chemistry and homology comparison with the up-dated NCBI/IgBLAST and IMGT/V-QUEST databases. Interphase FISH analysis was performed to screen for common cytogenetic aberrations and serum thymidine kinase (TK) levels were measured using a radioenzyme assay. *Results.* We identified IgVH1-69 gene usage in 31/270 CLL patients (11.5%), 30 of which were characterised further. Our results demonstrate that it is associated with un-mutated IgVH status (80% of cases) and a preponderance of males (73% of cases). Poor/intermediate prognosis cytogenetic abnormalities were detected in 10 of 15 (75%) cases analysed. Although 21 patients (70%) were found to have Binet stage A disease upon presentation, the majority have since progressed to stage B or C. The median TK level was 16.1 U/L (>8.5 U/L is associated with progressive disease). IgVH somatic hypermutation was found in 6 cases (20%) and was associated with shorter CDR3 regions, IGJH 4 gene usage and lower TK levels (median of 6.8 U/L). *Summary and conclusions.* We have found that IgVH1-69 gene usage is over-expressed in B-CLL, is associated with advanced disease and may have potential as a supplemental predictive marker of disease progression. However, it remains to be determined if IgVH1-69 gene usage is an independent poor prognosis marker, as its association with un-mutated IgVH status in most patients may be the overriding factor. We present evidence that IgVH1-69 B-CLL cases can be divided into mutated (n=6) and un-mutated (n=24) sub-groups and that mutated cases are defined by short CRD3 regions and biased IGJH 4 gene usage. We are currently investigating the possible common antigenic stimuli which may be involved in leukaemogenesis in this subgroup.

0102

NOTCH2 MIGHT BE INVOLVED IN THE PKC-DEPENDENT SURVIVAL OF B-CLL CELLS

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Notch2 plays a determining role in the development/homeostasis of murine Cd5+ B1 B-cells, suggesting a potential function for human NOTCH2 in B-CLL leukemogenesis. Here we show that freshly isolated B-CLL lymphocytes (n=30) express an activated form of NOTCH2 (N2IC) irrespective of their prognostic marker profile (ie. IgVH mutational status, CD38 expression, cytogenetics). Although the majority of cultured B-CLL samples downregulate their NOTCH2 activity within one day (24 out of 30), DNA-bound N2IC complexes could be maintained by the phorbol esther TPA (1 ng/mL) accompained by an upregulation of the NOTCH2 target gene CD23 and increased cell viability. These effects are sensitive to the Protein kinase C-delta (PKC-‰) inhibitor Rottlerin. The TPA induced NOTCH2 activity was found to be resistant to the g-secretase inhibitors (GSI) DAPT and compound E in 24 out of 30 B-CLL cases suggesting that the vast majority of B-CLL cases express an activated form of N2IC which is not tethered to the plasma membrane and, thus, does not require g-secretase for signaling. NOTCH2 inhibition either by DAPT in a GSI sensitive B-CLL case or by RNAi mediated NOTCH2 gene silencing suggests a functional relation between NOTCH2 dependent CD23 expression and B-CLL cell viability. Thus, deregulated NOTCH2 signaling seems to be a common phenomenon in B-CLL and might be critically involved in the PKC-dependent survival of B-CLL cells.

0103

BIALLELIC DELETION 13Q14.3: A NEW PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA?

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Background. Chronic lymphocytic leukemia (CLL), the most frequent type of adult leukemia in the Western world, is characterized by a highly variable clinical course with survival times ranging from months to decades. Genomic alterations in CLL are considered independent predictors of disease progression and survival. Monoallelic deletion of 13q14.3 (13q14x1) is the most common anomaly (55-60% of patients), associat-

ed to a favorable outcome. Biallelic deletion of 13q14.3 (13q14x2) alone or concomitant 13q14x1/13q14x2 has been observed in about 10-20% of patients with CLL. It has been scarcely evaluated in the literature. *Aim.* We analyzed the clinical, cytogenetic and FISH (fluorescence *in situ* hybridization) characteristics of 9 patients with 13q14x2 in order to evaluate its prognostic significance. *Patients and Methods.* From 1997 to 2006, a total of 100 patients with diagnosis of CLL were cytogenetically and FISH studied in our Institution. Among them, 9 cases (5 males; mean age 63 years; range 47-77 years) showed 13q14x2. Immunophenotypic analysis showed expression of pan-B antigen (CD19, CD20, CD22) with co-expression of CD5 and CD23. Rai clinical stages were: I-II: 5; III-IV: 4. Eight patients were under treatment, the remaining one did not accept to be treated. Five patients have already died at the moment of this analysis. Chromosome studies were performed on stimulated peripheral blood lymphocytes. G-banded technique was used. FISH analysis for trisomy 12, and deletions of D13S319 at 13q14 band, ATM at 11q22.3 and TP53 at 17p13 (VYSIS) was carried out according to standard protocols. *Results.* Six out of 9 patients with CLL showed normal karyotypes and 13q14x2 alone or concomitant 13q14x1/13q14x2. Mean time from diagnosis to treatment for this group was 26 months (range 3-61 months). The remaining three had other genomic alterations: trisomy 12 (5.5% of cells), i(17)(q10) and complex karyotype. The last two patients were studied at the progression of the disease after more than ten years of stable disease. The patient with +12 was studied at diagnosis and had a poor outcome with a very short survival (4 months).Two cases showed only biallelic deletion 13q14 (58% y 88% of cells). The other seven cases (78%) had concomitant 13q14x1/13q14x2, being biallelic clones larger than monoallelic ones in 5 cases (5/7:71.4%). Simultaneously, we used the defined set of hierarchical FISH risk categories to compare FISH results by stable versus progressive disease. FISH was abnormal for 27/46 (58.7%) patients with stable disease and 47/54 (87%) cases with progressive disease (p<0.003). Interestingly, all six patients with normal karyotypes and 13q14x2 showed progressive disease respecting to 38.7% of the cases with 13q14x1 alone (p<0.01). A high proportion of patients with progressive disease was also observed for anomalies +12, 17p- and 11q-. *Conclusions.* The high frequency of patients with concomitant 13q14x1/13q14x2 suggests that the biallelic deletion would result from clonal evolution. Our data indicate that 13q14x2 would represent a more aggressive anomaly than 13q14x1 and could be considered as a marker of possible prognostic value in CLL. More studies must be performed to confirm these results.

0104

GELDANAMYCIN HAS OPPOSING EFFECTS ON WILD-TYPE AND MUTANT P53 AND INDUCES P21CIP1 EXPRESSION AND CYTOTOXICITY IRRESPECTIVE OF P53/ATM STATUS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Wild-type (wt) p53 has a beneficial effect in cancer, whereas mutant p53 has a deleterious effect by virtue of its tumorigenic and anti-apoptotic properties and high level of expression. In keeping with this, p53 mutation/deletion in chronic lymphocytic leukaemia (CLL) is strongly associated with rapid clonal expansion, propensity to transform, and resistance to chemotherapy, and developing new and better ways of treating such patients has become a major priority. Cancer cells seem to depend more than normal cells on the Hsp90 chaperone protein, which can be inhibited by the ansamycin geldanamycin (GA) and its derivative 17-AAG. Previous studies have shown that GA or 17-AAG can kill CLL cells in a selective fashion via depletion of the Hsp90 client proteins Akt and Zap-70. However, there is also evidence to suggest that Hsp90 inhibition can induce changes in the p53 pathway that might be of potential therapeutic benefit in CLL patients with or without p53 defects. The present study was conducted to explore this possibility. *Aims.* To examine the effect of Hsp90 inhibition on the p53 pathway in CLL cells of defined ATM/p53 status, and to determine whether ATM/p53 defects are associated with resistance to *in vitro* cytotoxicity. *Methods.* CLL patients were characterized for their ATM/p53 status using a combination of p53 functional analysis, p53 gene sequencing, and FISH analysis for ATM/p53 deletion. Three groups were identified: no ATM/p53 defect; p53 defect (p53 mutation with deletion of the second allele); and ATM inactivation (no p53 mutation or deletion but p53 functional defect of the type associated with ATM mutation). CLL cells from these cases were treated *in vitro* with GA (10 nM-10 μM) for up to 5 days. Changes in the expression of specific proteins were detected with Western blotting. Cell viability was measured by FACS analysis of cells after staining with dihexolyloxacarbocyanine iodide (DiOC6) and propidium iodide (PI). *Results.* GA down-regulated mutant p53 protein and up-regulated wt p53 protein. The up-regulation of wt p53 by GA was accompanied by down-regulation of Akt and the active form of MDM2, indicating a possible mechanism. In addition to its effects on wt and mutant p53, GA also induced a biphasic increase in p21CIP1, a cyclin-dependent kinase inhibitor that is transcriptionally activated by wt p53. The late increase in p21CIP1 protein levels was seen in cases with no ATM/p53 defects or ATM inactivation but not in those with mutant p53, indicating that the effect was dependent on wt p53 but not ATM. In contrast, the early increase in p21CIP1 was not influenced by p53/ATM status. GA killed CLL cells in a dose-dependent manner irrespective of p53/ATM status. *Conclusions.* Our findings indicate that GA has opposing effects on wild-type and mutant p53 and induces p21CIP1 expression and cytotoxicity independently of ATM/p53 status, all of which should favour clonal regression *in vivo*. These findings reveal an important new dimension to the action of ansamycins in CLL and strongly support the further evaluation of these agents, especially in cases with p53 mutation or ATM inactivation.

0105

MONITORING OF THE STATUS OF BOTH P53 ALLELES IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA - RELEVANCE TO PROGNOSIS AND TREATMENT

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Background. Deletions and/or mutations of p53 gene, a key cell-cycle and apoptosis regulator, represent the most important prognostic factor related to treatment in B-cell chronic lymphocytic leukemia. The presence of aberrations (occurring in 10-15% of patients) almost inevitably leads to treatment requirement and poor outcome and the gene thus constitutes the strongest independent marker for disease-related death. A proportion of p53 mutations originates through spontaneous mutagenesis, however the suspicion exists that some mutations might be induced by DNA-damaging chemotherapy. *Aims.* In this study we determine the proportion of individual p53 aberrations (deletions, mutations, combination of both) in our set of unselected B-CLL patients and correlate the data to p53 protein level, indicating non-functionality of the gene. Further, we evaluate the influence of these alterations on prognosis, therapy requirement and the presence of previous treatment. *Methods.* The deletions of p53 locus (17p13.1) were detected using an interphase FISH and mutations in the gene were searched out using yeast functional analysis (FASAY), coupled to sequencing of templates from yeast colonies bearing the mutated p53 genes. Western blotting was used for determination of p53 protein level. *Results.* We screened 340 B-CLL patients for the deletions and mutations of the p53 gene and 12% of patients manifested a p53 alteration. Most of affected patients (61%) exhibited a complete gene inactivation (deletion/mutation), in a case of missense mutations mostly leading to high p53 protein level. However, single allele missense mutations or deletions also occurred (in 21% or 18% of p53-affected patients, respectively) and were only rarely accompanied by p53 protein over-expression. The requirement for therapy was prominent in p53-affected patients (85%) in comparison to the p53 wild-type group (54%). In this respect, we report a clear difference within the p53-affected patients relating to the treatment necessity and complexity of p53 aberration: all the patients in deletion/mutation subgroup underwent chemotherapy and a quarter of them has died within two years after diagnosis. In the single allele aberration subgroup only 64% of patients required a therapy and none of them died within the stated interval. In approx. a half of affected patients the p53 abnormality occurred before any treatment. We observed five patients changing from wild-type to mutant p53 status, always in connection to undergoing polychemotherapy. Moreover, in two intensively treated patients we detected a p53 mutation mosaic - i.e. each cell tested (n=10-15; cloned through FASAY analysis) beared different (mostly missense) mutation. *Summary and conclusions.* We suggest that monitoring of both p53 alleles might be useful in B-CLL patients, because the correlation among deletion, mutation and protein over-expression is not absolute and the course of the disease may be possibly grouped according to complexity of the p53 inactivation. Although a significant proportion of the p53 mutations is being confirmed as a result of spontaneous biological process, the possibility that DNA-damaging chemotherapy may induce or select some p53 mutations should not be rejected and needs further investigation.

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0106

THE ROLE OF IL-6 IN THE IMMUNE DYSFUNCTION OF B-CLL

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Immune dysfunction, manifest as susceptibility to infection and autoimmune disease, is a major clinical feature of B-cell chronic lymphocytic leukaemia (B-CLL). Although this is in part directly caused by the tumor load, defective T-cell function is also observed including patients with early stage disease. In this study we investigated the role of tumor derived soluble factors in the pathogenesis of immune dysfunction in B-CLL. Supernatants from B-CLL tumor cells (TSN) inhibited a third party allogeneic mixed lymphocyte reaction between T cells from two normal unrelated donors in 9/9 cases (p=0.002) and reduced both cell cycle entry and division of normal T cells in response to CD3/CD28 ligation (p=0.0096 and 0.02). TSN also inhibited CD40L upregulation and IL-2 secretion by activated T cells in 17/17 cases (p=0.0001). This is contrary to previous work that suggests loss of CD40L is exclusively due to tumor cell contact and we show that TSN inhibits by a different mechanism. We also demonstrate that the CD40L defect in B-CLL is reversed by separation of T cells from leukemic cells. Following the inhibitory effect of TSN on the Th1 cytokine IL-2 we investigated whether this was due to Th2 polarization of the T cells. Activation of normal T cells in TSN induced a 7-10 fold increase in IL-4 secretion. To identify the component of TSN causing this we did a cytokine bead array assay for IL-2, γ-Interferon (γ-IFN), Tumor Necrosis Factor-α, IL-10, IL-4, and IL-6. The levels of IL-6 were >85 ng/mL in all cases studied (n=5). The remaining cytokines were consistently present at <0.5 ng/mL except γ-IFN which was found in 2/5 cases at 1.5 and 3.0 ng/mL respectively. Neutralization of IL-6 reversed the inhibitory effect of TSN on T cell activation and IL-2 production whilst addition of the recombinant cytokine reproduced the findings. Blockade Of the IL-6 receptor on T cells restored CD40L expression by normal T cells, activated in the presence of TSN, from a mean of 41±4.3% to 63±4.3% of that observed in CM (p=0.0067). Our results demonstrate that IL-6 secretion is one of the mechanisms by which B-CLL cells modify the immune system. These findings have a number of implications for the treatment of B-CLL. Immunotherapy of B-CLL whether by allogeneic hematopoietic stem cell transplantation or more experimental strategies is most likely to be successful in the context of minimal residual disease. When this is not possible, inhibition of the IL-6 effect may be a strategy to improve the graft versus leukaemia effect in this disorder. In addition and perhaps more importantly, blockade of IL-6 signaling might both reduce the systemic symptoms and immune dysfunction that are such a central feature of B-CLL and slow disease progression by removing one of the factors in the cytokine network that supports the survival and proliferation of the tumor. Tocilizumab, a humanized antibody which blocks the IL-6 receptor, is well tolerated and effective in a number of IL-6 mediated disorders and our data suggest it as a potential adjuvant to the treatment of B-CLL.

0107

B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) WITH 14Q32 TRANSLOCATIONS: HAEMATOLOGICAL FEATURES AND CLINICAL OUTCOME

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Background. Translocations of 14q32 involving the IgH locus occur at a low incidence (approximately 2-5%) in B-CLL. The prognostic significance of 14q32/IgH translocation is still uncertain. *Methods and Aims*. 600 cases of B-CLL seen at 3 institutions over a 20-year period were assessed by conventional cytogenetic analysis and by FISH with the following panel of probes (17p13/TP53, 11q22.3/ATM, 6q21, chromosome 12 centromere, 13q14) and with a probe spanning the IGH locus. Patients with no detectable aberration or with isolated 13q- were included into a favourable cytogenetic group (group 1), those with +12, 6q- into an intermediate risk group (group 2) and those bearing 17p-, 11q-, or complex karyotype into an unfavourable group (group 3). Patients with 14q32/IgH translocation as primary chromosome change (i.e. absence of additional aberrations, presence of other aberrations in a minority of cells) were included into a specific category (group 4). Cases with 14q32/IgH translocation by conventional cytogenetics and/or by FISH were studied with probes spanning BCL1, BCL2, BCL3, BCL6, c-MYC, BCL11A, PAX5, CCND3 and CDK6 for the detection of translocation partners. Cases with t(11;14)(q13;q32)/BCL1 rearrangement were excluded. The aim of the study was to isolate cases with 14q32/IgH aberration as a possible primary abnormality in order to identify translocation partners and to study define the clinicobiological features associated with this abnormality. *Results.* We found 29/600 cases (4.8%) with 14q32/IGH break in interphase FISH (46-83%). Two cases carrying 11q- and 2 with 17p- were allocated into group 3. Twenty-five cases were included in group 4: an additional abnormality was present in a minority of nuclei (13-40%) in 11/25 cases: 7 cases with 13q-, 3 cases +12, 1 case 17p-. The latter 4 cases were included into the 14q32/IgH group (group 4) because additional aberrations were confined to a sideline. Translocation partners of 14q32/IGH translocations were so far identified in 8/25 cases (2p13/BCL11A: 1 case; 6p21/CCND3: 1 case; 18q21/BCL2: 6 cases). The study of the remaining cases is ongoing. We then evaluated the clinical impact of 14q32/IGH rearrangement in a series of 411 patients with complete clinicobiological data. Cases with 14q32/IGH translocations were characterized mainly by typical morphology and classical immunophenotype (immunophenotypic score 5 in 92% of the cases). Mutational analysis of IgVH gene showed an intermediate incidence of unmutated cases (60%). Patients with 14q32/IgH translocation did not differ significantly in terms of interval between diagnosis and treatment as compared with other CLL. However, in univariate analysis patients carrying the 14q32/IGH had a shorter interval between diagnosis and treatment when compared with group 1 (p=0.0084), while no significant difference was observed with respect to patients in groups 2 and 3. In multivariate analysis the presence of 14q32/IGH translocation maintained a significant independent impact (p 0.012) on the interval between diagnosis and treatment, along with stage (p<0.001) and CD38 positivity (p< 0.001). *Conclusions.* Clinical and biological features support the concept that the presence of 14q32/IGH rearrangement as primary abnormality could identify a subset of CLL patients which should be allocated into an intermediate risk category.

0108

COMPREHENSIVE PROTEIN AND RNA EXPRESSION ANALYSIS OF GENETIC SUBGROUPS OF CHRONIC LYMPHOCYTIC LEUKAEMIA

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Chronic lymphocytic leukemia is a disorder with a highly variable clinical course. The VH mutation status and genomic aberrations such as deletion 11q, trisomy 12, deletion 13q, and deletion 17p identify subgroups with different survival times. Protein expression levels of candidate genes involved in cell cycle and apoptosis (AKT1, Apaf-1, ATM, BAX, BCL-2, CDK2, CDK4, cIAP2, Cyclin-D1/D2/D3, MCL-1, MDM-2, p21, p27, p53, PI3K, RB, SMAC, Survivin, XIAP) were studied by immunoblotting. 97 CLL cases divided into subgroups with 11q- (n=23), +12q (n=24), 13q- (n=19), 17p-/p53 mutation (n=15) or a normal karyotype (n=16) were examined and compared to the cell lines EHEB, JVM-2 and Granta-519. In 25 cases, mRNA expression data (studied by RQ-PCR) was compared for AKT1, ATM, BAX, BCL-2, CDK4, Cyclin-D1/D2/D3, MCL-1, p21, p27, p53, PI3-K to assess if protein expression and mRNA levels are correlated. Expression levels were related to EHEB for standardization. VH-mutation status was available for 91 cases. 28 cases had mutated VH genes. In the normal karyotype subgroup a highly homogenous expression pattern for all proteins studied was observed independently of the VH-status. CLL samples with +12q, 13q- or normal karyotype had equal levels of ATM protein, whereas the 11q-subgroup showed reduced levels in 5 cases and absent ATM protein in 1 case (among 15 evaluable cases). The 17p-subgroup included 13 cases without deletion 11q showing ATM protein levels comparable to the normal karyotype group. 17p-cases showed stronger expression of p53 compared to all other cases, except for 4 cases with normal karyotype or deletion 11q. In all of these a p53 mutation could be detected in exons 2-11 by sequencing. Survivin protein and mRNA was absent or low in all cases. CDK4 protein expression was high in cases with 17p-, 11q- and 13q- but low in the subgroup with +12q and normal karyotype. MCL-1 expression was highest in the 17p-subgroup. Regarding PI3K, MDM-2, p21, p27, CDK2, Cyclin-D1, -D2, -D3, BAX, BCL-2, Apaf-1, SMAC, XIAP, cIAP2, RB, E2F, AKT1, ARF and STAT6 no variation in protein expression levels were observed across the subgroups. P27-expression was higher in all CLL cases compared to the cell lines contrary to

stronger levels of p21 in the cell lines. Clear concordance between RNA and protein expression was seen for BCL-2, Cyclin-D2, p21 and p27 regardless of the subgroup affiliation. However, the exact levels of RNA expression could not be retrieved by immunoblotting. In conclusion, the 17p-subgroup was the only group with a high level of p53 and MCL-1 protein expression suggesting that p53 is the affected gene in this subgroup. In contrast, the ATM protein levels are reduced only in a part of 11q- cases indicating a possible role of additional candidate genes. Cases with trisomy 12 and normal karyotype showed weak expression of CDK4 pointing to a functional relevance in these subgroups. Protein levels and mRNA levels correlated only for a subset of the genes investigated. This may be due to posttranscriptional mechanisms regulating gene expression or a lower sensitivity of immunoblotting to detect subtle expression differences.

0109

DOUBLING TIME OF SOLUBLE CD23 (SCD23DT) ALLOWS TO IDENTIFY A GOOD PROGNOSTIC SUBGROUP IN ZAP+ STAGE A UNTREATED CLL PATIENTS

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Background. Chronic lymphocytic leukemia (CLL) is a disease with unpredictable natural history in stage A patients (pts). Recently, it has been suggested that ZAP-70 expression could be a surrogate marker for IgVH mutational status as a biological prognostic factor. Indeed, analysis of mutational status is a heavy procedure but ZAP 70 expression is not always reproducible and the optimal technique has still to be validated. We previously reported that the doubling time of soluble CD23 (sCD23DT) is a major prognostic factor for progression of disease in untreated stage A CLL. Therefore we prospectively evaluated ZAP-70 protein expression and IgVH mutational status in untreated stage A B-CLL pts and correlated these markers with sCD23DT. *Methods.* sCD23 level was evaluated by a commercially available enzyme-linked immunosorbent assay (ELISA). ZAP-70 expression is determined in leukemic cells by flow cytometry, and positive expression was defined as >20% positive cells. When tests became available, ZAP-70 gene expression and IgVH mutational status were also evaluated in all pts included in the sCD23 study. *Results.* 60 pts with untreated stage A B-CLL were included in the study. Median age was 62 (42-82) years. The median follow-up was 58 (16-129) months. The sCD23DT was clearly associated with a progression of the disease: median Treatment-Free Survival (TFS) was 20 Mos in pts with sCD23DT inferior to one year comparing to 141 months in pts with sCD23 DT superior to one year (p<0.0001) with a median overall survival (OS) of 80 mos when the sCD23DT was inferior to one year compared to 176 months for the other group (p<0.0001). Median TFS and OS were respectively 20.7 months and 84 months in ZAP+ pts versus 104.2 mos (p=0.0003) and 176 months (p=0.0003) in the group of pts with negative ZAP-70. According to the mutational status the median TFS and OS were 20.7 mos and 84.8 months for pts with unmutated IgVH and 104 mos (p<0.0001) with a median OS not reached (p=0.0002) for pts with mutated status. Among pts with ZAP+ expression, the analysis of sCD23DT allows to detect a population with a better prognosis with a median TFS of 14 mos in the group of ZAP+ with sCD23DT inferior to one year comparing to a median TFS not reached in ZAP+ pts with sCD23DT superior to one year (p=0.0003). The median OS was 82 mos for pts ZAP+ with a sC23DT inferior to 1 year compared to a median OS not reached for ZAP+ pts with sCD23DT (p=0.0194). Among pts with ZAP– expression, sC23DT allowed also to isolate pts with worse evolution: median TFS of 60 months vs 141 mos (p=0.0017) but without difference in median OS. In pts with ZAP+ expression, the mutational status allowed also to detect a difference in terms of TFS: 14 months for ZAP+ unmutated pts versus median not reached for ZAP+ mutated pts, but we there was no difference in term of OS. In the group of ZAP– pts, there were no differences according to the mutational status. Even among pts with unmutated status and ZAP+ expression, we identified with sCD23DT two subgroup with different evolution in terms of median TFS: 16 mos with a sCD23DT inferior to one year and 54 months for the other pts (p=0.019), but in this small population there is no statistical difference in terms of OS according to sCD23DT (84 versus 112 mos, p=0.087). *Conclusions.* A) We confirmed that sCD23DT is a biological prognostic marker for EFS and OS in untreated stage A CLL. B) Mutational status is correlated with ZAP-70 expression and is an additional prognostic factor in ZAP+ pts. C) SCD23DT is even more reliable to identify a good prognostic group in ZAP+ stage A untreated CLL.

0110

HLA-DR ASSOCIATIONS AND CELL CONTACT IMPLICATE SPECIFIC T-CELL RESPONSES IN THE PATHOGENESIS OF SPLENIC MARGINAL ZONE LYMPHOMA

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Background. There is now strong evidence that interactions between the B-cell receptor (BCR) and antigen are central to the pathogenesis of a number of chronic lymphoproliferative disorders including B-cell chronic lymphocytic leukaemia (B-CLL) and splenic marginal zone lymphoma (SMZL). Compared to normal B-cells, B-CLL cells use a restricted range of immunoglobulin variable heavy chain (Vh) genes which, along with other structural similarities, suggests that these tumours arise from cells that have responded to a limited number of antigens. A particularly marked homology has been noted in the Vh3-21 subset with a short conserved complementarity determining region 3 and expression of the same Vlamba2-14 light chain strongly suggesting recognition of a common antigen. Restricted Vh gene usage has also been observed in SMZL; for example in one series, Vh1-02 expression was reported in 18/40 cases. In addition to signals through the BCR, there is also evidence that T-cell costimulation plays a role in the pathogenesis of many low grade lymphomas. In gastric marginal zone lymphoma for example, T-cell responses to Helicobacter pylori derived peptides provide signals that sustain the developing B-cell tumour and responses to autoantigens and other infectious organisms have been implicated in extranodal lymphomas at other sites. In B-CLL, activated CD40L+ T-cells neighbour proliferating leukaemic cells in so called pseudofollicles within the bone marrow and lymph nodes and, although the reason for their presence is unclear, it is suggested that they play a key role in the tumour microenvoronment. *Aims.* To investigate whether antigen specific T-cell responses promote the progression of B-CLL and SMZL. *Methods.* HLA typing was performed on subsets of B-CLL and SMZL and a group of 1667 normal controls. Sections of SMZL spleen were analysed by multiparameter confocal immunofluorescence microcopy. *Results.* Of 36 patients with SMZL, 30 (83%) expressed either HLA-DR15, HLA-DR4 or both compared to only 931 (56%) of the normal controls (p=0.001). The significance of this association was maintained when corrected for the analysis of multiple HLA-DR alleles (corrected p=0.013). Surprisingly, the nature of the HLA-DR association was dependent on the Vh gene utilised by the tumour. Of the 18 cases of Vh1-02 SMZL, 11 (61%) expressed HLA-DR15 compared to 26% of normal controls (p=0.0022, corrected p=0.029) whilst 13 (72%) of the 18 non Vh1-02 cases expressed HLA-DR4 compared to 36% of the controls (p=0.0023, corrected p=0.03). An excess of HLA-DR4 expression was also observed in patients with Vh1-02 SMZL that did not express HLA-DR15 (71% vs 19% of controls, p=0.015). No clear association between any of the subgroups of B-CLL and HLA type was observed. Analysis of SMZL spleen showed that proliferating tumour cells frequently contact T lymphocytes whereas non-proliferating cells do not (Figure 1. T-cells red, B-cells green, Ki67 white). *Conclusions.* Taken together these results suggest that a specific T-cell response to antigen plays a role in the pathogenesis of this B-cell disorder. The observation that the Vh gene used by the tumour specifies the type of HLA restriction implies that the BCR and costimulatory T-cells respond to the same antigen.

Figure 1. Confocal image of SMZL spleen.

Chronic lymphocytic leukemia and related disorders - Clinical

0111

ZAP-70 MRNA EXPRESSION QUANTIFIED IN B CELLS BY REAL TIME PCR IS A POWERFUL PROGNOSTIC FACTOR IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease with respect to prognosis and clinical outcomes. Two different groups in terms of overall survival and clinical characteristics are now classified on the IgVH mutational status. However, this costly analysis is very laborious and surrogate markers have been investigated. *Methods.* We developed a standardised and quantitative PCR (qPCR) method to measure Zap-70 mRNA (ζ-associated protein 70) expression in sorted CD19+ cells. The comparison of this method with others (Zap-70 and CD38 by flow cytometry, and lipoprotein lipase (LPL) mRNA by qPCR) was performed in a cohort of 108 patients with a median follow up of 82 months to evaluate their association with IgVH mutational status, overall survival (OS) and treatment-free survival (TFS). *Results.* The association between Zap-70 by qPCR and IgVH mutational status was clearly significant [$\tilde{}X^2$=50.95; p<0.0001] and characterised by a Cramer's V statistic of 0.72 indicating a very strong relation. This method also presents 87.8% sensitivity, 85.7% sensibility, 87.5% positive predictive value and 86% negative predictive value. Zap-70 expression was significantly associated with OS [p=0.0021] and TFS [p<0.0001]. Zap-70-positive patients had a significantly shorter median TFS (24 months) than Zap-70-negative patients (157 months). Moreover, Zap-70 by qPCR seems to have a better prognostic power than IgVH mutational status and the other prognostic marker tested. *Conclusions.* Zap-70 mRNA expression quantified by real time PCR is a strong surrogate marker of IgVH mutational status and a powerful prognostic factor.

0112

RITUXIMAB COMBINED WITH CLADRIBINE OR CLADRIBINE AND CYCLOPHOSPHAMIDE IN HEAVILY PRETREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. The results of preclinical and clinical studies suggest that rituximab can increase an antineoplastic activity of purine nucleoside analogs in CD20-positive B-cell lymphoid malignancies. *Aims.* The aim of current study was to determine the feasibility, effectiveness and toxicity of combined regimens consisting of rituximab and cladribine (2-CdA) (RC) and RC plus cyclophosphamide (RCC) in the treatment of patients with recurrent or refractory chronic lymphocytic leukemia (CLL). *Methods.* The RC regimen consisted of RIT at a dose of 375 mg/m^2 on day 1 and 2-CdA at a dose of 0.12 mg/kg in 2-hour infusion on days 2 to 6. The RCC protocol consisted of RIT at a dose of 375 mg/m^2 on day 1, 2-CdA at a dose of 0.12 mg/kg/day in 2-hour infusion on days 2 to 4, and cyclophosphamide at a daily dose of 250 mg/m^2 on days 2 to 4. The courses were re-administrated at time intervals 4 weeks or longer if severe myelosuppression occurred. If a response was documented, patients were treated until maximal response or prohibitive toxicity. Guidelines for response developed by the NCI-WG were used. *Results.* Fourty six patients with CLL entered the study. 33 (72%) patients had recurrent disease and 13 (28%) patients were refractory to prior therapy. Eighteen patients were treated with RC regimen and 28 with RCC regimen. The median number of courses administrated were 3 cycles (range 1-6 cycles). Three (6.5%) patients (95% CI 1-14%) achieved a complete response (CR) and 30 (65%) patients (95% CI 51-79%) a partial response (PR). According to the particular regimen, the overall response rate (OR) was obtained in 12 (67%) patients treated with RC (95% CI 45-89%) and in 21(75%) patients treated with RCC (95% CI 59-91%). Noteworthy, out of 2 patients who were previously treated with alemtuzumab and then relapsed, both responded to RCC. The median failure free survival (FFS) of responders was 9 (3-46) months. Hypersensitivity to rituximab (fever, chills, rush, hypotonia) was the major toxicity of RC/RCC regimens and occurred in 16 (33%) patients mostly during the first infusion of the drug. Grade 3/4 neutropenia was seen in 6 (13%) patients, grade 3/4 thrombocytopenia in 3 (9%) patients, and grade 3/4 infections were observed in 10 (28%) patients. *Conclusions.* The data indicate that both RC and RCC regimen have high therapeutic activity and relatively low toxicity in heavily pretreated patients with CLL.

0113

ALEMTUZUMAB AS CONSOLIDATION THERAPY AFTER FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB REGIMEN (FC-R) FOR THE TREATMENT OF YOUNG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (CLL) is a clonal hematopoietic disorder characterized by proliferation and accumulation of small lymphocytes. CLL has traditionally been considered indolent, with a prolonged clinical course. However, a large proportion of patients with CLL have severe symptoms, a poor prognosis, and often require more immediate treatment of their leukaemia. Several randomized studies indicate that cytotoxic therapy based on alkylating agents or new purine nucleoside analogs, such as fludarabine, in the indolent phase of disease, does not prolong the survival time of CLL patients. The monoclonal antibodies directed against CD52 antigen (alemtuzumab, Campath-1H) and CD20 antigen (rituximab) demonstrate also considerable activity in CLL patients. These agents have significant single-agent activity, distinct mechanism of action and generally, favorable toxicity profiles. 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. Moreover, monotherapy with alemtuzumab has also been shown to achieve a complete response with undetectable MRD in several patients with relapsed/refractory disease. *Aims.* 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. *Methods.* 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/m^2 i.v. on days 1-3, cyclophosphamide at a dose of 250 mg/m^2 i.v. on days 1-3, and rituximab at a dose of 375 mg/m^2 on day 0). One month after the last cycle all patients were subjected to a disease re-staging that showed a clinical CR, but 9 out of 12 patients showed the presence of MRD in the bone marrow. Thereafter 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. *Results*. At a clinical re-staging performed after one, three and six months from the end of therapy all patients showed a CR with undetectable MRD (molecular CR). At the present, (month +14) all patients are alive and in molecular CR. *Summary and conclusions.* 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.

0114

WHAT CAN WE CURRENTLY LEARN FROM NEW PROGNOSTIC MARKERS OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN THE EVERYDAY HAEMATOLOGICAL PRACTICE?

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Introduction. The opinions about the pathogenesis and prognostic factors of CLL have undergone significant changes over the past decade and a variety of new prognostic factors have been discovered. On the other hand, however, indication for the start of therapy continues to be based on the conventional hematological and clinical findings, while a range of new and effective therapeutic procedures is now used in the CLL therapy. The exact current role of the new prognostic markers of CLL therefore remains undefined. *Aims and Methods.* The aim of our work was to analyze the impact of following new prognostic markers on the fate of CLL patients who were diagnosed and treated at 4 independent

hematological centers in one country: a flowcytometric analysis cZAP70; a cytogenetic analysis of ATM; a cytogenetic, molecular genetic and functional analysis of p53; and a mutational status of IgVH. *Results.* The total of 501 patients (median age 61 years, 35-86) suffering from CLL who have been treated and monitored at the haematological centres referred to above underwent the analysis The median follow-up time was 53 months (1'325). del/mut p53 and mutational status of IgVH were the only prognostic markers of those listed above with a significant impact on the overall survival (OS). The median OS in patients with del/mut p53 (33 patients) was 90 months, while in patients without this aberration was 280 months (p<0.001). The median OS in patients with unmutated IgVH (173 patients) was 155 months, while in patients with mutated IgVH was not reached (p<0.001). The remaining prognostic markers did not significantly influence the OS. The patients with unmutated IgVH (173 patients) and patients with del ATM (56 patients) were treated significantly more frequently than patients without these changes: 66.5% vs. 19.5% of patients with unmutated vs. mutated IgVH received therapy, and 68.3% vs. 25.9% of patients with vs. without del ATM required therapy. In patients with unmutated IgVH, the presence of del ATM or del/mut p53 had no impact on the requirement of therapy. ZAP70 expression had not statistically significant influence on OS. When considering also a mutational status, the cZAP70 expression had not influence on the need of therapy. *Conclusions.* According to our analysis, the condition of p53 and mutational status of IgVH are the only prognostic factors influencing the OS. Condition of ATM has no impact on the OS in the current times of modern therapeutic procedures. The importance of the cZAP70 expression has been evaluated as the least significant of all studied markers. This analysis showed which markers have the strongest predictive value also in an unselected large group of patients from a r*eal hematological practice* and moreover, stresses the need for (inter)national standardization of new prognostic markers analysis.

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0115

AUTOLOGOUS TRANSPLANTATION PROLONGS EVENT FREE SURVIVAL AND OVERALL SURVIVAL IN B-CLL PATIENTS WITH B AND C BINET STAGES: RESULTS OF THE PROSPECTIVE GOELAMS LLC 98 TRIAL

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Background. The role of high dose chemotherapy with autologous progenitor cell support in first line therapy in patients with B-CLL remains to be defined. *Aims.* The aim of the prospective randomized GOELAMS LLC 98 trial was to compare two therapeutic strategies in previously untreated advanced B-CLL patients younger than 60 years. *Methods.* Conventional chemotherapy (Arm A) consisted of six monthly courses of ChOP, (i.e. vincristin IV 1 mg/m^2 on day 1, doxorubicin IV 25 mg/m^2 on day 1, cyclophosphamide (Cy) 300 mg/m^2 and prednisone 40 mg/m^2 both given orally from day 1 to day 5, followed by 6 ChOP courses every other 3 month in case of response. Fludarabine (25 mg/m^2 /d IV for 5 consecutive days) was used in case of non response (stable disease or progression) after 3 ChOP. Conventional therapy was compared to high dose therapy with autologous CD34$^+$ progenitor cell support (Arm B), using as consolidation of Complete Remission (CR) (NCI criteria) or Very Good Partial Response (VGPR, defined by >50% tumoral response and <30% bone marrow lymphocyte count) obtained after 3 monthly courses of CHOP. In case of absence of CR or VGPR, 3 to 6 monthly-courses of fludarabine were realized before mobilization with Cy 4 g/m^2 + G-CSF administration. Conditioning regimen included TBI 12 Gy and Cy 60 mg /kg /d for 2 days. *Results.* Between March 1999 and December 2004, 86 patients were randomized of which 82 were evaluable (39 in arm A and 43 in arm B). Sex-ratio, Binet stages and median age were similar in the two groups, as well as the number of unmutated patients (data available in 61 patients). In Arm B, 14 out of 43 patients were not transplanted because of disease progression (n=9), death during the first CHOP course (n=1), hepatitis C (n=1), mobilization failure (n=2) and graft contamination (n=1). One graft was not used because of contamination. Finally, 29 grafts were performed and CD34$^+$ cells purification was realized in 14 patients. Post transplant grade 3-4 non-hematological toxicity was infectious (2 CMV and 1 aspergillus infections). Second cancers occurred in 6 patients in Arm A (2 skin cancers,1 prostatic cancer, 1 multiple myeloma, 1 breast cancer and 1 acute myeloid leukaemia) and 5 patients in arm B (2 skin cancers, 1 bladder cancer, 1 breast cancer and 1 myelodysplasia). Cumulative number of death was 13 in arm A and 4 in arm B (p=0.013), due to 16 cases of progression (13 in arm A, 3 in arm B) and one case of toxicity in arm B. As an intent-to-treat analysis and with a median follow-up time of 49.7 months (range 1-94), median EFS was 22 months in Arm A and 53.1 months in Arm B (p<0,001) ; moreover, overall survival was significantly better (p=0.017) in intensive arm. *Conclusions.* We have shown that front-line high dose therapy with autologous progenitor cell support is feasible and prolongs EFS and overall survival in advanced B-CLL patients.

0116

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

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Background. (B-CLL) is a clinically heterogeneous disease with some patients exhibiting an indolent and others an aggressive course. Markers such as cellular expression of ZAP-70 or CD38 and IGVH gene mutational status have been used to assess prognosis. *Aims*. We investigated the CD38, ZAP-70 and IGVH status in a representative cohort of 179 Binet stage A B-CLL patients in order to define subgroups in terms of disease progression based on the time to first treatment (TTT). Gene expression profiling analysis was also performed to identify specific transcription profiles in B-CLL groups with a specific TTT. *Methods*. The proportion of CD38+ leukemic cells was determined by triple staining for CD19 FITC, CD38 PE, and CD5 Cy-Chrome. CLL Ig VH gene usage and mutation was determined on cDNA according to reported methods. Determination of ZAP-70 was carried out by Western blot. Gene expression profiling was performed in 60 patients on U133A chips using the 7G scanner (Affymetrix). Supervised analyses were made using the SAM (Significance Analysis of Microarrays) algorithm. *Results*. Statistically significant difference in terms of TTT at 4 years were observed in patients showing ZAP-70 strong versus cases ZAP-70 neg/weak (clustered together) (log rank 10.5, p=0.001), CD38 expression >30% (log rank 27, p<0.0001) or non-mutated IGVH (log rank 29.8, p<0.0001). In multivariate analysis the factors that turned out to correlate significantly with TTT were CD38 and VH mutational status, while Zap-70 showed a borderline significance. Since the RR associated with each of the 3 factors was comparable, we constructed a new potential prognostic model by a combination of these biological variables assigning a score of 1 for each prognostic marker with a final score ranging from 0 to 3 with intermediate values of 1 and 2. Approximately 54%, 22%, 13% and 11% of cases scored 0, 1, 2, and 3, respectively. At 4 years, 94%, 92%, 47%, and 37% of cases scoring respectively 0, 1, 2, and 3, remained therapy-free. Pooling together score 0 with 1 cases (low risk), and score 2 with 3, (high risk) the final curves showed, at 4 years 2% and 58% of cases who necessitated treatment (chi-square 34.2, p<0.0001). Gene expression profiling analyses on a representative panel of 60 patients (15 score 0, 16 score 1, 15 score 2, and 14 score 3) revealed 37 genes differentially modulated between the four classes. Differences in expression were more significant when group 0 and 1 were evaluated against 2 and 3 (46 genes) whereas the major changes were found between patients with score 0 and 3 (119 genes). Functional analyses of differentially expressed genes revealed their involvement in several biological processes such as transcription regulation, electron transport, protein transport and metabolism, cell cycle regulation and apoptosis. *Discussion.* The combination of biological and molecular features of B-CLL prompted us to propose a scoring system that can contribute to assist in designing clinical trials. In particular, it could be helpful to test to what extent early treatment of high risk patients (e.g. score 2 and 3) confers clinical advantages.

0117

LONG TERM FOLLOW UP OF HAIRY CELL LEUKEMIA PATIENTS TREATED WITH INTERFERON-α. THE IMPORTANCE OF MAINTENANCE

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Background. HCL is a chronic B-cell lymphoproliferative disorder with specific clinical, morphologic and immunophenotypic features. Although purine analoges are the most recommended first-line treatment, they are associated with significant immune suppression and secondary malignancies. IFN-α is an alternative treatment with fewer long-term side effects and considerable efficacy. *Aims.* The analysis of the outcome of HCL patients (pts) treated with IFN-α as first-line therapy and as maintenance in a single Hematology Unit. *Methods.* 75 consecutive HCL pts diagnosed and followed in our Department between 1980 and 2005, treated with IFN-α were retrospectively analyzed. *Results.* 77.3% were males and their median age was 51 years (30-81). 57% had splenomegaly and 12% hepatomegaly at diagnosis. Their median complete blood counts were as follows: hemoglobin 11.3g/dL (4.5-14.3), leukocyte and absolute neutrophil counts 3.1×10^9/L (0.9-26) and 0.77×10^9/L (0.03-3.9), respectively, platelets 79×10^9/L (20-295). 33% had hypergammaglobulinemia and the median percentage of bone marrow infiltration at diagnosis was 75%. 10% of the pts were splenectomized before IFN-α treatment. The median time from diagnosis to IFN-α initiation was 0.7 months. The majority of pts (68%) received IFN-α induction at a dose of 3MU/day, while 15% 3MU every other day. Median duration of IFN-α induction was 23.5 months. 8% of pts achieved a complete response with a negative bone marrow, while 73% had a partial remission with a >50% reduction of bone marrow infiltration and a complete restoration of all blood counts. The median time to complete hematologic restoration was 10.7 months. 79% of pts received IFN-α maintenance, the majority of whom (58%) at a dose of 3MU/week. 69% of pts never needed a 2d line treatment, while among those who did, 65% were retreated with IFN-α. The median follow-up was 95 months (9-287) and the 10-year overall survival was 80%. There were 2 cases of secondary neoplasia. At last follow-up 9% of pts were dead due to unrelated causes, 3% due to 2ary neoplasia, while none died due to disease. Two factors were of prognostic significance for freedom from 2d treatment (FF2T). FF2T was significantly longer for pts older than 51 years old ($p<0.0004$) and for those receiving IFN-α maintenance compared to those who did not ($p<0.03$). *Conclusions.* IFN-α has significant efficacy in HCL with a slow but sustained response and a low incidence of secondary neoplasia. Continuous maintenance seems to be important in maintaining response. Younger patients might benefit less from IFN-α.

0118

THE ACTIVITY OF ALEMTUZUMAB IS INDEPENDENT OF P53 MUTATIONAL STATUS IN FLUDARABINE REFRACTORY CLL: INTERIM ANALYSIS FROM THE CLL2H STUDY OF THE GCLLSG

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Background. The prognosis of fludarabine-refractory CLL is poor with a median survival time of 10 months. Campath-1H (Alemtuzumab) is approved for fludarabine refractory CLL and there is growing evidence that Alemtuzumab is active irrespective of high-risk genetic subgroups. *Methods.* In order to assess the incidence of TP53 mutations in fludarabine refractory CLL we studied TP53 mutations in the CLL2H trial of the GCLLSG. We used DHPLC to screen for TP53 mutations (Exons 2-11). Aberrant DHPLC profiles lead to sequencing of the respective exons. In addition, detailed clinical history and genetic studies (VH-mutation status, FISH) were available for all patients. *Results.* At an interim analysis we found 19 TP53 mutations in 58 patients with completed mutational analysis. DHPLC results on all 109 patients suggest the presence of further mutations which will be updated at the meeting. There was a high concordance rate between 17p deletions and mutations (15 of 19 analysed patients with 17p- also had a TP53 mutation (79%)). A preliminary analysis of the impact of the presence of a TP53 mutation showed no difference in overall survival for the subgroup with currently confirmed TP53 mutation, suggesting that alemtuzumab is active irrespective of p53 status. The overall response (CR and PR) rate for patients with TP53 mutation was 31% compared to 34% percent at this interim analysis. *Conclusions.* Campath-1H appears to be effective in genetic high risk subgroups including those patients with TP53 mutations. The functional consequences of 17p deletions with and without TP53 mutation are under investigation.

Figure 1. Overall survival for fludarabine refractory CLL.

0119

DOES REDUCED-INTENSITY ALLOGENEIC TRANSPLANTATION CONFER A SURVIVAL ADVANTAGE TO PATIENTS WITH POOR PROGNOSIS CHRONIC LYMPHOCYTIC LEUKAEMIA? A CASE-CONTROL RETROSPECTIVE ANALYSIS

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Background. Reduced-intensity allogeneic haematopoietic cell transplantation (HCT) is increasingly considered as a therapeutic option for several haematologic malignancies. However, relatively few patients with chronic lymphocytic leukaemia (CLL) have been treated with this approach. The disease has a long natural history, so there is reluctance to offer a procedure with a significant morbidity and mortality. However, up to 40% of CLL patients are younger than 60 years old at diagnosis, and these patients almost invariably die of their disease. Unfortunately, no prospective randomised trial comparing allogeneic HCT with conventional chemo- or immunotherapy has ever been performed. *Aims.* To assess retrospectively the outcome of CLL patients undergoing reduced-intensity allogeneic HCT compared with a group of matched controls that were candidates for transplantation but did not have a suitable donor or refused the procedure. *Methods.* Our databases were screened for cases having information available on the following variables: CLL diagnosis according to the WHO classification; age; time and type of CLL-specific treatment; and survival. Information on VH mutational status, cytogenetic abnormalities (i.e. 13q deletion, trisomy 12, 11q23 deletion, p53 deletion), CD38 and ZAP-70 expression was also available in 50% of patients. Cases comprised 30 patients diagnosed from 1992 to 2003, who underwent reduced-intensity allogeneic HCT at Barcelona and Birmingham. Unmanipulated haematopoietic cell grafts were harvested from HLA-matched siblings (23) and HLA-matched (3) or mismatched (4) unrelated donors. Controls consisted of 64 patients diagnosed from 1986 to 2005 at both institutions who received conventional therapy only. Matching variables were age at diagnosis and time to first CLL-specific therapy. *Results.* Both patient groups were well balanced in terms of CD38 and ZAP-70 expression, cytogenetic abnormalities and VH mutational status. Median age at diagnosis was 51 years (range 32-61) for the HCT group and 54 years (range 28-64) for the control group ($p= 0.138$). All patients required CLL-specific therapy within 4 years of diagnosis, with no significant differences between groups ($p=0.988$). The median number of therapy lines was not significantly different between groups ($p=0.163$) and 23% of cases and 27% of controls received an autograft as part of their therapy ($p=0.805$). Finally, median overall survival was 115 months for HCT patients and 93 months for controls, a difference that was not statistically significant ($p=0.59$, Fig-

ure 1). *Conclusions.* Results for reduced-intensity allogeneic transplantation are promising for selected patients with poor prognosis CLL. There is, however, insufficient data at this point in time to recommend the procedure outside clinical trials. International multicentre studies with greater numbers of patients could help clarify this issue.

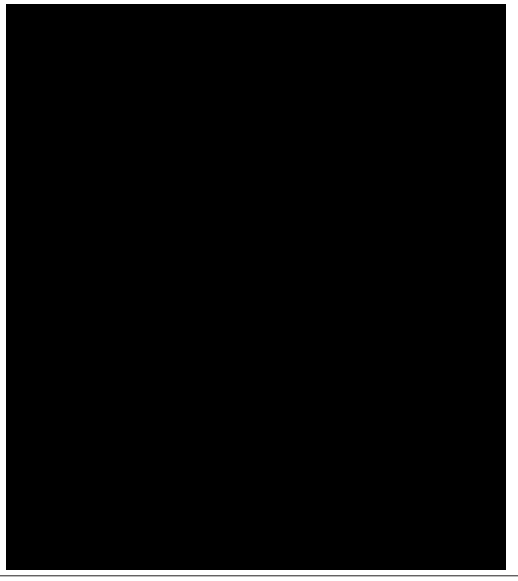

Figure 1. Overall survival according to therapy.

0120

PHASE III STUDY OF CLADRIBINE PLUS CYCLOPHOSPHAMIDE COMPARED WITH FLUDARABINE PLUS CYCLOPHOSPHAMIDE FOR PATIENTS WITH PROGRESSIVE CHRONIC LYMPHOCYTIC LEUKEMIA: REPORT OF PALGCLL3 TRIAL

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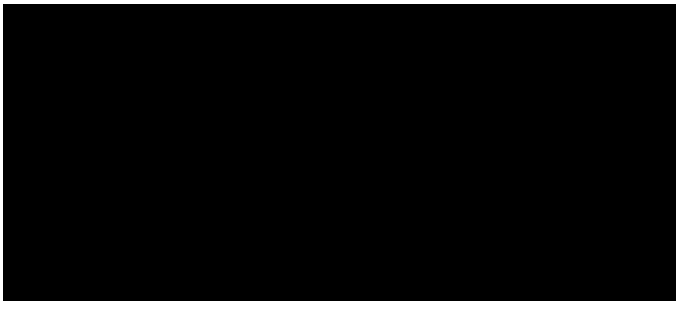
Background. Purine analogues, Cladribine (2-CdA) and Fludarabine (FA), are highly effective in treatment of chronic lymphocytic leukemia (CLL). *Patients and Methods.* This prospective randomized phase 3 trial was designed to compare the efficacy and toxicity of 2-CdA and cyclophsophamide (CC regimen) with FA and cyclophsophamide (FC regimen) in previously untreated progressive CLL. The primary end points of the study were complete response (CR) and overall response (OR) after completion of the therapy.

Table 1.



The secondary end points were progression free survival (PFS), overall survival (OS) and treatment related toxicity. Eligible patients were assigned to receive 6 courses of either 2-CdA 0.12 mg/kg/d i.v. with cyclophosphamide 250 mg/m^2/d i.v. for 3 consecutive days or FA 25 mg/m^2/d i.v. with cyclophosphamide 250 mg/m^2/d i.v. for 3 consecutive days administered at 28 day intervals. The treatment response and toxicity were evaluated according to NCI-SWOG guidelines. Minimall residual disease (MRD) was evaluated in patients with CR using three-color cytometry. *Results.* The study was started in January 2001 and here we present updated results from the 296 evaluated patients performed in December 2006. There were no significant differences in the rates of overall response (OR), complete response (CR), grade 3/4 neutropenia, thrombocytopenia and infections, MRD negativity and number of died patients. PFS was also similar in both groups (p=0.77). *Conclusions.* CC and FC regimens produced similar CR, OR and PFS, as well as have similar toxicity in previously untreated, progressive CLL.

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0121

IMMUNE THROMBOCYTOPENIA IMPAIRS SURVIVAL OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Immune thrombocytopenia (IT) is the most frequent autoimmune disorder during CLL, second only to hemolytic anemia. However, the real prevalence and the natural history of this complication are largely unknown. *Aims.* To determine main clinical features and overall survival (OS) of patients developing IT in the course of CLL. *Methods.* We retrospectively analyzed clinical records of 1233 consecutive patients with CLL admitted for the first time to three tertiary hematological center between 1 January 1995 and 31 December 2004. All patients met the diagnostic criteria for CLL of the National Cancer Institute. To be considered as affected by IT, patients had to fulfill the following criteria: rapid (<2 weeks) and severe fall (at least half of the initial level and below 100×10^3/μL) of the platelet count; normal or augmented number of megakaryocytes in the absence of extensive (>90%) lymphoid infiltration; no or limited splenomegaly; no recent (less than one month) cytotoxic treatment. Complete response (CR) to treatment for IT was defined by a platelet count ≥150×10^9/L, while partial response (PR) by a platelet count > 50×10^9/L or at least doubling the initial level. Remaining patients were considered as no responders (NR). *Results.* Sixty-four patients (5,1%) had IT at presentation or developed it during the course of the disease. IT could be established concomitantly to CLL diagnosis in 14 cases, while median time to IT for the remaining 50 patients was 30 months (range 2-117). The median platelet count at IT diagnosis was 14×10^9/L (range, 1-71). Twenty-six patients (41%) presented with moderate bleeding signs at IT diagnosis, with 5 patients (8%) experiencing severe hemorrhagic episodes. Fifty-six of the 64 patients (87%) received at least one treatment for IT.

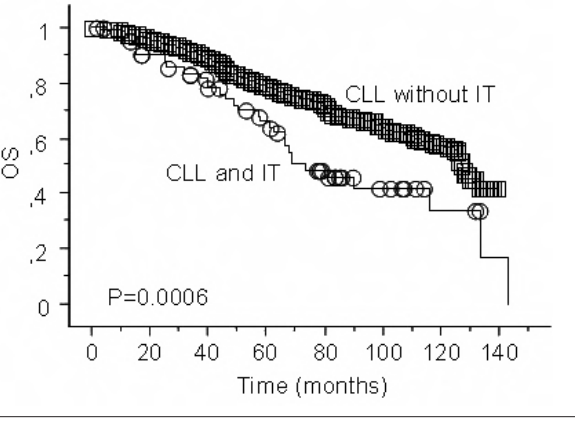

Figure 1.

Of the 33 patients who received i.v. Ig alone or in combination with steroids, 39% had at least a PR; 10 patients underwent splenectomy and 7 (70%) experienced a durable CR; 73% of patients who were treated with chemotherapy (Chlorambucil, COP, CVP) ± steroids obtained at least a PR. Two spontaneous remissions were observed. With a median follow-up of 63 months from IT onset 16 of the 56 treated patients are still NR (29%) and 13 of them still require treatment. No significant clinical differences were observed at CLL presentation between patients

with IT and patients without IT (RAI stage 0-2 in 91% vs 84% respectively, p=0.16). First line treatment for CLL was similar in the two groups consisting of Chlorambucil alone in 70% of patients. Five- and 10-year overall survival (OS) for all patients was 73% and 50%. Patients with CLL and IT experienced a significantly worst OS than other patients with CLL (p=0.0006), as shown in the Figure 1. Refractoriness to treatment for IT had an additional negative impact on survival. *Conclusions.* Our study, for the first time, demonstrates that occurrence of IT in CLL has a significant negative impact on survival, at variance with common belief.

0122

CT SCAN GIVES ADDITIONAL INFORMATION OF CLINICAL VALUE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. CLL is the most common chronic leukemia in adults and shows an extremely variable prognosis. Traditional staging systems (Rai and Binet) are based on clinical examination and presence of anemia and/or thrombocytopenia. However, lymph node stations that are not readily available for clinical examination such as abdominal and thoracic lymph nodes are not incorporated in these staging systems, nor is spleen enlargement not noted by palpation. These systems were developed before CT scan became a routine method for obtaining information about lymph node status in hematologic malignancies. We therefore decided to analyse if CT scan would provide additional prognostic information in CLL patients. *Aims.* To find out whether CT scan of thorax and abdomen could provide additional prognostic information in patients with CLL requiring first-line therapy. *Methods.* We identified 77 patients who had been included in four phase II studies at Karolinska University Hospital between 1990 and 2000. In these studies a CT scan was mandatory before start of therapy and also for assessment of response at the end of therapy. Data was retrospectively collected and analysed. Assessment of response was based on information from the charts of the patients using the NCI criteria. To obtain more detailed information of lymph node status we also used the GELF criteria originally developed for follicular lymphoma. *Results.* Time from CLL diagnosis to institution of first-line therapy was 12 (0-269) months. The median age at this time point was 66 (40-85) years and Rai stage was I (n=24), II (n=6), III (n=33) or IV (n=14). With the addition of a CT scan lymphadenopathy and/or splenomegaly was detected in totally 74 patients, leading to a modified Rai stage in 9 patients, in all cases from I to II. CT scan also revealed bulky (>7cm) abdominal lymphadenopathy in 11 patients. In total 54 patients had CT verified splenomegaly, in 22 cases this was not detected during clinical examination. The time from first-line treatment to start of next therapy was 29 months (1-156). Patients with a high lymphadenopathy tumor burden according to the GELF criteria had a significantly shorter time to therapy requirement than those with less advanced lymphadenopathy (p=0.038). *Summary and conclusions.* Clinical examination alone is not sufficient to rule out splenic enlargement and abdominal lymphadenopathy in CLL. The degree of lymphadenopathy appears to be of importance to predict duration of response to therapy. CT might also be of value for evaluation of progression and the need of further therapy. A larger study, preferably made at the time of CLL diagnosis is warranted.

0123

THE COMBINATION OF LUMILIXIMAB AND FCR (FCRL) PRODUCES HIGH RATES OF COMPLETE RESPONSE AND HAS COMPARABLE TOLERABILITY TO FCR IN PATIENTS WITH RELAPSED CLL: RESULTS OF A PHASE I/II STUDY

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Background. Lumiliximab, an IgG1 chimeric monoclonal antibody targeting CD23, has been shown to enhance fludarabine- and rituximab-mediated apoptosis in chronic lymphocytic leukemia (CLL) cells in a caspase-dependent manner. *Aims.* A phase I/II open-label dose-escalation multicenter study was initiated to evaluate the safety and efficacy of lumiliximab in combination with fludarabine, cyclophosphamide, and rituximab (FCR) in the treatment of relapsed $CD23^+$ B-cell CLL. *Methods.* Thirty-one patients with relapsed $CD23^+$ B-cell CLL whose disease was not refractory to FCR were enrolled after informed consent was obtained. They were treated with 375 mg/m^2 (n=3) or 500 mg/m^2 (n=28) of lumiliximab in combination with FCR for a maximum of 6 cycles. Response was assessed using National Cancer Institute Working Group criteria at weeks 13 and 25, and patients were to be followed for 48 months. *Results.* The median age of patients was 58 years. The median absolute lymphocyte count was $41\times10^3/\infty$L and the median number of previous regimens was 2 (range 1-9). Twenty-two patients (71%) responded to treatment with FCR plus lumiliximab (FCRL); the complete response rate was 52%. There were responses in 6 of 8 patients with del(11q22.3), 5 of whom achieved a complete response. One of 4 patients with del(17p13.1) had a partial response to treatment. Grade 3 or 4 adverse events were reported in 65% of patients, but these events were manageable and were expected with FCR therapy. The most common adverse events (all grades) were nausea (77%), pyrexia (61%), neutropenia (58%), chills (55%), and fatigue (48%). Data from the phase I/II trial were compared with published data from a study in 177 patients with relapsed or refractory CLL who were treated with FCR alone (Wierda W, *et al.* J Clin Oncol. 2005;23:4070-4078). The characteristics of patients treated with FCRL or FCR alone were comparable, with the exception of Rai stage I/II disease (74% vs 47%) and previous exposure to rituximab (60% vs 12%). The overall response rates were similar with the 2 regimens (71% vs 73%), but FCRL produced a complete response rate that was double the rate observed with FCR alone (52% vs 25%). The regimens had similar incidence rates and levels of severity of myelosuppression and other toxicities; approximately 45% of patients in both studies completed 6 cycles of treatment. *Conclusions.* These data suggest that lumiliximab administered in combination with FCR has a high level of activity in the treatment of relapsed CLL. The FCRL regimen has an acceptable safety profile and does not appear to add to the toxicity of FCR. The complete response rate achieved with FCRL appears encouraging, even in patients with del(11q22.3). A randomized trial comparing FCRL with FCR alone has been initiated and is currently enrolling patients.

0124

POST-REMISSIONAL RITUXIMAB ADMINISTRATION FOR THE TREATMENT OF OLDER CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS RESPONSIVE TO FIRST-LINE THERAPY WITH CHLORAMBUCIL AND PREDNISONE

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Background. Chlorambucil treatment is manageable and well tolerated in CLL patients, but is associated with partial responses and short response duration. *Aims.* With the aim of improving the quality of response, the anti-CD20 monoclonal antibody Rituximab was given as post-remissional therapy to older CLL patients (≥60 years) responsive to first-line treatment with 6 monthly courses of chlorambucil (C: 10 mg/sqm/day, d1-d5) and prednisone (P: 25 mg/sqm/day, d1-d5). *Methods.* This study included 19 CLL patients in PR after 6 courses of CP treatment who received 4 weekly doses of Rituximab (R: 375 mg/m^2). Median age was 65 years (range, 61-81 years). Five patients showed unmutated IgVH, while 14 were mutated. Prior to starting Rituximab treatment, the median number and the rate of residual PB $CD5/CD20^+$ lymphocytes were respectively 638×10^9/L (range: 129-8134$\times10^9$/L) and 54% (range: 13-87%) of lymphocytes, while the median rate of residual BM $CD5/CD20^+$ lymphocytes was 26% (range: 7-55%). Moderately (≥2cm) enlarged nodes and/or spleen were present in 8 patients (42%). Clinical and cytometric responses were assessed 4 weeks after the last Rituximab administration and, thereafter, every 3 months up to disease progression. *Results.* After Rituximab, the median number and the rate of PB $CD5/CD19^+$ lymphocytes decreased to 110×10^9/L (range: 6-1700 $\times10^9$/L) and 9% (range: 1-61%) of lymphocytes, respectively, and the median rate of BM $CD5/CD19^+$ lymphocytes to 5% (range: 0-50%). In all cases but 2, no enlarged nodes or splenomegaly were observed. Overall, the reduction of residual disease produced an upgrade of the previous response from PR to CR in 13 cases (68%), while no significant changes were documented in 6. Two of the 13 (15%) patients who achieved a CR after Rituximab showed less than 1% $CD5/CD19^+$ lymphocytes both in the PB and the BM. No evidence of a clinical benefit of Rituximab administration was observed in 3/5 IgVH unmutated

patients. After a median follow-up of 65 months (range 32-76), all patients have relapsed. The median time free from disease was 16 months (range: 3-46). A second-line therapy has been required by 12 patients (63%), with a median time to the next treatment of 29.5 months (range: 15-68). Rituximab infusion was well tolerated; mild infusion-related side effects were observed in 3 cases. No patient showed hematologic toxicity or infection. *Conclusions.* The results of this study indicate that Rituximab, given as post-remissional therapy in older CLL patients treated with CP, produced a clinical benefit with a good safety profile in the majority of cases. Additional studies need to be designed to explore new Rituximab schedules, the association of Rituximab with C and post-remissional treatment strategies in CLL patients not eligible for aggressive treatment modalities.

0125

COMBINATION OF IGVH MUTATIONAL STATUS AND GENETIC ABNORMALITIES MAY FURTHER REFINE PROGNOSTIC STRATIFICATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. Chronic lymphocytic leukemia (CLL) is a disease with an extremely variable clinical course. New prognostic factors such as mutation status of immunoglobulin heavy chain variable region (IgVH) or genetic aberrations detected by fluorescent *in situ* hybridization (FISH) can identify patients with high risk disease. However, a single prognostic factor is not sufficient to assess the prognosis of an individual patient accurately enough. *Aims.* To assess the prognostic impact of combination of IgVH mutational status and genetic aberrations in a single-center CLL cohort. *Methods.* 91 patients with CLL diagnosed according to NCI-WG criteria were included in the study. IgVH mutation status was analyzed using cDNA transcribed from B-CLL RNA for touch-down reverse transcriptase polymerase chain reaction (RT-PCR) with degenerate primers for VH1-7 families; IgVH sequences were aligned to the nearest germline using the Ig BLAST database. Forty-two patients carried mutated IgVH and 49 unmutated IgVH genes. Genetic aberrations were detected using commercially available FISH kits for del 13q, del 11q, del 17, and trisomy 12 by Abbott. There were 33 cases with del 13q as a sole abormality, 25 patients with del11q, 18 with trisomy 12 and 14 patients with del17p. More than 2 abnormalities were detected in 14 cases.

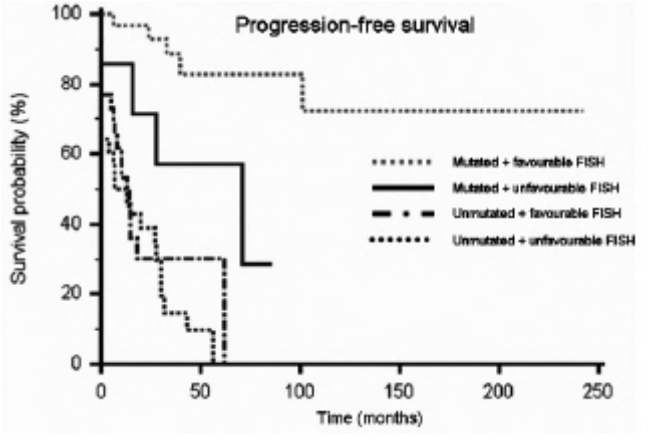

Figure 1. Combination of IgVH and FISH and PFS.

Results. As expected, patients with unmutated IgVH had significantly shorter PFS in comparison to mutated IgVH genes (median PFS 15 vs. 101 months, $p<0.0001$). According to FISH results, patients were divided into a subgroup with favourable (i.e., no abnormality or del 13q as a sole aberration) and unfavourable (del 11 q and/or del 17p and/or trisomy 12). The group with unfavourable abnormalities had significantly shorter PFS in comparison to favourable FISH (median PFS 16 vs. 101 months, $p=0.0002$). When we combined these two factors together, FISH results had significant impact on PFS in patients with mutated but not unmutated IgVH genes: unfavourable FISH significantly worsened PFS in the subgroup of patients with mutated IgVH (mutated + favorable vs. unfavourable FISH, median PFS not reached vs. 71 months, $p=0.011$). However, FISH did not have additional prognostic impact in IgVH-unmutated cohort (unmutated + favourable vs. unfavourable FISH, median PFS 13 vs. 13 months, $p=0.476$). *Conclusions.* This study confirms that IgVH mutation status and genetic aberrations play a major role as prognostic factors in CLL. Data obtained in our single center retrospective analysis show that results of FISH may refine prognosis in terms of progression-free survival in IgVH-mutated patients but fail to add prognostic information in patients with unmutated IgVH genes. Larger, prospective study with longer follow-up is needed to perform a more detailed statistical assessment including multivariate analysis and impact of these prognostic factors on overall survival. Supported by research project MZO 00179906 from Ministry of Health of Czech Republic.

0126

THE VALUE OF SERUM BETA 2-MICROGLOBULIN (β-2M) IN COMPARISON WITH CD38 AND ZAP-70 EXPRESSION, AND IG VH GENE STATUS IN PREDICTING THERAPY FREE INTERVAL IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) PATIENTS

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Background and Aims. Cellular expression of ZAP-70 or CD38, IgVH gene mutational status and chromosome abnormalities are well-established prognostic features in B-cell chronic lymphocytic leukemia (CLL). However, studies addressing the value of newer prognostic factors in comparison to older ones (i.e., β-2M]) are lacking. With this in mind we wondered whether serum levels of β-2M, when compared with cellular expression of ZAP-70 or CD38 and IgVH gene mutational status, might retain its role in predicting clinical outcome of B-CLL patients. The patient cohort included 194 previously untreated B-cell CLL cases with available information on either CD38, ZAP-70, IgVH status or β-2M. For the purpose of the present study 166 Binet stage A patients assigned to a wait and see policy were analyzed. Time to first treatment (TFT) was the end point taken into account. *Results.* Serum levels of β-2M did not correlate with CD38- or ZAP-70-expression while the same did not apply for mutational status of IgVH. As a matter of fact, higher levels of β-2M were more frequently found among patients with unmutated IgVH in comparison to patients with mutated IgVH ($p=0.05$). In univariate analysis patients with increased serum levels of β-2M ($p=0.001$), higher CD38- ($p<0.0001$) or ZAP-70-expression ($p<0.0001$) and unmutated IgVH ($p<0.0001$) showed a significantly shorter 4-year TFT. In multivariate analysis, β-2M together with CD38-expression and mutational status of IgVH ($p=$) retained their prognostic value in predicting TFT. Interestingly,a subset analysis carried out among patients with unmutated and mutated IgVH status, respectively, revealed significant different TFT curves as a function of β2-m only in patients with unmutated CLL. *Conclusions.* In conclusion, our results confirm the role of newer prognostic variables (i.e., ZAP-70 or CD38, IgVH gene mutational status) in forecasting the clinical outcome of patients with early CLL. Interestingly, β2-m may allow an improved prediction of TFT in patients with unmutated IgVH.

0127

PROGRESSION FREE SURVIVAL IS SUPERIOR WITH ALEMTUZUMAB VS CHLORAMBUCIL AS FRONT-LINE THERAPY FOR PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. CAM307 is a phase III, open-label, multinational, randomized controlled trial comparing alemtuzumab (CAM) to chlorambucil (CHLO) for previously untreated BCLL. *Aims.* To compare efficacy and safety of alemtuzumab (CAM) to chlorambucil (CHLO) as front-line therapy. *Methods.* Eligible patients had Rai Stage I-IV and were randomized 1:1 to either CAM 30 mg IV tiw up to 12 weeks or CHLO 40 mg/m^2 po q 28 days up to 12 cycles. CAM patients received prophylactic

trimethoprim/sulfamethoxazole DS and famciclovir treatment during therapy and until CD4+ counts were ≥200 cells/μL. The primary endpoint was PFS; secondary endpoints included response rate, time to alternative therapy, survival, and safety. A total of 297 patients were randomized (CAM n=149 and CHLO n=148; median age: 60 years); performance status 0-1: 96%; maximum lymph node size ≥5 cm: 22%; and Rai stage I-II: 63%. An independent response review panel (IRRP) confirmed diagnosis, Rai stage, response and disease progression that were used in the efficacy analysis. *Results.* PFS was superior for CAM (p=0.0001, stratified log-rank test) with a hazard ratio of 0.58 (95%CI: 0.43, 0.77). Response rate was higher for CAM than CHLO, previously reported (ASH, 2006;301). CAM was superior to CHLO in patients <65 years of age, <70 years of age, with maximum lymph node size <5 cm, and performance status <2 as measured by PFS and response rates. Kaplan-Meier median time to alternative treatment was significantly longer for CAM (23.3 months) compared to CHLO (14.7 months); p=0.0001 (stratified log-rank test), hazard ratio 0.54 (95%CI: 0.39, 0.74). With a median duration on treatment of 11.7 weeks for CAM and 28.3 weeks for CHLO, the difference in time off active treatment is even greater for the CAM patients. There was no overall difference in overall survival with 24 deaths in each arm and a median follow-up time of 2 yrs for the study. A trend towards improved PFS with CAM was observed in patients with 17p deletion, not statistically significant due to small sample size. Common drug related adverse events (AEs) (≥15%) in the CAM arm (n=147) were pyrexia (64%), CMV PCR positivity (53%), chills (50%) and urticaria (15%) and in the CHLO arm (n=147) were nausea (35%) and vomiting (18%). Symptomatic CMV infection occurred in 16% in CAM arm only, none grade 4. Excluding CMV, infections occurred in 46% of CAM and 50% of CHLO patients. Relevant grade 3/4 treatment-emergent AEs (CAM vs CHLO): pyrexia (8% vs 0%), CMV events (8% vs 0%) and chills (3% vs 0%). Treatment-emergent grade 3/4 thrombocytopenia (12% vs 12%) and anemia (11% vs 18%) were similar for CAM vs CHLO. Although grade 3/4 treatment-emergent neutropenia was more common with CAM vs CHLO (41% vs 25%), bacteremia/sepsis (3% vs 2%) and febrile neutropenia (5% vs 3%) were comparable. *Conclusions.* CAM307 demonstrates that CAM is superior when compared to CHLO in therapy-naïve BCLL patients with significantly longer PFS, time to alternative treatment, higher overall ORR, CR and MRD negative rate, with manageable toxicities.

Table 1.

IRRP	CAM (N=149)	CHLO (N=148)	p-value
PFS Months (K-M median (95% CI))	14.6 (12.3, 21.7)	11.7 (9.9, 13.2)	0.0001*
ORR	83%	53%	<0.0001**
CR Rate	24%	2%	<0.0001**
MRD negative Rate†	7%	0%	0.0008**
Time to Alternative Treatment Months (K-M median (95% CI))	23.3 (20.7, 31.0)	14.7 (12.6, 16.8)	0.0001*

*log-rank test stratified by Rai Stage (I-II vs III-IV)
**Pearson Chi-square test or Fisher's Exact test
† Measured by 4-color flow cytometry

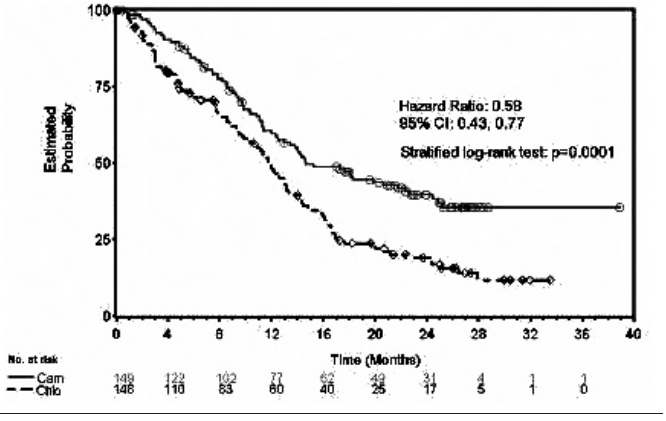

Figure 1. Kaplan-Meir Curve of PFS by Treatment Arm.

0128

CYTOGENETIC ABNORMALITIES AND ASSOCIATED EFFICACY FROM A PHASE III STUDY COMPARING ALEMTUZUMAB VS. CHLORAMBUCIL AS FIRST LINE THERAPY FOR B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. CAM307 is a randomized Phase III trial comparing the efficacy and safety of alemtuzumab (CAM) with chlorambucil (CHLO) in 297 previously untreated B-cell chronic lymphocytic leukemia (BCLL) patients requiring therapy according to NCIWG criteria. *Aims.* To evaluate outcome relative to cytogenetics at baseline using molecular cytogenetic analysis by fluorescent *in situ* hybridization (FISH) of peripheral blood cells. *Methods.* Patients were randomized 1:1 to CAM (n=149) vs. CHLO (n=148) using standard dosing regimens. Fluorescence *in situ* hybridization (FISH) on interphase nuclei of lymphocytes isolated from blood was analyzed for cytogenetic abnormalities prior to the start of therapy. FISH analysis was performed using 13 DNA probes to detect chromosomal aberrations in 17p13.1 (P53), 13q14 (RB1, D13S319 and D13S25), 11q22.3-11q23 (ATM and MLL), 6q27 (subtelomere), 6q21 (chromosome 6q21/alphasatellite 6 cocktail probe), trisomy 8q24 (MYC locus), trisomy 12 (CEP12) and translocations involving the locus of immunoglobulin heavy chain gene (IGH, 14q32.33). Samples were analyzed in 282 patients (95%); chromosomal aberrations were detected in 231 patients (82%) while 51 patients (18%) exhibited a normal interphase FISH pattern. *Results.* The most frequent abnormalities were deletions (del) at loci 13q (49%), sole 13q (24%), 11q (19%), 17p (7%), 6q (4%), and trisomies 12 (14%) and 8q (5%). Translocations involving the IGH gene were detected in 10 patients (4%). An exploratory analysis was performed to correlate time to event variables (assessed by an independent response review panel) with cytogenetics. Chromosomal aberrations are presented according to Döhner's hierarchical model (Döhner, 2000, N Engl J Med) that included del 17p, del 11q, trisomy 12, normal, and sole del 13q. Coexistence of del 17p and del 11q was not observed. Although del 13q was observed with all chromosome abnormalities, nearly half of the cases with del 13q14.3 (D13S25 and D13S319 loci) coincided with an ATM deletion (11q22.3). *Conclusions.* Overall, 82% of treatment naïve BCLL patients revealed cytogenetic aberrations by FISH and 59% of cases showed combination of abnormalities. In patients with del 17p, ORR, CR rate and PFS were over 3 times higher for CAM vs CHLO. Despite the significant improvement in ORR for patients with del 11q, when comparing CAM vs CHLO, no difference in PFS was seen. Further investigation of CAM therapy in high risk cytogenetic subgroups is warranted.

Table 1. Response and Kaplan-Meier Estimate of Median PFS (months) by Cytogenic Abnormality.

	CAM				CHLO				PFS	
Mutations	n	ORR %	CR %	Kaplan-Meier Median PFS (months)	n	ORR %	CR %	Kaplan-Meier Median PFS (months)	p-value[1]	Hazard Ratio (95% CI)[2]
del 17p	11	64	27	10.7 (2.7-22.7)	10	20	0	2.2 (1.5-12.2)	0.4066	0.63 (0.22, 1.87)
del 11q	23	87	13	8.5 (6.1-21.7)	31	29	7	8.5 (4.8-12.0)	0.4338	0.77 (0.41, 1.47)
Trisomy 12	24	83	25	18.3 (12.3, n/e)	10	80	0	12.9 (9.7-14.8)	0.0915	0.46 (0.18, 1.16)
No abnormality	25	84	16	19.9 (10.2, n/e)	26	69	4	14.3 (11.3, 20.1)	0.5582	0.81 (0.41, 1.63)
Sole del 13q	33	91	27	24.4 (13.8. n/e)	34	62	0	13.0 (9.4-16.1)	0.0170	0.45 (0.23, 0.88)

[1]Comparisons between treatment groups are based on the log-rank test stratified for Raistage (I-II vs III-IV).
[2]Hazardrations are calculated using Cox model stratified for Raistage (I-II vs III-IV).
n/e: not estimated

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0129

PARENTAL ORIGIN DETERMINATION I FISH A NEW METHOD TO CHARACTERIZE THE PARENTAL ORIGIN OF HUMAN CHROMOSOMES ON A SINGLE CELL LEVEL

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The discrimination of homologues (chromosomal) regions could up to now be done exclusively by molecular genetic *Aims.*using microsatellite or SNP-analysis. Only in exceptional cases a distinction on a single cell level by chromosomes was possible. Therefore, we developed a method called parental origin determination fluorescence *in situ* hybridization (pod-FISH) that is suited for a single cell analysis and can distinguish every homologues chromosome pair on the basis of copy number polymorphisms (CNP). Presently we selected 225 of over 2191 reported polymorphic regions in the human genome and used them as pod-FISH probes. pod-FISH results were verified by microsatellite analysis. We established pod-FISH probe sets for all 24 human chromosomes. To enable working with more than one polymorphic BAC probe at the same time, chromosome specific pod-FISH sets using 5 different fluorochromes were developed. For larger chromosomes like chromosomes 1, 2, 3, 4, 6, 9 and X it was more convenient to create chromosome arm specific pod-FISH sets for an easier analysis and to prevent double labeling. Up to now two cases with a known UPD, 2 cases with maternal contamination and three leukemia cases (AML with trisomy 8) were analyzed successfully. Also the method was applied on a unique case which is a chimera of two cell lines: one female cell line with a complete paternal isodisomy (97%) and one normal male cell line (3%). The results obtained by pod-FISH were in complete concordance with data obtained by microsatellite-analysis. The usefulness and feasibility of the new pod-FISH approach was compared to conventional microsatellite analysis and proved to be as reliable. Thus, the pod-FISH method will open new horizons for diagnostic and scientific fields that could not be questioned by now. E.g. the analysis of single cells will allow in leukaemia to get new diagnostic markers for therapy control after transplantations or to detect maternal contamination in prenatal diagnosis.

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0130

ALTERATIONS OF THE TP53 IN AML AND CLL ARE STRONGLY ASSOCIATED WITH A COMPLEX ABERRANT KARYOTYPE

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The TP53 gene is the most frequently mutated gene in human tumors identified so far. However, in leukemia TP53 alterations seem to be rare. It was the aim of this study to determine the incidence of TP53 mutations in AML and CLL and analyze their relation to cytogenetic and other molecular genetic aberrations. Overall 235 AML cases were analyzed for TP53-mutations, FLT3-length mutations (FLT3-LM), MLL partial tandem duplication (MLL-PTD) and NPM1 mutations. TP53 mutation screening of exons 3-9 was performed by denaturing high performance liquid chromatography (DHPLC). All mutations detected were verified by direct sequencing. The first 149 patients were unselected, the remaining were selected to increase the numbers in rare cytogenetic subgroups. In the unselected cohort a TP53 mutation was detected in 13.4% (20/149 cases). In the total cohort a TP53 mutation was observed in 33 of 235 cases (14%). 29 of these 33 cases (87.9%) showed a complex aberrant karyotype. In the four remaining cases with TP53 mutation a normal karyotype, a trisomy 8, a trisomy 13, and a t(8;21)(q22;q22) each as the sole abnormality were observed, respectively. This data confirms results from our previous independent series demonstrating a high incidence of TP53 mutations in AML with complex aberrant karyotype (1. series 78%, current series 69%). In all other cytogenetic subgroups TP53 mutations are very rare (4/193, 2.1%). Two cases withTP53 mutation showed also a FLT3-LM and one a MLL-PTD. TP53-mutations were not found together with NPM1 mutations. In addition 194 consecutive CLL patients were screened for TP53-deletion by fluorescence *in situ* hybridization (FISH) and for TP53-mutations. A TP53 deletion was detected by FISH in 18 cases (9.3%). 16 of these 18 cases also showed a TP53 mutation. In four further cases without a TP53 deletion a TP53 mutation was observed. Therefore, the total incidence of TP53 alterations in CLL was 11.3% (22/194) with a significant association between TP53 deletions and TP53 mutations ($p<0.0001$). In 160 of 194 CLL cases including 15 cases with a TP53 mutation a chromosome banding analyses had been performed. 11 of 15 cases (73.3%) with TP53 mutation showed a complex aberrant karyotype (≥ 3 aberrations). The remaining 4 cases with TP53 mutation showed a 13q deletion, 3 of these with one other abnormality in addition. Therefore, the frequency of TP53 mutations in CLL with complex aberrant karyotype was 50% (11/22) as compared to only 2.9% in the remaining cytogenetic subgroups (4/138). In conclusion, 13.4% of AML cases and 10.3% of CLL cases show a TP53 mutation. In both leukemia subtypes a high frequency of TP53 mutations was found in cases with a complex aberrant karyotype (69% and 50%, respectively), while TP53 mutations were rare in all other cytogenetic subgroups (2.1% and 2.9%, respectively).

0131

CHROMOSOME 3 ABERRATIONS IN MYELOID MALIGNANCIES REVEALED BY MOLECULAR CYTOGENETIC TECHNIQUES AND THEIR PROGNOSTIC SIGNIFICANCE

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Background. Structural rearrangements of the long arm of chromosome 3 (3q) have been described in approximately 2,5% of patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) as well as in lower percentage of patients in the blastic crisis of chronic myeloid leukemia (CML BC). They are connected with unfavorable outcome and poor prognosis. Deletions of the short arm (3p) were described in solid tumors, however, they are rather rare in myeloid neoplasias. *Aims.* Precise identification and description of chromosome 3 rearrangements in bone marrow cells of patients with myeloid malignancies by means of various molecular cytogenetic techniques. Evaluation of the relationship between chromosome 3 aberrations and hematological parameters including outcome and survival of the patients. *Methods.* Using conventional cytogenetic techniques we examined 37 patients (18 males, 19 females; average age 58,3 years) with myeloid malignancies (21 MDS, 13 AML, 3 CML BC), with chromosome 3 rearrangement in bone marrow cells. They represented 4,2% out of all the patients with myeloid malignancies, examined in our Department during the years 2000-2006. Multicolor banding technique for chromosome 3 (mBAND, MetaSystems™) was carried out in 30 patients, deletions of the telomeric regions were proved using TelVysion 3p and 3q Probes (Abbott-Vysis™). Chromosomes involved in complex translocations were identified by multicolor FISH (mFISH, MetaSystems™). *Results.* The clinical outcome was very poor in this cohort with short survival time (median 5 months). No significant difference in survival of patients with MDS and AML was found, no difference in male/female ratio. Abnormal chromosome 3 was found at the time of diagnosis in 34 patients, in three others (two with MDS and one with CML BC) clonal evolution with 3q rearrangements was seen during the course of the disease and treatment. In all cases it occurred together with other chromosomal abnormalities. Complex karyotypes were confirmed in 35 patients, in 21 patients monosomy 7 or rearrangement of chromosome 7 were ascertained. On the contrary, in our cohort inversion inv(3)(q21q26) was proved in one patient only. Non-reciprocal translocation accompanied by deletion 3p or 3q was the most frequent aberration. Deletion of the short arm was detected in 14 patients and in12 of them deletion of the 3p subtelomeric region was ascertained. As the most of published studies were done by conventional cytogenetic techniques on G-banded chromosomes, cryptic deletions could be easily overlooked. Thus high sensitivity of mBAND and FISH techniques increased radically identification of 3p deletions. *Summary.* Using molecular cytogenetic methods we detected cryptic aberrations of chromosome 3. High percentage of 3p deletions (37,8%) in our cohort of patients is the most important finding not described previously. Studies of these rearrangements could provide new information about genes possibly involved in genomic instability during progression of leukemias.

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0132

ANALYSIS OF AML/MDS CELL LINES HIGHLIGHTS GENES TARGETED BY CONCOMITANT 5Q DELETION AND 11Q23 AMPLIFICATION

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Background. In addition to chromosome rearrangements causing a variety of gene fusions, MLL1 may be targeted by regional chromosomal copy-numberr gains amplifying the 11q23 region (amp-11q23) in acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS). However, conflicting reports suggest that other genes at 11q23 may be targeted. Amp-11q23 is invariably partnered by deletions affecting the long-arm region of chromosome 5 (5q-) implying collaboration of genes at these loci. *Aims.*To identify genes targeted by 5q-/amp-11q23 and characterize AML/MDS cell line models for this disease entity. *Methods.* Fluorescence *in situ* hybridization with tilepath clones covering 11q2 and 5q; genomic (G) and reverse transcription (RT) q(uantitative)-PCR. *Results.* Key candidate target genes, notably, DDX6, FLI1 and MLL1, displayed mean 4.5x genomic amplification by G-qPCR. Of these, only MLL1 displayed consistently elevated gene expression by RT-qPCR, averaging 5.6x upregulation overall. We then analyzed the expression of known downstream targets of MLL1-gene fusion rearrangements in an AML context, including, MEIS1, HOXA7, HOXA9, CDKN1B, CDKN2C - where only the last named displayed consistent upregulation. All amp-11q23 cell lines displayed 5q- with a common deleted region at 5q31 extending from 133-140 Mbp. Of 5q genes recently identified as possible deletion targets, only BRD8 and KCT2 were consistently downregulated, with expression levels at ~0.25x, and ~0.4x, control levels, respectively. In addition, although genomic copy number increases at 8q24 were present, MYC was not consistently upregulated. *Summary and Conclusions.*Taken together, these results highlight MLL1 as a salient target of 11q23 amplification and implicate CDKN2C as a possible downstream target. Furthermore, our data imply that leukemogenic overexpression requires downregulation of BRD8 (a nuclear receptor coactivator) and KCT2 (a membrane receptor protein) in AML/MDS cells. These findings highlight candidate loci to serve as potential therapeutic targets in AML/MDS, together with cell lines which may be useful in further investigations.

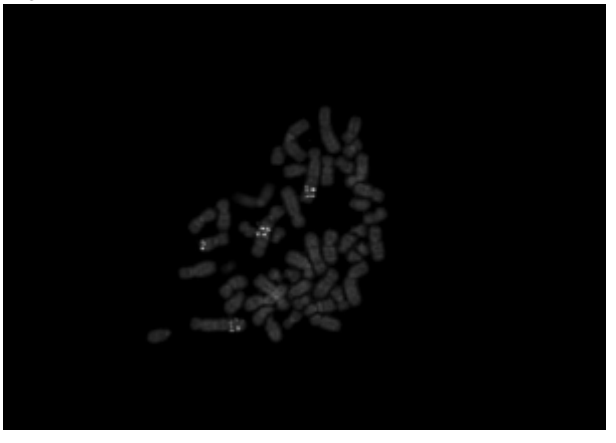

Figure 1. MLL1 amplification.

0133

ANALYSIS OF 1Q21 COPY NUMBER CHANGES IN PATIENTS IN PROGRESSION AND RELAPSE OF MULTIPLE MYELOMA

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Chromosomal abnormalities have biologic and prognostic significance in multiple myeloma (MM), especially among patients with relapsed and refractory disease. The primary translocations occur as early and perhaps as initiating events during the pathogenesis of MM. However, these translocations are not sufficient for the malignant progression of this disease and the accumulation of additional genetic alterations are necessary for a fully malignant phenotype. Previous comparative genomic hybridization (CGH) studies have revealed, that the gain or amplification of 1q, consistently involving 1q21, is associated with a poor prognosis of MM. The aim of this study was to determine the 1q21 copy number changes in patients with a relapse and progression of the disease using molecular cytogenetic methods and to correlate these findings with other cytogenetic changes and clinical parameters. We analyzed bone marrow cells from 23 MM patients, 13 with a relapse of MM and 10 with a progression of MM. All patients (median of the age 60 years) were examined by conventional cytogenetics and by FICTION method with locus specific probes 1q21/1p36 (QBiogene, MP Biomedicals, CA), RB1, IgH, IgH/CCND1, IgH/FGFR3 and centromeric probes for chromosomes 7, 9, 11, 15, 17 (Abbott-Vysis, Downers Grove, IL, USA). Comparative genomic hybridization (CGH) (Abbott-Vysis, Downers Grove, IL, USA) and multicolor fluorescence *in situ* hybridization (M-FISH) (MetaSystem, Altussheim, Germany) were used to detect chromosomal changes in 2 patients with a complex karyotype. Using FICTION method we detected copy number changes of 1q21 in 12 (52%) out of 23 analyzed cases. All patients with copy number changes of 1q21 had other cytogenetic abnormalities: deletion of RB1 gene and t(4;14) were found in 4 (17%) patients, deletion of RB1 gene and deletion of IgH gene in 4(17%), trisomy of examined chromosomes in 2 (9%), a single t(11;14) in 1(4%) and a single t(4;14) in 1(4%) patient. The detected cytogenetic changes and clinical data were analyzed and will be presented.

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0134

FREQUENCY AND CLINICAL IMPLICATIONS OF ADDITIONAL CHROMOSOMAL ABERRATIONS IN ETV6/RUNX1 POSITIVE CHILDHOOD ALL

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Background. Cryptic translocation t(12;21)(p13;q22) which give origin to the hybrid gene ETV6/RUNX1 is one of the most reliable predictors of a favourable clinical outcome in childhood ALL. Despite of generally favourable prognosis of ETV6/RUNX1 positive ALL, late relapses may occur within this group of patients with current therapy. One of the reasons could be the high instability of the genome of leukemic cells, which is manifested at the chromosomal level by formation of secondary abnormalities. Additional chromosomal abberations were proved in about 50-70% of patients with ETV6/RUNX1 positive ALL. Genetic changes that are most frequently associated with t(12;21) are the deletion of the wild type ETV6 allele, trisomy of chromosome 21 and/or duplication of the ETV6/RUNX1 fusion gene. Also non-specific structural and/or complex chromosomal rearrangements could be found. *Aims.* The aim of the present study was to evaluate the frequency and clinical implications of additioanal and/or complex chromosomal aberrations for prognosis of chidren with ETV6/RUNX1 positive ALL. *Methods.* For the assessment of ETV6/RUNX1 fusion gene RT-PCR and/or double target interphase FISH (I-FISH) with locus-specific probe (Abbott-Vysisä) were used. Karyotypes were analyzed by conventional G-banding and by molecular cytogenetic methods. Structural and/or complex chromosomal aberration were proved by FISH with whole chromosome painting probes (Cambioä) and/or by mFISH with the *24XCyte* probe kit (MetaSystemsä). *Results.* We examined 126 children with ALL and ETV6/RUNX1 fusion gene detected by RT-PCR and/or I-FISH. Patients were diagnosed between 1995 and 2006, age ranged between 15 months and 16.6 years (median 4.2 years). Relapse appeared in 20 children (15.9%), five patients died. In 75 children (59.5%) we found besides t(12;21)(p13;q22) additional chromosomal aberrations, the most frequently trisomy or tetrasomy of chromosome 21 (23 cases), deletion of non-translocated ETV6 allele (29 cases), deletion of 6q (8 cases) and/or rearrangements of the long arm of chromosome X (6 cases). Complex karyotypes were identified in 40 children (31.7%). In twelve of them variant translocations of chromosomes 12 and 21 with other partners were observed. Univariate analysis indicated that patients with complex aberrations in ETV6/RUNX1 positive cells had significantly shorter event-free survival (p=0.01). *Conclusions.* In this cohort of children with ETV6/RUNX1 positive ALL complex karyotypes were connected with worse prognosis. Our findings suggest the importance of comprehensive molecular cytogenetic analysis in identifying additional and/or complex chromosomal aberrations in ETV6/RUNX1 positive cells.

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0135

ROUTINE DETECTION OF CYTOGENETIC ABNORMALITIES IN B-CELL LYMPHOPROLIFERATIVE DISORDERS USING INTERPHASE FISH TECHNIQUES

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Background. Specific cytogenetic abnormalities are increasingly used to segregate patients with B-cell lymphoproliferative disorders (B-LPD) into good and poor risk sub-groups which may influence clinical decision making. The critical indications are in the differential diagnosis of diffuse large B-cell lymphoma (DLBCL)/Burkitt lymphoma (BL); B-CLL/mantle cell lymphoma (MCL); the differential diagnosis of CD5- B-LPDs and the identification of prognostic variables such as a p53 deletion. Conventional karyotyping significantly underestimates the incidence of cytogenetic abnormalities in B-LPD and fails in a significant proportion of cases. The purpose of this study was to evaluate the applicability of interphase fluorescent *in situ* hybridisation (iFISH) in the routine diagnostic laboratory. *Methods.* iFISH analysis was carried out on 3,126 (2,643 B-LPD and 483 myeloma) cases (excluding Myeloma IX samples) from the Yorkshire and Humberside region presenting to HMDS between 2003 and 2006. B-LPD samples were initially categorised on the basis of CD5 expression, with $CD5^+$ cases (B-CLL and MCL) assessed for deletions of p53, ATM, 13q14, trisomy 12 and BCL-1/IgH translocation ($CD5^+CD23^-$) and CD5- cases (Burkitt/DLBCL, FL and MZL) assessed for rearrangements of cMYC, BCL-2, IgH, MALT-1(extranodal MZL), BCL-6 and p53 deletions. Myeloma cases were assessed for deletion/monosomy 13 and IgH rearrangements. Cases with an IgH rearrangement were further investigated to determine the translocation partner. The only selection criteria used in myeloma cases was that smears contained >10% plasma cells that had a neoplastic phenotype by flow cytometry. iFISH was carried out on blood or marrow smears, dab/imprint of fresh tissue, or cytospins of CSF/effusions. Probes were used according to the manufacturer's protocol (BCL1/IgH, cMYC/IgH, BCL2/IgH dual colour translocation probe sets; BCL6, cMYC, MALT-1 and IgH BreakApart's: Abbott Molecular Diagnostics and IgH, PAX-5, BCL-3; CCND1 Split-Signal: Dako: p53/α 17, ATM/α 11, 6q21/α6, QBioGene). *Results.* Successful results were obtained in 2,558/2,683 (96.9%) B-LPD cases, and in 459/483 (95.0%) myeloma cases. Abnormal results were detected in 86.1% of B-LPD cases and in 83.7% of myeloma cases. 271/1010 (26.8%) B-CLL cases were re-designated as adverse risk because of a p53 and/or an ATM deletion; 110/391 (28.1%) DLBCL cases were re-designated as adverse risk, based on rearrangements of either BCL-6, cMYC or BCL-2; 253/483 (52.3%) myeloma cases have deletion/monosomy 13 and 152/483 (31.5%) an IgH rearrangement, of these 33/483 (6.8%), had BCL-1/IgH; 22/483 (4.6%) had FGFR3/IgH; 6/483 (1.2%) had cMAF/IgH and 1/483 (0.2%) had a cMYC/IgH translocation. The number of patients with unidentified translocation partners (90/152, 59.2%) appears higher than that reported in the literature and is worthy of further study. *Conclusions.* This large series of cases provides evidence for the applicability and robustness of iFISH in a routine diagnostic setting. We conclude that iFISH is applicable to the routine assessment of patients with B-LPD or myeloma. The majority of patients have abnormal patterns and the failure rate is very low compared to conventional karyotyping. Interphase FISH performed directly on smears/dabs/cytospins is rapid, reliable and relatively inexpensive and can be integrated into diagnostic laboratories and should ultimately allow for risk stratification in real time.

0136

COMPARISON OF PROGNOSTIC IMPACT OF CHROMOSOME 1Q21 GAIN IN PATIENTS WITH MULTIPLE MYELOMA TREATED BY BORTEZOMIB, THALIDOMIDE AND ANY CONVENTIONAL THERAPY

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Background. Amplification of chromosome band 1q21 as well as increased expression of CKS1B gene in this area is a frequently mentioned prognostic factor for patients with multiple myeloma (MM). *Aims.* This study was aimed at comparison of prognostic impact of 1q21 gain in three selected groups of patients with diagnosed MM based on treatment regiment. *Methods.* Plasma cells were identified by cytoplasmic light-chain fluorescence *in situ* hybridisation (cIg-FISH), 1q21 amplification (Amp1q21) utilizing the 1q21/1p36 DNA probe. Amp1q21 was taken such as detection of one or more additional signals of 1q21 DNA probe. Cut-off level for presence of Amp1q21 was established to 20%. Patients with Amp1q21 and patients lacking Amp1q21 of each group were statistically correlated with clinical parameters. Up to date we have carried out analysis of 66 (n=66) patients. This group of patients with median of follow'up 8,6 months (range: 0,3-30,4) was divided according to the undergone therapy into 3 groups: *C-group* comprises 17 patients treated by any conventional therapy; *T-group* comprises 27 patients treated by thalidomide; *V-group* comprises 22 patients treated by Bortezomib. The response and other parameters such as time to progression (TTP) and overall survival (OS) were assigned by IMWG criteria. *Results.* Amp1q21 was found in 62.1% of all 66 patients. Percentage of patients with Amp1q21 in C/T/V-groups were as follows: 64.7%/40.7%/86.4%, respectively. Clinical parameters valid for patients with Amp1q21 (listed in C/T/V order) were as follows: overall response rate (ORR) 42.8% /83.3%/50%; TTP 8.8/12.1/8.0 months; OS 16.1/6.6 / not yet reached for V-group. The same parameters valid for patients lacking Amp1q21: ORR 33.3%/80%/66.7%; TTP not yet reached for C-group / 8.2/not yet reached for V-group; OS not yet reached for all groups. TTP median of patients with Amp1q21 vs. patients lacking 1q21 was: 8.2 vs. 12.1 months (p=0.269), OS 6.6 vs. not yet reached (p=0,072) in thalidomide group. We didn't find any other significant differences between patients with/without Amp1q21 and their parameters in V- and C-group. *Summary and conclusions.* Our results suggest that patients with Amp1q21 treated by thalidomide show a trend towards the worst prognosis based on overall survival. We are currently investigating whether or not our findings will be confirmed on a larger cohort of patients with longer follow-up.

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0137

ROUTINE DETECTION OF CYTOGENETIC ABNORMALITIES IN B-CELL LYMPHOPROLIFERATIVE DISORDERS USING INTERPHASE FISH IN PARAFFIN EMBEDDED TISSUE

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Specific cytogenetic abnormalities are increasingly being used in the differential diagnosis of B-cell lymphoproliferative disorders (B-LPD) and to segregate patients into risk sub-groups which may influence clinical decision making. Paraffin embedded tissue (PET) is often the only diagnostic material available for analysis and it is therefore essential that molecular techniques are applicable in this setting. The critical indications for performing FISH on PET are in the differential diagnosis of diffuse large B-cell lymphoma (DLBCL)/Burkitt lymphoma (BL); B-CLL/mantle cell lymphoma (MCL); confirming the diagnosis of follicular lymphoma (FL), especially where BCL2 is negative; and in extranodal marginal zone lymphoma (ExMZL). The purpose of this study was to evaluate the applicability of interphase fluorescent *in situ* hybridization (iFISH) on PET in the routine diagnostic laboratory. Paraffin iFISH analysis was carried out on 329 B-LPD cases diagnosed in HMDS between 2003 and 2006, where only a paraffin block was available for analysis. Cases were investigated based on the provisional diagnosis following histology and immunohistochemistry. 221 cases with a differential diagnosis of BL/DLBCL were investigated for rearrangements of cMYC, BCL2, and BCL6; 22 FL and 81 ExMZL were assessed for t(14;18) and BCL6 rearrangements and IgH and MALT1 rearrangements respectively. 6 cases with a differential diagnosis of B-CLL/MCL were investigated for BCL1 rearrangements. Cases with an IgH rearrangement were further investigated to determine the translocation partner. An adaptation of our 'fresh' iFISH protocol was used, with an additional enzymatic digestion with Sigma Protease XXIV and a 90C pre-denaturation step. We have validated this method by comparison with iFISH on fresh tissue and PCR on the same sample. Probes were used according to the manufacturer's protocol (BCL1/IgH, cMYC/IgH, BCL2/IgH dual colour translocation probe sets; BCL6, cMYC, MALT-1 and IgH BreakApart's: Abbott Molecular Diagnostics and IgH, PAX-5, BCL-3; CCND1 Split-Signal: Dako). Successful results were obtained in 318/329 (96.6%) of cases, which is comparable to fresh material. 89/221 BL/DLBCL cases had a cMYC rearrangement as the sole abnormality and a final diagnosis of BL was made based on this basis. The remaining 132 cases were classed as DLBCL and had either a t(14;18), a BCL6 rearrangement, additional cMYC rearrangements, or extra copies of any combination of the probes assessed. The t(14;18) was seen in 8/12 FL-common type and 1/8 FL-BCL2neg cases had an unbalanced BCL2 rearrangement. 4/6 B-CLL/MCL cases had rearrangement of BCL1 and were therefore re-classified as MCL. 10 cases of ExMZL had rearrangement of MALT1, and 10 had IgH

rearrangements. This large series of cases provides evidence for the applicability and robustness of iFISH on paraffin embedded tissue in a routine diagnostic setting, where the presence of a specific abnormality is essential in confirming the diagnosis. A high proportion of patients have abnormal patterns and the failure rate is very low. iFISH performed on thin paraffin sections is rapid, very reliable and relatively inexpensive and can be integrated into diagnostic laboratories and allows for definitive diagnostic testing and risk stratification in real time.

0138

NPM1 HAPLOINSUFFICIENCY IN MDS/AML WITH DEL(5Q)/MONOSOMY 5

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Background. NPM1, a gene mapping at band 5q35, is implicated in different forms of leukaemia and NPM1 haploinsufficiency induces preleukemic MDS-like features in mice. It is not known whether NPM1 is involved in the pathogenesis of MDS/AML with del(5)(q)/-5 which occurs as an isolated change or as part of complex karyotypes in MDS and in AML. *Aims.* To study NPM1 genomic status in MDS/AML with del(5)(q)/-5 by fluorescence *in situ* hybridization (FISH) and denaturing high-performance liquid chromatography (DHPLC). *Methods*. We studied 58 patients with MDS and 47 with AML (total 105 patients), all bearing del(5q)/-5 which was isolated or associated with 1 other aberration in 51 cases and included in complex karyotypes in 54. FISH was done with the following DNA clones for the long arm of chromosome 5: centromere- LSI EGR1/D5S721/D5S23 5q31-LSI CSF1R/ D5S721/D5S23 5q34 (Vysis, Abbott, Italy) - RP11-117L6 (NPM1/5q35.1) - CTC-286C20 (FGFR4/5q35.2) - CTC-549A4 (NSD1/5q35.3) - RP1-240G13 (5qter) - telomere. Cut-off limits for monosomy/deletion in interphase analyses were the highest values in normal samples (4% for RP11-117L6, 7% for CTC-286C20, 6.5% for CTC-549A4, 8% for RP1-240G13). Exon 12 NPM1 mutations were studied by DHPLC in 57/105 patients (20 with isolated del(5q)/-5; 37 with complex karyotypes). *Results.* FISH detected NPM1 deletion in 10/58 (17%)patients with MDS and in 22/47 (47%) with AML (Fisher's exact test, $p<0.0014$). NPM1 deletion was found in 4/51 (7.84%) cases with isolated del(5q)/-5 and in 28/54 (51.85%) cases with complex karyotypes ($p<0.0001$). Recurrent cytogenetic abnormalities in complex karyotypes i.e. aneuploidy, -7/del(7q), +8, del(12p), translocation/del(17p), markers, rings, were analysed in relation to the NPM1 deletion. Only aneuploidy was more frequent in complex karyotypes with NPM1 deletion (85.7% cases) than in complex karyotypes with normal NPM1 (56% cases) ($p<0.0308$). NPM1 exon 12 mutations were detected only in 2 cases of MDS with isolated del(5q) and normal NPM1 at FISH analysis. *Conclusions*. For the first time, we establish NPM1 haploinsufficiency 1) is a rare event in MDS/AML with isolated del(5q)/-5 and does not play a major role in the origin of these malignancies and 2) is a frequent event in MDS/AML with del(5q)/-5 in complex karyotypes (>50% of cases) where it is significantly related to aneuploidy and is probably an early event generating a cascade of mitotic mistakes which underlie chromosomal instability and numerical changes.

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0139

GENETIC PATHWAYS IN LOW- AND HIGH-GRADE HCV-RELATED LYMPHOMAS ARE DIFFERENT

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Background. Chronic infection with hepatitis C virus (HCV) causes monoclonal B-cell lymphoproliferative disorders including type II mixed cryoglobulinemia, a benign monoclonal lymphoproliferation, and B-cell non-Hodgkin's lymphomas (NHL) with marginal zone lymphoma (MZL) and large B-cell lymphoma (LBCL) as the most common subtypes. Genetic information on HCV-associated lymphoproliferations is limited to detection of the IgH/BCL2 rearrangement in mixed cryoglobulinemia and less frequently in NHL. *Aims.* We studied 16 HCV-positive patients with NHL, with or without cryoglobulinemia, classified as follows: 8 case of MZL, 6 of which were splenic lymphomas; 4 cases of LBCL, 3 cases of small lymphocytic lymphoma (SLL) and 1 case of lymphoplasmacytic lymphoma (LPL). We also analysed 19 patient with type II mixed cryoglobulinemia without lymphoma to identify genetic pathways that are common to mixed cryoglobulinemia and B-cell NHL. *Methods.* DNA extracted from lymph nodes and/or spleen and/or bone marrow aspirate and/or peripheral blood lymphocytes of the patients with NHL was analysed by metaphase and/or array- Comparative Genomic Hybridization (CGH). Subsequently, Fluorescence *in situ* Hybridization (FISH) with a selected panel of probes for chromosomes 2 and 3 was applied to samples from patients with NHL and type II mixed cryoglobulinemia. *Results.* Genomic imbalances were detected in 11/14 cases (78%) with lymphoma. The most frequent changes were gains of 1q (19%) and 3q (25%) and loss of 2q (31%). Gain of 3q was observed only in low-grade splenic marginal zone lymphomas (SMZL) (4/6 cases). In three of these cases antiviral therapy led to lymphoma regression. In 1/19 patients with type II mixed cryoglobulinemia without lymphoma we found 80% of circulating B-cells carrying trisomy 3. Loss of 2q was observed in 4/5 patients with aggressive large B-cell type lymphomas, with or without a MZL component; was often associated with 1q gain and was mutually exclusive of 3q gain. These patients did not respond to antiviral treatment. Deletion mapping by FISH narrowed the minimal 2q lost region to 850 kb at 2q22.3. Cryptic duplication or deletion events in the other cases were excluded by FISH analysis. *Conclusions.* This first molecular cytogenetic investigation into B-cell lymphomas associated with HCV infection used an integrated CGH and FISH approach and identified two distinct, mutually exclusive events: 3q gain is a hallmark of B-cell clonal expansion rather than malignancy, as it occurs in low-grade SMZL as well as in the non-malignant lymphoproliferative phase of type II mixed cryoglobulinemia. Conversely, deletion of 850 kb at 2q22.3 emerges, for the first time, as a genomic aberration in HCV-related aggressive B cell lymphomas.

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0140

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 GIMEMA WP ANALYSIS)

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Aims. 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 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 1 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 50 pts in PCgR by CC, 52% were FISH positive with a range between 1 and 5% and 48% 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

(MMolR, defined as a BCR-ABL x 100 ratio <0.1%): 164 (91.6%) were FISH negative and 15 (8.4%) were FISH positive (1.3-10% positive cells). *Summary and conclusions* We suggest that interphase FISH is a very releable 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, AIL grants, Fondazione del Monte di Bologna e di Ravenna.

Table 1.

	N°	FISH negative	FISH 1-5%	FISH 6-10%	FISH > 10%
CCgR > 20 met	263	209 (79.5%)	44 (16.7%)	7 (2.7%)	3 (1.1%)
CCgR < 20 met	102	74 (72.5%)	21 (20.7%)	4 (3.9%)	3 (2.9%)
PCgR > 20 met	50	0	26 (52%)	15 (38%)	9 (10%)
CCgR and MMolR	179	164 (91.6%)	12 (6.7%)	3 (1.7%)	0

0141

NUP98 RECOMBINATIONS TARGET CD34+ HEMATOPOIETIC CELLS IN AML AND T-ALL

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Introduction. NUP98, a versatile gene, recombines with various partners in myeloid disorders, such as acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), chronic myeloid leukaemia (CML), and in T-cell acute lymphoblastic leukaemia (T-ALL), where to date, four different fusions have been reported. As the cell of origin of NUP98+ leukaemia is unknown we hypothesized involvement of a totipotent hematopoietic stem cell. To study hematopoietic lineages affected by NUP98 changes in MDS/AML and in T-ALL we used a FICTION approach, combining simultaneous immunophenotyping and interphase FISH. *Materials and Methods.* Patients: We analyzed one case of MDS (refractory anemia with excess of blasts) bearing add(11)(p15)/NUP98-NSD1, one case of AML with inv(11)(p15q22)/NUP98-DDX10, one case of AML carrying t(4;11)(q1?;p15)/NUP98-RAP1GDS1 and one case of T-ALL with t(4;11)(q21;p15)/NUP98-RAP1GDS1. In all cases metaphase FISH identified NUP98 disruption; RT-PCR showed fusion products from NUP98/NSD1, NUP98/DDX10, and NUP98/RAP1GDS1. FICTION: The following monoclonal antibodies were used: Anti-CD34, anti-CD133, anti-CD33, anti-CD13, anti-CD14, anti-glycophorin A , anti-CD19, anti-CD20, anti-CD3, anti-CD7. Clone RP11-348A20, encompassing the entire NUP98 gene, was used to detect NUP98 rearrangements. Immunophenotype (in red) and hybridization signals (in green) were simultaneously identified and counted under an Olympus fluorescence microscope with filter sets for Cy-3 (red) and FITC (green). At least 15 cells (range 15-100) were analysed for each antibody. Cytospins with bone marrow cells from two healthy donors were used as normal controls for each antibody. The cut-off for NUP98 split was established at the upper limit from normal controls. *Results.* The percentage of cells bearing split NUP98 overlapped in patients with MDS and AML: CD34+ from 36% to 93%; CD133+ from 58% to 85%; CD33+ from 60% to 76%; CD13+ from 31% to 76%; CD14+ from 60% to 90%; Glycophorin A+ from 33% to 78%; CD 19+ from 35% to 69%; CD20+ from 39% to 69%; CD3+ from 41% to 70%; CD7+ from 50 to 76%. In the patient with T-ALL split NUP98 was found in 90% CD34+ cells, in 67% CD133+ cells, in 100% CD3+ T cells and in 83% CD7+ T cells. Compared with normal controls no significant differences emerged in erytroid precursors, B lymphoid cells, CD33+, CD14+ and CD13+ cells. *Conclusions.* In both AML and T-ALL NUP98 rearrangements target a CD34+ totipotent hematopoietic stem cell. In the hematopoietic differentiation cascade both myelomonocytes and lymphocytes are involved in NUP98+ AML while only T-lymphocytes are involved in T-ALL. Consequently, we hypothesize that microenviromental and/or genetic factors other than NUP98 fusions determine phenotypic features in NUP98+ leukemias.

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0142

PROGNOSTIC SIGNIFICANCE OF COMPLEX KARYOTYPE IN PHILADELPHIA CHROMOSOME-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS

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Background and Aims. Different from AML, the prognostic significance of complex karyotype is not known in adults with ALL. The aim of study was to analyze the possible prognostic influence of complex karyotype in Ph- adult ALL patients treated with risk-adapted protocols from the Spanish PETHEMA Group. *Patients and Therapy.* The cytogenetic studies were reviewed following the ISCN criteria (2005). Complex karyotype (CK) was defined as the finding of 3 or more structural chromosomal aberrations. Patients were included in two different trials: ALL-96 for standard-risk (SR) ALL, and ALL-93 or ALL-AR03 for high-risk (HR) ALL. *Results.* 266 evaluable patients. SR: n=49, 26 males, WBC count 12×10^9/L (SD: 14), 24 patients with normal karyotype (NK)(54,5%), 15 non-complex karyotype (non- CK)(34,1%) and 5 complex karytotype (CK)(11,4%). HR: n= 217, 119 males, WBC count 58×10^9/L (SD: 77), 104 with NK (53,9%), 64 non-CK (33,2%) and 25 CK (12,9%). CR, DFS and OS according to karyotype group and trial (Table 1). *Conclusions.* Complex karyotype did not have any prognostic relevance in adults with Ph- high-risk ALL, whereas a significant short survival observed in standard-risk patients with complex karyotype.

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Table 1.

CR	ALL - 96 trial (n= 49)			ALL - 93/03 trials (n=217)		
NK	13/24 (79%)		p=0.05	88/104 (85%)		p=0.05
Non- CK	13/15 (86%)			57/64 (89%)		
CK	4/5 (80%)			16/25 (70%)		
DFS	Median (yr)	2 yr (95% CI)	p	Median (yr)	2 yr (95% CI)	p
NK	-	63% (37-89)	0.014	1.9	47% (34-60)	0.019
Non- CK	-	100%		1.8	44% (29-59)	
CK	1.6	33% (0-86)		1.3	34% (7-61)	
OS	Median (yr)	2 yr (95% CI)	p	Median (yr)	2 yr (95% CI)	p
NK	3,7	59% (37-81)	0.07	1.7	42% (32-54)	0.999
Non- CK	3.6	65% (36-94)		1.2	45% (31-59)	
CK	0.6	20% (0-55)		1.8	44% (21-67)	

0143

CYTOGENETIC ABNORMALITIES OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL IN LEUKEMIC PATIENTS WHO HAD RECEIVED TOTAL BODY IRRADIATION BEFORE ALLOGENEIC TRANSPLANTATION

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Background. High dose chemotherapy or chemoradiotherapy including total body irradiation (TBI) is routinely used before hematopoietic stem cell transplantation and may result in many adverse effects on normal tissue, including secondary malignancies. Nevertheless, the effect of TBI on bone marrow stromal cells, the mesenchymal stem cells (MSCs), has never been investigated. *Aims.* To evaluate the cytogenetic status of MSCs isolated from leukemic patients who had received myeloablative chemotherapy or chemoradiotherapy as conditioning treatment before allogeneic peripheral blood stem cell transplantation (PBSCT). *Methods.* Bone marrow-derived MSCs were isolated and *ex vivo* expanded from 8 patients of leukemia who had received Busulfan / Cyclophosphamide or Cyclophosphamide / TBI before sex-mismatched, allogeneic PBSCT. Immunophenotyping using flow cytometry and induction of adipocytic and osteocytic differentiation were conducted in all specimens to confirm the characteristics of MSCs. Cytogenetic studies were conducted using traditional G-banding. For further investigation the effect of irradiation on MSCs, MSCs isolated from 2 normal adults and had normal karyotypes were subjected to irradiation with blood irradiator (Cs137) using the same regimen as TBI before allogeneic transplantation (200cGy twice daily for consecutive 3 days; 1200cGy total radiation dose). *Results.* MSCs can be successfully isolated and *ex vivo* expanded from bone marrow of all the 8 patients who had received myeloablative treatment before allogeneic PBSCT. Despite the intensive chemotherapy or chemoradiotherapy, they were all recipient-origin after allogeneic transplantation, in contrast to complete donor chimerism of bone marrow blood cells. Interestingly, some of the MSCs had cytogenetic abnormalities after transplantation using TBI as conditioning treatment (Table 1). One of these patients (Case No. 7) had a normal Robertsonian variant der (13;14)(q10;q10) before allogeneic transplantation, and get a new, clonal t(1;17)(q21;p13) after transplantation. For 2 MSCs isolated from normal adults and had normal karyotype, complex chromosomal abnormalities developed after direct irradiation using blood irradiator Cs137. *Conclusions.* This is the first report showing that irradiation, either using TBI before transplants or using blood irradiator in experiments, may result in chromosomal abnormalities of bone marrow-derived MSCs. Since the clinical application of bone marrow-derived MSCs increased rapidly, we strongly suggest that MSCs isolated from patients who had received TBI should be avoided from further clinical usage. Besides, further study is needed to observe the tumorogenesis of these cells.

Table 1.

Cytogenetic Status of BM-MSCs Before / After Allogeneic PBSCT					
Case No	Disease	Conditioning Treatment	Chromosome of BM-MSCs		Chromosome of BM-blood cells after PBSCT
			Before PBSCT	After PBSCT	
1	AML	TBI/Cy	Not available	46 XY, t(7;22)(p22;q11), del (13)(q12q22), del (15)(a15?)	Normal XX
2	CML	BuCy2	Not available	Normal XX	Normal XY
3	ALL	TBI/Cy	Not available	47 XY, del (1)(p36), +2, t(3;13)(p21;q12), t(5;10)(q33;p15)	Normal XX
4	AML	BuCy2	Not Available	Normal XY	Normal XX
5	AML	TBI/Cy	Normal XX	Normal XX	Normal XY
6	AML	BuCy2	Normal XX	Normal XX	Normal XY
7	ALL	TBI/Cy	Robertsonian variant, 46 XY	46 XY, Robertsonian variant, t(1;17)(q21;p13)	Normal XX
8	ALL	TBI/Cy	Normal XX	Normal XX	Normal XY

Epigenetics, transcription and signalling

0144

TRANSCRIPTIONAL REGULATION OF SONIC HEDGEHOG EXPRESSION BY NF-κB

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Ligand-dependent hedgehog pathway activation has recently been implicated in carcinogenesis and progression of human cancers. However, the mechanisms controlling Shh expression are poorly understood. Here, we report for the first time that Shh is regulated by the transcription factor NF-κB through direct and indirect mechanisms. Electro mobility shift assays revealed that NF-κB complexes comprising NF-κB subunits p50 and p65 specifically bind to putative NF-κB binding sites identified in the human Shh promotor region by computer-assisted analysis. Furthermore, NF-κB activation by TNFa or p65 overexpression causes increased Shh promoter activity. NF-κB-mediated transcriptional activation of Shh was mapped to a minimal NF-κB consensus site at position +139 of the Shh promotor by deletion and mutation analysis. TNFa also activates the Shh promotor via NF-κB but independent of the minimal NF-κB binding site. Moreover, NF-κB activation by TNFa results in increased Shh mRNA and protein expression in pancreatic carcinoma cells. Importantly, specific NF-κB inhibition by IκBa superrepressor also blocks TNFa-induced Shh promotor activation and Shh expression. By demonstrating that NF-κB regulates Shh expression, our findings have important implications. Thus, NF-κB inhibition may provide a novel strategy to target aberrant Shh activation in human cancers, which warrants further investigation.

0145

POST TRANSLATIONAL TAX MODIFICATIONS CONTROL CRITICAL CYTOPLASMIC AND NUCLEAR STEPS OF NF-κB ACTIVATION: UBIQUITYLATED TAX ACTIVATES IKK ON A CENTROSOME-ASSOCIATED IKK SIGNALOSOME AND SUMOYLATED TAX MEDIATES THE FORMATION OF RELA-ENRICHED TAX NUCLEAR BODIES AND TRANSCRIPTIONAL ACTIVATION

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Background. The Tax oncoprotein plays a crucial role in the proliferation and transformation of HTLV-I infected T lymphocytes through various mechanisms, including activation of the NF-κB pathway. *Results.* We found that cytoplasmic ubiquitylation of Tax C-terminal lysines is critical for Tax binding to the IκB kinase complex and subsequent nuclear translocation of RelA. Conversely, we demonstrate that the same lysines are sumoylated in the nucleus, an event required for the formation of RelA/p300-enriched Tax nuclear bodies and full NF-κB transcriptional activation. In contrast, Tax ubiquitylation and sumoylation are dispensable for its activation of CREB-dependent genes. We further show that ubiquitylated Tax is not associated with active cytosolic IKK subunits, but binds endogenous IKK-α,β,γ and targets them to the centrosome. K63-ubiquitylated Tax colocalizes at the centrosome with IKKγ while K48-ubiquitylated Tax is massively stabilized upon proteasome inhibition. *Summary and Conclusions.* Altogether, these results support a model in which K63-ubiquitylated Tax activates IKK in a centrosome-associated signalosome, leading to the production of Tax-free active cytoplasmic IKK. Thus, ubiquitylation and sumoylation of the same residues of Tax regulate two essential steps controlling NF-κB activation, demonstrating how these post-translational modifications can cooperate to promote Tax-induced transformation. These observations highlight an unsuspected cellular and biochemical complexity in Tax-induced NF-κB activation.

0146

ARSENIC TRIOXIDE INDUCES ACCUMULATION OF CYTOTOXIC LEVELS OF CERAMIDE IN ACUTE PROMYELOCYTIC LEUKEMIA AND ADULT T-CELL LEUKEMIA/LYMPHOMA CELLS THROUGH *DE NOVO* CERAMIDE SYNTHESIS AND INHIBITION OF GLUCOSYLCERAMIDE SYNTHASE ACTIVITY

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Background. Arsenic trioxide (As) is an effective treatment for acute promyelocytic leukemia (APL) and potentially for HTLV-I associated adult T cell leukemia/lymphoma (ATL). Many cytotoxic drugs induce apoptosis through the generation and accumulation of the sphingolipid breakdown product, ceramide, a coordinator of the cellular response to stress. *Aims.* We investigated the contribution of ceramide to the mechanism of action of As in APL and ATL. *Results.* Treatment of APL and ATL derived cells with clinically achievable concentration of As induced accumulation of cytotoxic levels of ceramide. Arsenic effects on ceramide levels in APL cells were more potent compared to all trans retinoic acid (ATRA) effects. We also show that As downregulated neutral sphingomyelinase activity and that, in contrast to ATRA, As-induced ceramide accumulation was not due to induction of acidic sphingomyelinase, but rather resulted from both de novo ceramide synthesis and inhibition of glucosylceramide synthase activity. Interestingly, As effects on de novo ceramide synthesis were similar in APL and ATL derived cells despite the defective pathway in ATL cells. Summary and *Conclusions.* These results indicate that As-induced ceramide accumulation may represent a general mediator of As effects, which paves the way for new therapeutic interventions that target the metabolic pathway of this important sphingolipid secondary messenger.

0147

INSULIN-LIKE GROWTH FACTOR II: A NOVEL AUTOCRINE GROWTH FACTOR IN THE A POPTOSIS, PROLIFERATION, AND MATURATION OF ERYTHROID PROGENITORS IN UMBILICAL CORD BLOOD

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Human umbilical cord blood (CB) contains a higher proportion of erythroblasts compared with adult peripheral blood (PB). The increased number of erythroid progenitor cells (EPCs) in CB could compensate for intrauterine hypoxia. On the other hand, it remains unclear how the abundant EPCs contribute to the fetal hematopoiesis by themselves. To search the novel function of EPCs, CB-derived $CD36^+$ EPCs were subjected to cDNA microarray. It revealed 124-fold higher levels of insulin-like growth factor II (IGF-II) gene expression in CB-EPCs than those in stimulated lymphocytes of adult PB. Then, the following experiments were conducted to determine whether IGF-II exerts as an autocrine erythropoietic factor, which suggests a novel function of EPCs. *Methods/ Results.* The real-time PCR verified that IGF-II mRNA levels were highest in CB-EPCs of all other CB- or adult PB-fractionated cells studied. When CB-EPCs were cultured with erythropoietin (EPO) in serum-free medium, the addition of anti-IGF-II-antibody (Ab) reduced the number of erythroid colonies. To reveal the role of IGF-II in erythropoiesis via an autocrine mechanism erythroid colony-forming cells (ECFCs) were purified from CB and adult PB for further analysis. In brief, CB samples were obtained from normal full-term deliveries. Mononuclear cells (MNCs) were separated by density gradient centrifugation. After depletion of adherent cells, nonadherent cells were collected and negative selection was performed using anti-CD3, CD11b, CD15, and CD45RA antibodies and immunomagnetic beads. The remaining cells were cultured with FCS, human AB serum, stem cell factor (SCF), interleukin-3 (IL-3), and EPO (day0). Day3 ECFCs were collected and incubated under serum-free condition without IL-3. Day7 ECFCs were collected and used in the following experiments. When CB- and adult PB-ECFCs were cultured with IL-3, SCF and EPO, the mRNA levels of IGF-II and type 1 or type 2 IGF receptor increased with the maturation of both ECFCs. The increasing rate of ECFC maturation by IGF-II was higher in CB-ECFCs than in adult PB-ECFCs. Immunocytochemistry demonstrated IGF-II protein in the majority of CB-ECFCs. The addition of anti-IGF-II Ab, but not anti-IGF-I Ab, reduced the number of ECFCs in the liquid culture with EPO. Anti-IGF-II Ab effectively inhibited the proliferation, and accelerated the apoptosis of ECFCs, assessed by MTT and BrdU assays, and flow cytometry. The addition of anti-IGF-II Ab reduced the proportion of glycophorin-A+ cells in ECFCs, which showed a larger in size with a less nuclear condensation, indicating immature erythroid cells. *Summary.* The microarray analysis and quantitative PCR demonstrated the high expressions of IGF-II mRNA in EPCs. The expression levels of IGF-II, type 1 or 2 IGF receptor in the mature erythroid cells were higher than those in the immature erythroid cells. We confirmed that IGF-II is produced by EPCs by themselves, and has a crucial role in fetal erythropoiesis by modulating the apoptosis, proliferation and maturation in an autocrine fashion.

0148

BCL11B KNOCKDOWN LEADS TO APOPTOSIS OF MALIGNANT T-CELLS

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Background. The B-cell CLL/lymphoma 11B gene (BCL11B) encodes a Krüppel-like zinc finger protein which plays a crucial role in thymopoiesis and has been associated with hematopoietic malignancies. A tumor suppressor function of BCL11B has been hypothesized, but the precise protein function has not yet been elucidated. *Aims.* To get more insights in the biological function of BCL11B we analyzed the effects of BCL11B knock down in Jurkat and HUT-78 T-cell lines, Raji B-cell line and in normal T-lymphocytes. *Methods.* Upon BCL11B downregulation using siRNA the DNA content was analyzed by propidium iodide incorporation assay, apoptosis was determined by Annexin V binding assay and Caspase 3 activation was measured by FACS. The genes regulated by Bcl11b were screened by comparing the expression profile of the siRNA and mock transfected T-cell lines using the Affymetrix microarray Genome U133 Plus 2.0. and subsequently confirmed by quantitative real-time PCR. *Results.* We demonstrated that the survival of human T-cell leukemia and lymphoma cell lines depends critically on Bcl11b. Suppression of Bcl11b by RNA interference selectively induced apoptosis in transformed T-cells while normal mature T-cells remained unaffected. The apoptosis was executed by simultaneous activation of death receptor-mediated and intrinsic apoptotic pathways, most likely as a result of TRAIL up-regulation and suppression of the Bcl-xL antiapoptotic protein. Our data indicate an antiapoptotic function of Bcl11b. The resistance of normal mature T-lymphocytes to Bcl11b suppression-induced apoptosis and restricted expression pattern make it an attractive therapeutic target in T-cell malignancies. *Summary and Conclusions.* Our study provides for the first time clear evidence for Bcl11b being an anti-apoptotic protein in T-cell malignancies. The resistance of normal mature T-lymphocytes to Bcl11b suppression-induced apoptosis and restricted expression pattern make it an attractive therapeutic target in T-cell malignancies.

0149

P19 (CDKN2D) PLAYS AN IMPORTANT ROLE IN THE ENDOMITOTIC ARREST LINKED TO THE ACCELERATION OF THE MEGAKARYOCYTE MATURATION

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Ploidization, which appears during normal megakaryocyte differentiation, is linked to a modification in gene expression profile. One of the genes which transcription is positively regulated during ploidization, is a cell cycle inhibitor, p19(CDKN2D). p19, p18, p16 and p15 are members of INK4 family of cyclin dependent kinase (CDK) inhibitors that function in G1 to block the activity of CDKs 4 and 6. Except the role of p19 in cell cycle, this protein can regulate neuronal and macrophage differentiation and has been found to be expressed in erythroid cells. p19 acts also as a protector against apoptotic cell death. In this work, for the first time we have evidenced the role of p19 in megakaryopoiesis. Here we demonstrated that the expression level of p19 in megakaryocyte lineage is 10-fold higher than in the erythroid lineage. Moreover, the expression of p19 increases not only during ploidization after sorting the MKs according to their DNA content but also during MK differentiation (in diploid $CD34^+CD41^+CD42^-$, $CD34^+CD41^+CD42^+$ and $CD34^-CD41^+CD42^+$ populations) both at the mRNA and protein level. Transduction of $CD34^+$ cells at day 1 and 2 of culture by two different shRNAs p19 leads to a moderate increase (31.7±5%) in mean ploidy level suggesting a role of p19 in the arrest of endomitosis as well as mito-

sis during differentiation. Surprisingly, the repression of p19 induced a decrease in MK differentiation. Indeed, the about 70% diminution in p19 expression leads to a 38.6%±17% decrease in $CD41^{high}CD42^{high}$ MKs. This decrease was not linked to a differentiation blockage but to a delay in differentiation. The number of proplatelet forming MKs was diminished when cells were transduced with shRNA p19. However, when mature $CD41^{++}CD42^{++}$cells were analyzed, no difference in the number of proplatelet forming MK cells was detected suggesting that p19 did not play a direct role in platelet formation. Knowing that polyploidization is linked to MK differentiation, we tried to dissect the role of p19 in the arrest of endomitosis and in the regulation of MK maturation. To perform such experiments, polyploid MKs were transduced with shRNA p19 and 72 hours later, 22.3±6.6% increase in mean ploidy level was observed. However, when each ploidy class was analyzed separately, a slight decrease in CD42 expression in polyploid MKs was observed suggesting that these cells will accelerate their maturation once the cell cycle is achieved. To confirm our results, $CD34^+$ cells were transduced by a lentivirus encoding for p19 cDNA, a 59.7±6.8% decrease in mean ploidy level was detected. Furthermore, in each ploidy class, an important increase in CD41 and slight increase in CD42 expression is observed. All together our results demonstrated that p19 has an important role in the arrest of endomitosis allowing the acceleration of MK maturation.

0150

THE LEUKEMOGENIC CALM/AF10 FUSION PROTEIN ALTERS THE LOCALIZATION OF THE EPIGENETIC REPRESSOR IKAROS

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The t(10;11)(p13;q14) translocation leads to the fusion of the CALM and AF10 genes. This translocation can be found as the sole cytogenetic abnormality in acute lymphoblastic leukemia, acute myeloid leukemia and also in malignant lymphomas. The expression of CALM/AF10 in primary murine bone marrow cells triggers the development of an aggressive leukemia in a murine bone marrow transplantation model. Here we show that AF10 interacts with the epigenetic repressor Ikaros in yeast-two-hybrid assays. Interestingly, Ikaros is required for normal development of lymphocytes and aberrant expression of Ikaros has been found in leukemia. In a murine model, the expression of a dominant negative isoform of Ikaros causes leukemias and lymphomas. The Ikaros interaction domain of AF10 was mapped to the leucine zipper domain of AF10, which is required for malignant transformation by both the CALM/AF10 and the MLL/AF10 fusion protein. The interaction between AF10 and Ikaros was confirmed by GST-pulldown and co-immunoprecipitation. In contrast to AF10, CALM/AF10 alters the nuclear localization of Ikaros. The transcriptional repressor activity of Ikaros is reduced by AF10. These results suggest that CALM/AF10 might have a dominant negative effect on Ikaros, and thereby block differentiation of the leukaemia propagating cell in CALM/AF10 positive leukemias.

Figure 1. *In vivo* localization in co-transfected NIH 3T3 fibroblasts (Confocal Laser Scan).

0151

BRAIN-EXPRESSED X-LINKED-2 (BEX2): EPIGENETIC REGULATION OF A POTENTIAL MARKER FOR ACUTE MYELOID LEUKEMIA WITH MIXED LINEAGE LEUKEMIA REARRANGEMENTS

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In epigenetically regulated genes, methyl-CpG recognizing proteins like MeCP2 may bind to methylated CpG-rich areas and contribute to silencing of these genes by recruitment of histone deacetylases (HDAC) and histone methyltransferases. Previously, we identified BEX2 as candidate gene for the diagnosis of acute myeloid leukemia (AML) with mixed lineage leukemia translocations (MLLmu), similarly as extensively described for HOX gene expression in MLLmu acute lymphoblastic leukemia. Human brain expressed X-linked (BEX) is a novel gene family consisting of at least six family members. Human BEX1 and BEX2 are highly expressed in various brain-derived tissues, show diverse expression patterns in peripheral tissues like the liver or pancreas, but are not expressed in hematopoietic tissues like spleen, thymus, lymph node or peripheral blood lymphocytes. We show here that a strict correlation exists between the methylation status of the BEX2 5´ CpG-rich area and expression of BEX2 mRNA: BEX2-negative MLL wild-type (MLLwt) cell lines showed hypermethylation, BEX2-positive MLL mutant (MLLmu) cell lines showed hypomethylation of this specific CpG-rich area. Supporting the view that the expression of BEX2 is epigenetically regulated, we found that treatment of MLLwt cell lines with the demethylating agent Aza-2´deoxycytidine (Aza) induced BEX2 expression in MLLwt cell lines. Furthermore, treatment of MLLwt cell lines with the HDAC inhibitor trichostatin provoked upregulation of BEX2 mRNA in these cells, alone and in combination with the demethylating agent Aza. Chromatin immunoprecipitation assays confirmed the specific binding of acetylated histone H3 to the BEX2 5´region in BEX2-positive, but not in BEX2-negative cells. Methylated CpG-rich areas may not only silence genes by recruitment of HDAC and histone methyltransferases, but also by preventing binding of specific transcription factors to their DNA target regions. The CpG-rich area in the 5´region of BEX2 contains an ARNT-1 binding site. Stimulation of cells with 3-methylcholantrene (3-MC) leads to binding of the receptor AhR to ARNT-1, translocation of the AhR/ARNT-1 complex from the cytoplasm into the nucleus, and induction of ARNT-1 target genes like CYP1A1. A clearly positive effect of 3-MC on BEX2 expression was detectable only, if MLLwt cells were preincubated with Aza. These data suggest that BEX2 negativity in MLLwt cell lines is the consequence of CpG hypermethylation, recruitment of HDAC and prevention of ARNT-1 binding to its cognate binding site.

0152

DOWN REGULATION OF DLX3 EXPRESSION IN MLL/AF4 CHILDHOOD LYMPHOBLASTIC LEUKEMIAS IS MEDIATED BY PROMOTER REGION HYPERMETHYLATION

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Background. Chromosomal translocations that inactivate or create new fusion genes are a common hallmark of acute lymphoblastic leukemia (ALL) and may serve as diagnostic and prognostic markers in subtypes of ALL. Aberrant methylation of clustered cytosine-guanosine motifs (CpG), especially in CpG islands located in gene promoter regions, is an early and essential step in tumour development and methylation has been proved to be a mechanism of gene silencing as common as the disruption of tumour-suppressor genes by mutation or deletion. Moreover, DNA hypermethylation and transcriptional silencing of genes involved in tumor invasiveness, cell growth and apoptosis, may also influence recurrence after treatment and overall survival. Inactivation of cancer-related genes by DNA methylation is a frequent event also in paediatric and adult ALL and, by looking at differences in the methylation pattern of multiple genes, specific risk groups among patients have been identified. The DLX genes implicated in haematopoiesis and in a number of other processes with highly dynamic spatio-temporal expression patterns and well-defined CpG islands in their promoter regions, are attractive targets for methylation studies in leukemia subtypes. In particular, the down-regulation of DLX2, 3 and 4 had been described in a group of paediatric B-ALL characterized by the t(4;11)(MLL-AF4) chromosomal rearrangement and, in several cancer cell lines, is directly connected to an increased resistance to apoptosis. The presence of extended CpG islands at the 5' end of DLX2, 3 and 4 genes, their possible role in resistance to apoptosis, and the recent finding of DLX5 promoter methylation as one of the epigenetic markers of chronic lymphoblastic leukemia, prompted us to study the methylation and gene expression pattern of DLX2, 3 and 4 in specific paediatric ALL subtypes. *Aims.* Our main purpose was to understand if, in pediatric leukemias, the methylation of DLX2, 3 and 4 genes could have a functional role in their gene and protein expression and if differential methylation patterns were able to dis-

tinguish between genotypic B-cell leukemia subgroups with different responses to chemotherapy. *Methods.* Methylation Specific PCR, RQ-PCR, 5'-Aza-2'dC treatment, Western Blot. *Results.* Analysis of methylation and gene expression patterns of DLX3 in 64 specimens of B-lineage ALL revealed that DLX3 presents aberrant methylation in paediatric B-ALL patients. *in vitro* experiments with 5'-Aza-2'dC on leukemia cell lines, confirmed by western blot analysis, indicated that the methylation of DLX3 CpG islands has a functional role and interferes with DLX3 gene and DLX3 protein expression in B-ALL cells. To validate this data we studied two groups of patients with the two better known paediatric chromosomal rearrangements: MLL-AF4 and TEL-AML1 respectively. Hypermethilation of DLX3 significantly reduces its expression in MLL/AF4 rearranged leukemias while methylation is almost absent in TEL-AML1 positive ALL specimens. *Conclusions.* Our results show for the first time the aberrant methylation of DLX3 gene and its functional inactivation role in the specific cohort of paediatric B-ALL with the MLL-AF4 chromosomal aberration. This finding proposes DLX3 as a new epigenetic candidate marker involved in leukemiogenesis of this high-risk acute lymphoblastic leukemia.

0153

LBH589, A NOVEL DEACETYLASE INHIBITOR (DACI), IN THE TREATMENT OF CUTANEOUS T-CELL LYMPHOMA (CTCL): CHANGES IN TUMOR GENE EXPRESSION PROFILES RELATED TO CLINICAL RESPONSE AFTER THERAPY

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Background. In preclinical and Phase I studies, deacetylase inhibitors (DACIs) have shown promise in the treatment of hematological malignancies, in particular cutaneous T-cell lymphoma (CTCL); however, the target genes affected by DACIs remain unknown. LBH589, a novel DACI, is currently undergoing clinical trials for the treatment of CTCL. *Aims.* To determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of oral LBH589 in patients with CTCL, and to characterize the biologic activity of oral LBH589 when administered to this patient population. *Methods.* This is an ongoing Phase I study of LBH589 in patients with advanced-stage CTCL. Patients were entered into either an oral DLT regimen of 30 mg three-times weekly (M, W, F; n=1), or an MTD regimen of 20 mg three-times weekly (M, W, F; n=9). Treatment was discontinued in the event of disease progression or unacceptable toxicity. Gene expression profiling using Affymetrix U133A plus 2.0 GeneChip? with 54,675 probe sets was carried out in 3-mm punch biopsies of CTCL skin lesions taken from patients 0, 4, 8, and 24 hours after LBH589 administration. F-statistics were used to adjust for differences between the subjects, allowing for the prioritization of genes that responded consistently over time to LBH589. Assessment of hyperacetylation of histone H3 as a biological marker of LBH589 activity in patient samples was conducted by immunohistochemistry and assessment of blood mononuclear cells. *Results.* All 10 patients were evaluable for response: 2 achieved a complete response (CR), 4 achieved a partial response (PR), and 2 had stable disease (SD) (RR = 6/10; 60%). Median duration of treatment was 4 weeks in the DLT arm and 23 weeks in the MTD arm. Duration of response was 178.5 days for both arms. One patient in the DLT arm experienced grade 4 diarrhea (n=1). Patients in the MTD arm experienced grade 3 diarrhea (n=1), grade 3 neutropenia (n=3), and grade 3 thrombocytopenia (n=1). Microarray data from 6 patients demonstrated that within individual patient tumors, alterations in gene expression were observed at all time points in the first 24 hours post-treatment. Global changes in gene expression patterns were also observed when all patients and all time points were investigated. The following were major findings following LBH589 treatment: there were rapid changes in gene expression; more genes were repressed than were activated; gene expression changes were observed in both responders and non-responders. qRT-PCR confirmed array analysis. Hyperacetylation levels were observed up to 72 hours following LBH589 administration. *Conclusions.* LBH589 induces CR in patients with CTCL. Ongoing disease regression is apparent weeks after discontinuation of therapy. CTCL provides a unique scenario in which the effect of a drug on genes can be observed over time using microarray analysis. Treatment with LBH589 results in the differential expression of numerous genes, which may help elucidate the biologic activity of LBH589 in the CTCL patient population.

0154

WNT PATHWAY MUTATIONS IN ACUTE LEUKEMIA PATIENTS

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Aims. WNT signaling pathway proteins function as haematopoietic growth factors and regulate proliferation in normal T-cell and B-cell development. Not much is known about activation of this pathway, its ligands and receptors in leukaemia pathogenesis. Genetic alterations in WNT pathway associate with different types of cancers as well as acute leukemias. Here we determined β-catenin expression levels and mutational alterations in the β-catenin, AXIN1 and APC genes in acute leukemia patients. Patients and Method; Peripheral blood and/or bone marrow samples were taken at diagnosis. β-catenin mRNA levels were determined by quantitative real time PCR (QRT-PCR). Acute lymphoblastic (n=126) and myeloblastic leukemia patients (n=30) showed ~4 fold increased β-catenin mRNA levels. Using denaturing high-performance liquid chromatography (DHPLC) analysis and DNA sequencing, acute lymphoblastic leukemia and acute myeloblastic leukemia patients were screened for inactivating WNT pathway mutations. Sequence variations of AXIN1 (exons 1-5, APC, MKK, GSK3 β and β-catenin binding domains) and APC (exon 15, AXIN and β-catenin binding domains) were determined. Results and Conclusion; These variations were found to be 35% for AXIN1 (exons 1-5) and 50% for APC (exon 15) in ALL patients and 19% for AXIN1 and 54% for APC genes. There was no β-catenin (exon 3) mutation in AML patients, and one ALL patient have this mutation. Cytoplasmic B-catenin accumulation frequently occurs in leukemia. According to our findings β-catenin mutations are rare in leukemia samples. Our preliminary results also support that, abnormal β-catenin accumulation via ligand-independent manner in acute leukemias seem to closely associated with AXIN1 and APC mutations.

0155

WNT5A GENE EXPRESSION AND PROMOTER METHYLATION IN ACUTE LEUKEMIA PATIENTS

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WNT5A which is a member of WNT family, functions as a tumor suppressor gene in haematopoietic tissue. It promotes intracellular Ca^{++} release and activation of protein kinase C. WNT5A was found to be over-expressed in various cancer types, where as acute lymphoblastic (ALL) and acute myeloblastic (AML) leukemia patients showed loss of WNT5A gene expression. Previous results indicated that WNT5A/Ca^{++} dependent pathway suppresses cyclin D1 expression and WNT5A null mice develop B-cell lymphoma and chronic myeloid leukemia. We determined WNT5A and its downstream targets c-MYC and cyclin D1 expression levels in acute leukemia patient. Bone marrow and/or peripheral blood samples were obtained from ALL (n=126) and AML (n=34), at the time of diagnosis. Also normal bone marrow (n=6), normal peripheral blood (n=10) and $CD19^{+}$ cells samples were used as controls. The relative expression levels of WNT5A, c-MYC and cyclin D1 genes were detected by quantitative-real time PCR (QRT-PCR). Methylation status of WNT5A promoter is determined by methylation specific PCR (MSP). Our results indicated that the mRNA levels of WNT5A and cyclin-D1 were decreased, but level of c-MYC was increased. This confirms the finding that c-MYC expression is independent of WNT5A presence, but cyclin-D1 is WNT5A dependent. To investigate the decrease in WNT5A mRNA expression levels, we performed MS-PCR and found hypermethylation of WNT5A promoter in 86% of ALL's and 85,7% of AML's. Loss of WNT5A gene dosage was observed in several primary tumors depending on the homozygous deletions of WNT5A, RNA or promoter silencing. Here we determined decreased WNT5A expression depending on the WNT5A promoter hypermethylation in ALL and AML patients.

0156

ONCOGENETIC FUNCTION OF KTS+ ISOFORM OF WT1 IN ACUTE AND CHRONIC LEUKEMIAS

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Background. The Wilms' tumour gene (WT1) is overexpressed in a variety of hematological malignancies including acute and chronic leukemias, myeloproliferative disorders and myelodysplastic syndromes and nowadays it is considered a sort of universal marker of leukaemia. WT1 was originally identified as responsible for the kidney tumour of Wilms and described as a tumour suppressor gene but, in the setting of leukaemia, it seems to function as an oncogene. WT1 has different isoforms. In particular, KTS$^+$ and KTS$^-$ are 2 isoforms derived from alternative spicing of exon 9. In normal cells the two isoforms are approximately equally represented. *Aims.* the aim of the study was to analyze the different function and distribution of the two isoforms. *Methods.* After informed consent, 132 BM samples were collected from 86 AML and 46 CML patients at diagnosis and 20 samples from healthy subjects. 62 patients were also evaluated during follow-up. WTS$^+$ and KTS$^-$ isoforms were quantified by capillary electrophoresis The relative amount of two isoforms was calculated by measuring the picks area of electropherogram . NIH3T3 and 293T cell lines were transfected with WT1 KTS$^+$ or WT1 KTS$^-$ plasmids. WT1 protein was studied by Western blot and immunofluorescence in BM cells and transfected cell lines. Downstream genes transcriptionally activated by WT1 such as Spred-2 and E-Cadherin were evaluated by Real Time PCR. *Results.* We demonstrated that AML and CML patients have an unbalanced KTS$^+$/KTS$^-$ ratio with a significant increase of KTS$^+$ isoform as compared to KTS$^-$. The ratio observed ranges from 1.6 to 6.1 in AML from 1.6 to 9.5 in CML. In 10% of the patients we observed a complete disappearance of the KTS- isoform . Western blot and immunofluorescence carried out in BM cells and transfected cell lines allow to establish that the KTS+ isoform is mainly localized in the cytoplasm and KTS$^-$ isoform is mainly nuclear localized. In BM cells carrying the isoform KTS$^+$ or in cells transfected with KTS$^+$ isoform we observed the lack of transcription of downstream genes such as Spred1 or E-cadherin. In addition, in patients who achieved a complete remission after chemotherapy, WT1 KTS$^+$/KTS$^-$ ratio returned within the normal range and Spred1 and E-cadherin transcript and protein were significantly upregulated. Finally, cells transfected with KTS$^+$ isoform presented morphology changes, altered adhesion properties and increased proliferation as compared to KTS- transfected cells. *Conclusions.* This study demonstrates that in leukemic cells there is a disruption of the normal transcription activity of WT1 and this is mainly due to the unbalanced ratio between the two isoforms KTS$^+$ and KTS$^-$ with different localization and function. This alteration results in a defective transcriptional activity of WT1 which can probably play an oncogenic role in leukemic cells.

0157

ANKHD1 PROTECTS LEUKEMIA CELLS FROM APOPTOSIS AND BINDS TO SIVA, A PROAPOPTOTIC PROTEIN

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Background. Ankyrin-repeat-containing proteins regulate multiple cellular functions including transcription, cell-cycle, cell survival and participate in protein'protein interactions via their repeat motifs. Ankyrin Repeat and KH Domain Containing 1, ANKHD1, has been recently described, in humans, as a cytoplasmic protein overexpressed in prostate cancer cell line and in leukemia cells compared to normal hematopoietic cells. Its homologous protein, MASK, was described in Drosophila melanogaster as an essential protein for differentiation, proliferation and cell survival. However, the role of ANKHD1 in leukemia cells has not been fully elucidated. *Aims.* The aim of this study was to identify new proteins associated with ANKHD1 and the role of ANKHD1 in the apoptotic process of leukemia cells. *Methods.* In order to identify possible targets of the ANKHD1 protein, we performed a yeast two-hybrid screen using ANKHD1 protein (amino acids 1130-1243) in pGBKT7 vector, as the bait, and a Matchmaker pACT2-cDNA library from normal human bone marrow (Clontech), as the prey. The protein interaction detected was confirmed using the yeast two-hybrid assay, through co-transfections of AH109 yeast with the ANKHD1-pGBKT7 bait and the new candidate for protein interaction identified in pGADT7 vector. Post-transcriptional ANKHD1 gene silencing was done using small interfering RNA, SMARTpool siRNA duplexes (Dharmacon), at a concentration of 400 nM. Transient transfections of Jurkat cells were performed by electroporation in a Bio-Rad Gene Pulser II (300V, 975 microfarads). Cells were cultured for 48 h after transfections and then submitted to Western blotting and apoptosis analysis. Apoptotic cell death was evaluated using Annexin V-FITC/PI staining and FACS analysis. *Results.* The yeast two-hybrid screening identified the new protein interaction between ANKHD1 and SIVA. Co-transfections of AH109 with pGBKT7-ANKHD1 and different SIVA-pGADT7 constructs (SIVA1, SIVA2, SIVA C-terminal, SIVA N-terminal, SIVA Dead Domain) confirmed the association between ANKHD1/SIVA1 and ANKHD1/SIVA2, and the need for both the N-terminal and C-terminal regions of SIVA for the interaction with ANKHD1. Western blotting confirmed that ANKHD1 expression was reduced by 80% in the Jurkat cells transfected with ANKHD1 siRNA compared with controls cells (electroporated cells). Treatment of Jurkat cells with the ANKHD1 siRNA resulted in increased apoptosis (27% of apoptotic cells) compared with control cells (14% of apoptotic cells). *Conclusions.* The association between ANKHD1 and SIVA isoforms suggests that ANKHD1 participates in the apoptotic signaling in leukemia cells, since we know that SIVA1 and SIVA2 are overexpressed in acute lymphoblast leukemia cell lines and induce apoptosis in Jurkat cells. The increased apoptotic rate after posttranscriptional ANKHD1 gene silencing indicates an anti-apoptotic function of ANKHD1 in Jurkat cells. In conclusion, ANKHD1 protects leukemia cells from apoptosis and binds to SIVA, possibly inhibiting the proapoptotic function of SIVA. These results indicate that ANKHD1 is associated with the abnormal phenotype of leukemia cells; the identification of new disease-specific targets for acute leukemia immunotherapy expands treatment options and increases our chances of successfully treating this heterogeneous disease and lowering the unacceptably high mortality rate.

Genomics and proteomics

0158

PLASMA PROTEOMIC PROFILES MAY PREDICT EARLY ACUTE GRAFT-VS.-HOST DISEASE FOLLOWING REDUCED INTENSITY CONDITIONING ALLOGENEIC HLA-IDENTICAL SIBLING TRANSPLANTATION

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Background. Modern approaches to predict the occurrence/severity of aGVHD are needed. *Aims.* This study aimed to identify a plasma protein signature correlating with occurrence of early aGVHD. *Methods.* We performed Surface-Enhanced Laser Desorption/Ionization time (SELDI) of flight mass spectrometry profiling of plasma from 88 pts who received RIC allo-SCT from HLA-identical siblings. *Results.* Median age of pts was 51 (range, 18-70) y. 41 pts (47%) had a myeloid malignancy, whereas 30 (34%) had a lymphoid malignancy. 17 pts (19%) were treated for metastatic non-hematological malignancies. The RIC regimen included fludarabine, busulfan and ATG in 53 pts (60%) and low dose irradiation in 35 pts (40%). With a median FU of 400 (range, 127-829) d, 20 pts (23%) had early (prior to day 35 after allo-SCT) grade 2-4 acute GVHD (12 grade 2 and 8 grade 3-4). Denatured plasma samples (collected at a median of 28 d.) were incubated with H50 and CM10 ProteinChip arrays and subjected to SELDI analysis. Pts population was divided into a training (n=59) and a validation set (n=29). In the training set, 36 protein peaks were differentially expressed according to early aGVHD occurrence. By combining partial least squares and logistic regression methods, we built a multiprotein model that correctly predicted outcome in 96% of pts (14/14 patients with early aGVHD; specificity, 96%). The observed correct prediction rate in the validation set was 69% with a sensitivity of 67%, and a specificity of 70%. While negative predictive value of the model was only 36%, predictive positive value was estimated to 89% in the validation set. The performances of the model remained very similar after iterative (500 times) random resampling (correct prediction rate: 74%, median sensitivity: 48%, median specificity: validation set: 83%). Univariate and multivariate analyses of known risk factors (demographic features, diagnoses and transplant procedures) for early grade 2-4 aGVHD did not show any statistically significant difference between the group of 20 patients who had early grade 2-4 aGVHD as compared to the remaining patients, and suggested that the multiprotein index is likely to be the only independent prognostic parameter. Major components of this multiprotein index are currently being characterized and will be presented. *Conclusions.* Obviously, larger prospective studies are still needed, but our results already suggest that proteomic analysis of plasma will prove increasingly important in the early and clinical diagnosis of aGVHD.

0159

PROTEOME PROFILING IDENTIFIES APOLIPOPROTEIN A1 AS A SERUM MARKER CORRELATED TO JAK2 V617F BURDEN IN POLYCYTHEMIA VERA AT DIAGNOSIS

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Background. Polycythemia Vera (PV) is a myeloproliferative disorder (MPD) originating from a multipotent haematopoietic progenitor cell. The great majority of PV are characterized by a recurrent acquired gain-of-function mutation of the JAK2 protein (JAK2-V617F). Modifications of JAK2-V617F burden are associated with changes of PV phenotype and probably impact on the risk of complications. However, we don't know whether if the proportion of JAK2-V617F allele modifies certain serum proteins. *Aims.* The purpose of our study was to generate serum proteome profiles of PV patients to discover and identify novel serum protein correlated with the levels of granulocyte JAK2-V617F. *Methods.* PV serums were collected in a multicenter study and were randomly affected in two independent learning and validation sets. Proteome profiles were generated by SELDI-TOF mass spectrometry. JAK2 V617F status was determined by quantitative-PCR analysis of purified granulocytes. *Results.* Unsupervised clustering analysis of the learning set showed that PV patients could be separated in two major subgroups tending to be different with respect to the mean percentage of mutated JAK2 (p=0.09), the number of PRV-1 transcripts (p=0.08) and Ht (p=0.09). Comparative analysis of proteome profiles found significant difference (p<0.05) for 27 protein or peptide masses (characterized by their m/z ratio) between these two subgroups. To validate the association of these proteins with JAK2-V617F levels, we applied the 27 markers to the validation set. Two subgroups with significantly different mean percentage of JAK2 V617F (p=0.04) were thus isolated. The two groups also differed by their number of PRV-1 transcripts (p=0.02), their hematocrit level (p=0.01) and their platelet number (p=0.05). Among the 27 protein markers, we selected a 28kDa m/z marker (p28) that retained the highest discriminative value between PV subgroups in each of the two independent sets; p28 expression being the highest for PV patients with more than 75% of mutated alleles. Separate fragmentation analysis in tandem mass spectrometer (nano-LC-MS/MS) of amino-acid sequence of 10 proteolytic peptides unambiguously identified Apolipoprotein A1 (Apo-A1) as the 28kDa m/z marker. Immuno-assay on an automated random immuno-analyser confirmed the correlation between serum Apo-A1 concentrations and JAK2-V617F percentages, and showed that serum Apo-A1 assay allowed the specific discrimination of PV patients with more than 75% of mutated alleles. *Conclusions.* Our results showed that serum proteome profiles of PV patients are influenced by JAK2 V617F burden. In particular we showed that Apolipoprotein A1 is a serum biomarker correlated to the percentage of JAK2-V617F in granulocytes. These results suggest that serum Apolipoprotein A1assay should provided useful information for identification of PV with high levels of mutated JAK2.

0160

GRAFT VERSUS HOST DISEASE AND OTHER COMPLICATIONS CAN BE DEPICTED BY SPECIFIC PROTEOMIC PATTERNS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for many hematologic malignancies or hematopoietic dysfunction syndromes, but the application is still limited due to major complications, such as severe graft versus host disease (GvHD) and infectious complications. Diagnosis GvHD is based on clinical features and biopsies, a non invasive, unbiased laboratory test does not exist. *Results.* Here we describe the blinded evaluation of a proteomic pattern useful for early diagnosis of acute GvHD, evaluated on 902 samples collected from 168 patients undergoing allo-HSCT at 5 different transplant centers. The majority of the patients included were transplanted for hematological malignancies (n=158), 10 for hematopoietic failure syndromes (sAA, n=6; PNH n=1; OMF, n=3). Forty-five patients were treated with dose-reduced conditioning regimens; GvHD-prophylaxis consisted of antibodies plus Cyclosporin (CsA) plus Metotrexat (MTX) or mycophenolic acid (MMF), respectively. Most patients were transplanted from matched unrelated donors (MUD, n=100), while 63 received stem cells from matched related (MRD, SIB, 1 syngeneic), 3 from haplo-identical, and 2 from mismatched donors. Using capillary electrophoresis coupled online with mass spectrometry (CE-MS), 170 potentially aGvHD-specific polypeptides were identified in a training set of 13 urine samples from 10 allo-HSCT patients with aGvHD (> grade II) and samples (n=50) of 23 patients without aGvHD. The application of the aGvHD specific pattern and calculated model allowed correct classification of blinded and prospectively collected urine samples with high accuracy: The model enabled the diagnosis of aGvHD > grade II with a sensitivity of more than 83% [95% CI 73.1 to 87.9]). High resolution proteome analysis with diagnostic peptide patterns may help to identify patients at risk of severe aGvHD development prior to clinical features (mean: 7 days, range: 1 to 13 days prior to clinical symptoms) in an unbiased laboratory based screening assay. Other patterns (bacterial infection/septicaemia, CMV and EBV reactivation and infection) will be shown. Application of proteomic based patterns may lead to the establishment of a diagnostic tool suitable for pre-emptive therapy of aGvHD based on proteomic patterns.

0161

MOLECULAR STUDY OF PORTUGUESE PATIENTS WITH CLINICAL DIAGNOSIS OF SHWACHMAN-DIAMOND SYNDROME

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Shwachman-Diamond syndrome (SDS; MIM# 260400) is a rare autosomal recessive disorder characterized by the association of exocrine pancreatic and bone marrow dysfunction. Other systemic findings (skeletal, liver and psychomotor) or problems secondary to bone marrow dysfunction may also be detected. Intermittent or persistent neutropenia is the most common haematological finding, but anaemia and thrombocytopenia are present in approximately 40% of the patients. The SDS locus was mapped to chromosome 7q11 where disease-associated mutations were later reported in the Shwachman-Bodian-Diamond syndrome gene (SBDS; MIM# 607444). The present report summarises the molecular analysis of 14 Portuguese patients with suspected diagnosis of SDS. Direct sequencing of all coding regions of the SBDS gene, including exon-intron boundaries, was conducted in all cases. Additional molecular studies including long-range PCR and cDNA analysis were performed to clarify the involvement of a refractory mutation in a case where only a heterozygous mutation had been detected by direct sequencing. Four patients were found to be compound heterozygous for the common c.181_184TA>CT and c.258+2T>C mutations. One of the patients was a compound heterozygote for the c.258+2T>C mutation and a previously unreported genomic deletion (c.258+374_459+250del). This novel mutation, predictably giving rise to an internally deleted polypeptide (p.Ile87_Gln153del), appears to have arisen from an excision event mediated by AluSx elements present in introns 2 and 3 of SBDS gene. In the remaining nine patients no pathogenic mutations were found. Eight previously reported polymorphisms were also detected in the course of this study (c.129-162TTGGGGGTAAGAAAdelinsGGGGGCGGGGG, c.129-71G>A, c.141C>T, c.201A>G, c.258+54T>G, c.459+92A>G, c.635T>G and c.651C>T). The previously reported mutations c.181_184TA>CT and c.258+2T>C, arising from gene conversion events, are the most frequent mutations associated with SDS in this group of patients. The detection of a large genomic deletion encompassing exon 3 illustrates the importance of screening for gross rearrangements, especially in patients where a single mutation is detected by routine methods. The considerable number of patients in which no mutations were found, may be explained by variations in intronic or regulatory regions that were not screened, the involvement of other genes acting in a common pathway, or broad selection criteria permitting inclusion of misdiagnosed patients.

0162

METHYLATION PROFILE IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Epigenetic (nonmutational) changes in the promoter region of multiple genes are very important in leukemia triggering and development including chronic myeloid leukemia (CML). To further characterize this event, the methylation status of the 5'-promoter region of multidrug-resistance (MDR-1) gene and calcitonin (CT) gene in CML patients was investigated. Totally 26 DNA samples obtained from mononuclear cells of peripheral blood and bone marrow of 18 CML patients were investigated. Ten patients were in chronic phase of the disease, 5 - in accelerated stage and 3 - in blast crisis. As a control we used genomic DNA obtained from 10 healthy donors. We applied methyl-dependent polymerase chain reaction (PCR) method, investigating the intensity of CpG-sites methylation of both genes in the 5'-promoter area. The methylsensitive (HpaII) and methyl insensitive (MspI) restriction endonucleases were used for the digestion of DNA-samples. The products of the PCR were scanned directly in agarose gel. According to the data of the experiments, the intensity of the MDR-1 gene methylation progressively decreased from stage to stage of CML. Conversely, the methylation level of 5'-CT-gene became higher. The CT/MDR-1 optical density ratio was used for the semiquantitative analysis. We revealed a sustained increase of this index during the disease progression. The mean donor (normal volunteers) index was 0.44±0.14, whereas the similar figures in CML were 0.82±0.21 in chronic phase; 1.35±0.17 in accelerated phase and 2.19±0.4 in blast crisis. It is interesting to notice, that this index was higher in patients (in chronic phase), who received imatinib, compare to those, who was under conventional (hydrea) therapy. It is well known, that DNA-methylation often reversely correlates with gene expression. Thus, the revealed hypomethylation state of 5'-region of the MDR-1 gene suggests that the progression of CML is likely accompanied by acquisition of multidrug-resistance phenotype. CT-gene hypermethylation, as it was stated earlier, strongly correlates with the loss of expression of tumor suppressor genes. Both components are crucial in the illness progression. These findings might be useful in the disease monitoring and also serve as a marker of treatment efficacy in CML.

0163

IDENTIFICATION OF NEW PROTEINS INVOLVED IN THE PATHOGENESIS OF THE ANTIPHOSPHOLIPID SYNDROME BY PROTEOMIC ANALYSIS: EFFECTS OF *IN VIVO* STATINS TREATMENT

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Background. Antiphospholipid syndrome (APS) is an autoimmune disease manifested by thrombotic or obstetrical events in the presence of antiphospholipid antibodies (aPL). In spite of the recent progresses, many aspects of this disease remain unclear, such as the molecular mechanisms leading to thrombosis. In addition to its anti-inflammatory and immunomodulatory properties, statins have been shown antithrombotic effects, although the molecular mechanisms leading to this effect are not yet fully understood. This study analyzed, by using proteomic techniques: a) changes in proteins expression of monocytes from APS patients related to the pathophysiology of the syndrome; and b) the *in vivo* effects of Fluvastatin on the pattern of protein expression in this cellular setting. *Patients and Methods.* Proteomic analyses were performed in 25 APS patients. Control groups included: 10 patients with aPL antibodies but without previous thrombosis, 10 patients with previous thrombosis but without aPL antibodies, and 10 healthy donors. Ten patients with APS and previous history of thrombosis further received Fluvastatin (20 mg/day) for one month. Blood samples were obtained before treatment and after one and three months of treatment. All patients were tested for the presence of anti-cardiolipin autoantibodies and lupus anticoagulant. Monocytes were isolated from peripheral blood mononuclear cells by magnetic depletion of non-monocytes. Proteomic analyses were performed using two-dimensional electrophoresis and MALDI-TOF mass fingerprinting analysis. *Results.* Approximately 500 protein spots were detected on the comassie-stained gels from the donors' material. Proteins identified as more significantly altered between monocytes from APS patients and controls' donors belonged to the group of signal transduction mediators (i.e. lipocortin I, Annexin II, Rho A proteins, Ubiquitin conjugating enzyme, zinc finger proteins), metabolic enzymes (i.e. α-enolase, ATP-synthase) and immunomudulators (i.e. Hsp60, disulfide isomerase). Some of these differentially expressed proteins (such as annexin II, Rho A proteins and Hsp60) have previously been shown to play a relevant role in the pathogenesis of the APS. *in vivo* statins treatment for one month reversed the changes observed in the expression levels of those proteins. These levels then suffered a slowly return, although remained significantly changed in relation to control values after three months of the end of the treatment. To confirm the results, more specific analytical techniques, such as real time RT-PCR and Western blot were used. Moreover, to assess the effect of aPL on monocyte proteomics, monocytes from normal individuals were treated with affinity purified patients' IgG, in the presence or in the absence of fluvastatin. All those studies further supported the data presented. *Conclusions.*Our study has identified, by proteomic analysis, for the first time: 1) some proteins that may be involved in the pathogenic mechanisms of the APS syndrome; and 2) the changes that protein patterns of monocytes from APS patients suffered when statins treatment was used for 1 month. These findings might provide new targets for rational pathogenesis-based therapies of APS.

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0164

EVALUATION OF MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) TO DETECT GENOMIC DELETIONS IN CHRONIC LYMPHATIC LEUKEMIA A COMPARISON WITH CYTOGENETICS, FISH AND PCR

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Background. MLPA is a new method to detect losses or gains of genes or gene fragments. CLL ist the most common leukemia in the western world. Genetic variances, like deletions, are important prognostic factors in this form of leukemia. *Aims.*Was to test the clinical applicability of MLPA in CLL-cases in comparison with established standards for risk evaluation like cytogenetics and FISH. *Methods.* Peripheral blood and bone marrow samples were collected from 70 CLL patients at various stages of disease, 22 healthy persons and a human B cell lymphoma cell line (DOHH-2) showing characteristic chromosomal aberrations as a control. If possible, karyotyping by GTG banding was performed. The samples were checked for CLL typical chromosomal aberrations with a FISH panel containing probes for 11q, 13q, 17p and CEP12. For MLPA, 20-500 ng of genomic DNA were used. After hybridisation of the probes to their target sequences, a ligation reaction was carried out. The ligation products were amplified by PCR. The CLL optimised MLPA-Kit contains probes for the detection of del(11q) (ATM), del(17p) (TP53), Trisomy 12, Trisomy 19, del(13q), amplification of 2p24 (MYCN), and 6q rearrangements. 1 μL of the PCR product was analysed on an ABI3130 Genetic Analyzer and evaluated with the SeqPilot v1.3.1 program. In cooperation with the Institute for Bioinformatik of the Johannes Kepler University of Linz a Feature Selection Analyse with the P-SVM (Potential Support Vector Machine) program was performed to detect markers predicting CLL. For this, the raw data from the ABI 3130 was converted into Excel files and analysed with this program. *Results*. The relative peak area (RPA) represents difference of the peak area of the patient file and the median of the peak area of 19 control data. All RPA data below 75% were scored as deleted, whereas all data over 125% were scored as amplified. The present MLPA data generated by the SeqPilot Program show significant correlation to FISH results and karyotyping. Furthermore we have tested the sensitivity for detecting aberrations in cell mosaics because this is a common observation in CLL. At least 30 percent mosaicism is well detectable in cell dilution experiments with the DOHH-2 cellline; the limited sensitivity has to be considered in the clinical setting. The (P-SVM) feature selection analyses showed that MYC (localised on chromosome 8) was the best marker to predict CLL in samples of unknown origin, 49 of the 70 analysed CLL patient samples are predicted right (70%). 20 of the 22 control samples were predicted as negative (91%). *Summary and Conclusions.* This study shows that MLPA may be a helpful tool for the assessment of losses or gains of gene fragments in CLL, although a solitary application of this method in routine diagnostic is not practical at the moment. The result of the P-SVM shows that MYC may be an interesting marker in the diagnosis of CLL.

0165

HEMOCHROMATOSIS GENOTYPES AND RISK OF 31 DISEASE ENDPOINTS - META-ANALYSES INCLUDING 66,000 CASES AND 226,000 CONTROLS

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Background. Hemochromatosis genotypes have been associated with liver disease, diabetes mellitus, heart disease, arthritis, porphyria cutanea tarda, stroke, neurodegenerative disorders, cancer, and venous disease, but studies have reported conflicting results. *Aims.* We examined associations between hemochromatosis genotypes C282Y/C282Y, C282Y/H63D, C282Y/wild type, H63D/H63D, H63D/wild type vs. wild type/wild type and 31 disease endpoints. *Methods.* Meta-analyses including 202 studies with 66,263 cases and 226,515 controls were conducted. Potential sources of heterogeneity were explored. *Results.* For liver disease, the odds ratio for C282Y/C282Y vs. wild type/wild type was 3.9(99% CI: 1.9-8.1) overall, 11(3.7-34) for hepatocellular carcinoma, 4.1(1.2-14) for hepatitis C, and 10(2.1-53) for nonalcoholic steatohepatitis. For porphyria cutanea tarda, the odd ratios were 48(24-95) for C282Y/C282Y, 8.1(3.9-17) for C282Y/H63D, 3.6(1.8-7.3) for C282Y/wild type, 3.0(1.6-5.6) for H63D/H63D, and 1.7(1.0-3.1) for H63D/wild type vs. wild type/wild type. Finally, for amyotrophic lateral sclerosis the odds ratio was 3.9(1.2-13) for H63D/H63D vs. wild type/wild type. These findings were consistent across individual studies. *Conclusions.* In aggregate, C282Y/C282Y associated with liver disease, all 5 hemochromatosis genotypes associated with porphyria cutanea tarda, while H63D/H63D associated with amyotrophic lateral sclerosis.

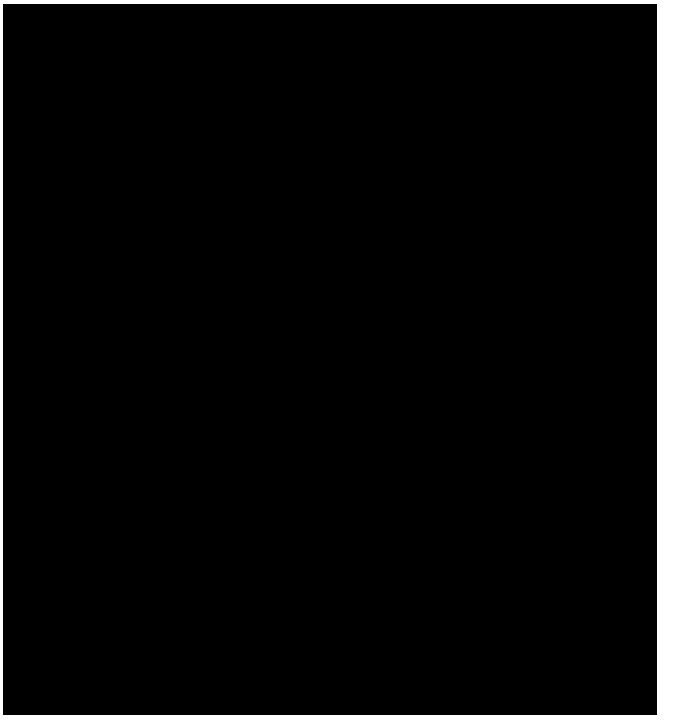

Figure 1.

0166

IDENTIFICATION OF HISTONES H2B AND H4 OVEREXPRESSION IN MANTLE CELL LYMPHOMA BY MASS SPECTROMETRY-BASED PROTEOMIC STUDY

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Background. Mantle cell lymphoma (MCL) is a non germinal centre small B-cell lymphoma that represents about 6-10% of all non-Hodgkin lymphomas (LNH). The MCL genetic hallmark is the chromosomal translocation t(11;14)(q13;q32) leading to the overexpression of cyclin D1 an important regulator of the G1 phase of the cell cycle. However, the cyclin D1 overexpression is not sufficient to induce MCL. Additional genetic alterations may occur in subsets of MCL. Most of these alterations appear to disturb the cell cycle machinery / interfere with the cellular response to DNA damage, thus making MCL a paradigm for cell cycle and DNA damage response deregulation in cancer in general. *Aims.* Our goal was to expand the current understanding of the molecular pathogenesis of MCL using proteomic strategy and to provide the basis for identification of biomarkers specific to MCL compared to other small B-cell lymphomas. *Methods.* Using Surface Enhanced Laser Desorption/Ionization - Time of Flight (SELDI-TOF) technology the MCL proteome was compared to the proteome of 2 other non germinal centre small B-cell lymphomas, the small lymphocytic lymphoma (SLL) and the marginal zone lymphoma (MZL). Whole cell lysates obtained from 18 MCL (17 nodes and 1 spleen), 20 SLL (20 nodes), 20 MZL (1 node and 19 spleens) fresh frozen biopsies and 7 traumatic normal spleens were applied on two different ProteinChip array surfaces (CM10 and Q10) that were bombarded by 4 different laser intensities. All spectra were aligned and peak intensities were normalized to the total ion current (M/Z<50,000 Da) after baseline subtraction. All protein peaks detected were analyzed at the same time with Eisen's hierarchical clus-

tering software to identify specific proteomic signatures. SELDI-TOF-assisted purification followed by in gel trypsin digestion and LC-MS/MS peptides identification was used to identify proteins overexpressed in MCL tumors. *Results.* The combination of all data obtained with the two different ProteinChip generated 1300 analyzable protein peaks. The protein patterns were first analyzed using hierarchical clustering in an unsupervised fashion revealing a very homogenous protein pattern among all lymphoma samples. The second analysis using hierarchical clustering in a supervised method (discriminating score) pointed out tissue specific protein signatures (node and spleen). Those tissue signatures were subtracted from the data and specific protein signatures for SLL, MZL and MCL were found based on the expression level of 34 protein peaks. SELDI-TOF-assisted purification was used to optimize protein purification before in gel digestion and LC-MS/MS identification. We identified two core histones as overexpressed proteins in MCL tumor biopsies: histone H2B and histone H4. *Conclusions.* The proteomic profiling of MCL tumor biopsies using SELDI-TOF technology leads to the characterization of a specific MCL protein signature. Among proteins overexpressed in MCL, we identified histones H2B and H4. The overexpression of these two core histones in MCL was concordant with the cell cycle deregulation previously reported in this lymphoma entity.

0167

HIGH FREQUENCY OF MICROSATELLITE INSTABILITY IN LYMPHOID TUMOUR CELL LINES

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Background. The mismatch repair (MMR) system is specialized in removing replication errors which are thought to be recognized by the heterodimers MutSα (MSH2 and MSH6) and MutSβ (MSH2 and MSH3). Defective MMR can be detected via microsatellite instability (MSI) and causes diseases like HNPCC. *Aims.* With our studies we wanted to gain insights in the frequency of MSI in tumour cell lines from different tissues and in the contribution of defective expression of MSH2, MSH3 and MSH6 to the MSI phenomenon. *Methods.* Fluorescence PCR in combination with capillary electrophoresis was used to analyse 500 human tumour cell lines for length shortening of BAT26, a marker for MSI detection. MSI cell lines were further investigated for presence of transcripts and proteins of MSH2, MSH3 and MSH6 by RT-PCR and western blot analysis, respectively. cDNAs of MSH2, MSH3 and MSH6 were inserted into a bicistronic vector allowing expression of each protein fused to EGFP for localization studies by fluorescence microscopy. *Results.* MSI was proven in 8% (41/500) of tumour cell lines. The relative amount of MSI cell lines among cell lines of special tumour groups was significantly highest in T-cell leukaemia (59%) and ovarian adenocarcinoma (50%), followed by colon adenocarcinoma (25%) and B-cell leukaemia cell lines (24%). Further investigation revealed presence of full-length transcripts for MMR genes in 81% of MSI cell lines, while only 51% expressed the corresponding proteins. In addition to MSH2, MSH3 and MSH6 full-length transcripts seven alternatively spliced variants were obtained from MMR proficient cell lines. Cloning and stable expression of EGFP fusion proteins with the wildtype transcripts of MSH2, MSH3 and MSH6 and their variants showed that only MSH2 WT, MSH3-WT, MSH3-V3, MSH3-V5, MSH6-WT and MSH6 V1 fusion proteins are restricted to the nucleus, whereas expressed MSH2 V1, MSH6-V2 and MSH6-V3 are mainly localized in the cytoplasm and MSH3-V4 fusion protein is distributed all over the cell. For functional characterization we set a UV-A beam to a defined area of the nucleus and live imaging revealed an accumulation of MSH2-WT and MSH6-V1 fusion proteins but not MSH3-WT fusion protein at the site of the UV-A damage. *Summary and Conclusions.* The MSI phenomenon could be shown in an unexpected high number of T- and B-cell leukaemia cell lines. Genomic deletions or epigenetic silencing of MSH2, MSH3 and MSH6 genes are not the predominant mechanisms responsible for absence of MMR activity, indicating that loss of function mutations may play a bigger role. 10 wildtype and variant transcripts for MSH2, MSH3 and MSH6 demonstrated diverse localization properties upon heterologous expression, suggesting additional functions or regulatory mechanisms. MutSα but not MutSβ seems to be involved in DNA repair after UV-A damage.

0168

METALLOPROTEINASE POLYMORPHISMS IN PH-NEGATIVE CHRONIC MIELOPROLIFERATIVE DISORDERS: NEW GENETIC FACTORS?

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Background. Philadelphia-negative chronic myeloproliferative disorders (MPDs) are characterized by clonal proliferation of haematopoietic progenitor cells in the bone marrow and by different degrees of extramedullary haematopoiesis, which is associated with increased $CD34^+$ circulating cells. The transient mobilization of haematopoietic stem cells and progenitor cells has been attributed to the induction of a highly proteolytic environment within the bone marrow. Matrix metalloproteinases (MMPs) are central factors in the control of extracellular matrix turnover and have been implicated in connective tissue destruction and remodelling associated with cancer invasion and metastasis. A common insertion polymorphism (an additional guanidine) in the nucleotide sequence of the MMP-1 gene promoter has been reported. The 2G homozygotes show an increased transcription activity compared to 1G homozygotes ad controls. On the promoter of the MMP-3 gene has been described a polymorphism that *in vitro* assays demonstrate that the promoter activity of the 5A allele had 2-fold higher promoter activity than the 6A allele. *Aims.* We investigated whether the MMP-1 and/or the MMP-3 promoters polymorphisms are associated with susceptibility and/or progression of MPDs. *Methods*. 46 patients: 21 with Polycythemia Vera (PV); 18 with Essential Thrombocythemia (ET) and 7 with Idiopathic Myelofibrosis (cIMF) and 133 healthy controls were genotyped for the two polymorphisms. Genotypes were determined with PCR-RFLP methods. Results MMP-1 genotype was statistically different between patients and controls (patients vs controls ;1G/1G vs 1G/2G+2G/2G 6% vs 26%; OR 4.78; CI% 1.35-25.22; p=0.007); for MMP3 the 5A/5A genotype was less represented (13% vs 27%; OR 2.59: CI% 0.97-8.07; p=0.04)).No correlation was observed with spleen size, $CD34^+$ percentage, and JAK2-V617F mutation. *Conclusions.* Our data suggest a role of MMP1 and MMP3 in the matrix remodeling associated with CMD. However, no firm conclusion regarding the clinical significance of these Metalloproteinase promoter polymorphisms can be drawn until much larger cohorts have been analysed.

0169

NEW DATA ON ROBUSTNESS OF GENE EXPRESSION SIGNATURES IN LEUKEMIA: COMPARISON OF THREE DISTINCT TOTAL RNA PREPARATION PROCEDURES

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Background. Gene expression microarrays had been used to classify known tumor types and various hematological malignancies enforcing the objective that microarray analysis could be introduced soon in the routine classification of cancer. However, there are still doubts about gene expression experiments performance in clinical laboratory diagnosis. For instance, the quality of starting material is a major concern in microarray technology and there are no data on the variation in gene expression profiles ensuing from different RNA extraction procedures. *Aims.* Here, as part of the internal multicenter MILE Study program, we want to assess the impact of different RNA preparation procedures on gene expression data, analyzing 27 patients representative of nine different subtypes of pediatric acute leukemias. We compared the three currently most used protocols to isolate RNA for routine diagnosis and microarray experiments. *Methods.* The methods are named as method A: lysis of mononuclear leukemia cells, followed by lysate homogeniziation, followed by total RNA isolation; method B: TRIzol RNA isolation, and method C: TRIzol RNA isolation followed by total RNA purification step. The methods were analyzed in triplicates for each sample (24) and additional three samples were performed in technical replicates of three data sets for each preparation (HG-U133 Plus 2.0). *Results.* Method A results in better total RNA quality as demonstrated by 3'/5' GAPD ratios and by RNA degradation plots. High comparability of gene expression data is found between samples in the same leukemia subclasses and collected with different RNA preparation methods thus demonstrating that sample preparation procedures do not impair the overall signal distribution. Unsupervised analyses showed clustering of samples first by

each patient's replicate conditions, then by leukemia type, and finally by leukemia lineage. In fact, B-ALL samples are clustered together, separately from T-ALL and AML, demonstrating that clustering reflects biological differences between leukemias and that the RNA isolation method is a secondary effect. Also, supervised cluster analyses highlight that samples are grouped depending on intra-lineage features (i.e. chromosomal aberrations) thus confirming the clustering organizations as reported in recent gene expression profiling studies of acute leukemias. Our study shows that biological features of pediatric acute leukemia classes largely exceed the variations between different total RNA sample preparation protocols. However, technical replicates analyses reveal that gene expression data from method A have the lowest degree of variation, are more reproducible and more precise as compared to the other two methods. Furthermore, compared to methods B and C, method A produces more differentially expressed probe sets between distinct leukemia classes and is therefore considered the more robust RNA isolation procedure for gene expression experiments using high-density microarray technology. *Conclusions.* We therefore conclude that method A (initial homogenization of the leukemia cell lysate followed by total RNA isolation) combined with a standardized microarray analysis protocol is highly reproducible and contributes to robustness of gene expression data and that this procedure is most practical for a routine laboratory use.

0170

GENOMIC IMBALANCE PROFILES IN MAJOR SUBSETS OF B-NHL: A DESCRIPTIVE META-ANALYSIS OF 1831 PUBLISHED CASES ANALYZED BY CGH

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Background. In the search for disease specific genomic imbalance patterns and genomic aberration hot spots, Comparative Genomic Hybridization (CGH) has been applied to virtually all types of malignant neoplasias. Since single publications of genomic screening data can only describe a limited set of cases, for statistically meaningful comparisons of several entities data has to be derived from large-scale data collections. *Aims.* This study compares overall aberration profiles in different subsets of B-cell non-Hodgkin's lymphomas (NHL), with the aim to identify disease-specific aberration hot spots and imbalance patterns. This data will be useful when evaluating presumptive oncogenetic targets, as well as for diagnostic marker identification. *Methods.* For the Progenetix molecular-cytogenetic database, genomic aberration data from currently 15810 cases has been collected, including approximately 50% of all published chromosomal CGH data. For the application of data mining methods, the ISCN related karyotype annotations of all cases were converted to a band specific data matrix. From this collection, 1831 B-NHL analyzed by CGH were selcted for subset specific analysis. *Results.* B-NHL cases were assigned to clinico-pathological subsets: DLBCL (695 cases), CLL (549), MCL (217), FCL (175), marginal zone lymphoma (MZL and SMZL, 93 cases), Burkitt lymphoma (66) and PMBCL (26).

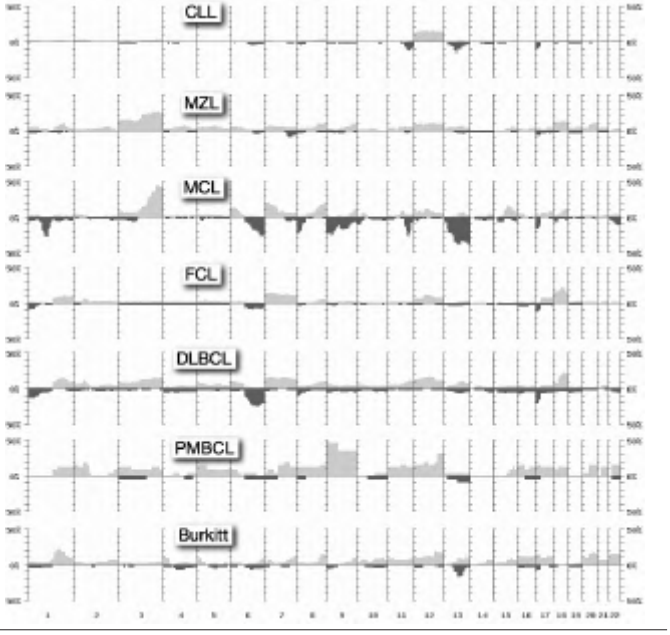

Figure 1. CGH in B-NHL: genomic gains (up) and losses (down).

For all subsets, imbalance profiles were generated, capturing the band-specific frequencies of genomic gains and losses. As an approximation for chromosomal instability, the median number of aberrant chromosomes was derived, ranging from 1 (CLL, FCL, MZL) to 5 (MCL). Average imbalance profiles showed remarkable differences between different diagnostic groups in frequency and distribution. Frequent gain hot spots were found on 3q26q27 (MCL and MZL), 9p (only in PMBCL), 1q (BL, DLBCL, PMBCL, FCL), 12q (several), 7 (MCL, FCL, DLBCL, PMBCL) and 18q21 (most in FCL, DLBCL). Frequent losses were found on 6q25q27 (MCL) and 6q16q21 (DLBCL), 13q (most in MCL, BL, CLL) as well as on 1p, 8p, 9, and 11q in MCL (Figure 1). *Summary*. Genomic imbalance patterns in B-NHL entities are disease-specific and may reflect varying pathogenetic mechanisms. With the possible exception of 9p gains in PMBCL, regional hot spots occur in several entities, but with varying frequencies and in different combinations. This meta-analysis supports the existence of separate regions *targeted* by lossen on 6q in FCL/DLBCL (6q16q21) and MCL (6q25q27). Of all entities, DLBCL case show the largest variation in individual imbalance patterns.

0171

EVI1 OVEREXPRESSION IN A SERIE OF 212 HEMATOLOGICAL MALIGNANCIES

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Background. The ecotropic viral integration site 1 (EVI1), located in chromosome 3q26, has been recognized in the last years as one of the most aggressive oncogenes associated to human leukemias. The inappropiate expression of EVI1 in hematopoietic cells has been implicated in the development or progress of myeloid disorders. Previous studies, applying microarray technology, indicate that high levels of EVI1 expression are detected in 10% of patients with AML .The correlation between overexpression of EVI1 in bone marrow and poor outcome in AML and MDS is a frecuent issue of discussion in the literature. *Aims.* To analyze the incidence of EVI1 expression in haematological malignancies and its value as prognostic factor. *Methods.* The study was performed restrospectively on a total of 212 patients (118 male/94 female, age range 1-92) 122AML, 23 MDS, 38 CML and 29 ALL. Also 7 healthy volunteers were studied. Expression of EVI1 gene (EVI1+) was examined in bone marrow samples and/or peripheral blood at diagnosis and during follow-up by RT-PCR .Survival curves in the cases of AML, LLA and MDS patients were plotted following the Kaplan Meier method and differences between the curves were analysed with the Log Rank test and Breslow test. *Results.* Of the 212 patients 53 overexpressed EVI1.The relation of EVI1+ in the different pathology groups was: 27/113 (23, 8%) AML (FAB subtypes: 2M0, 1 M1,3 M2, 6 M4, 7 M5, 1 M6, 4 AML 2ªMDS,3 unclassificated); 14/55 (25,4%) CML; 8/25(32%) MDS (1 RA; 1CRDM; 1 CRDM-SA; 3AREBI; 2 AREBII) and 4/29(13, 8%)LLA(2 ALL-B, 1 ALL-T and 1 ALL-Phi +). Survival curves in the different pathology groups didn´t show any significant differences in overall survival and disease free survival when comparing EVI1+ to EVI1- population. When survival curves were analyzed in the AML group with age range >14 to <60 all treated with similar chemotherapy schemes, no significant difference was observed. However, in ALL patients with the same age range , and a small samples size, a shorter survival was observed in the EVI-1+ group. *Conclusions.* 1) Although the higher expression of EVI1 gene was clearly associated with myeloid malignancies, is not restricted to this group. It is note worthy that 13, 8% of EVI1 overexpression in the LLA patients group was observed.2) In AML samples a greater than expected incidence of overexpression of EVI1 was observed (22,8% vs 11% previosly described), without a statistically significant prognosis.3) EVI1 overexpression could have a marked relevance in the development and progression of hematological malignancies but probably without a clear implication in the outcome (prognostic) of the illness.

0172

SNP ARRAY PROFILING OF FOLLICULAR LYMPHOMA REVEALS NOVEL REGIONS OF ACQUIRED UPD

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Background. The use of Single Nucleotide Polymorphism (SNP) array profiling has uncovered extensive regions of acquired uniparental disomy (aUPD) in the cancer genome undetected using cytogenetics or array-CGH platforms. These usually arise by mitotic recombination and can render a cell homozygous for a pre-existing abnormality. *Methods.* SNP array analysis was performed using the Affymetrix 10K Gene-chip mapping array on DNA extracted from a series of Follicular Lymphoma (FL) lymph nodes biopsies from 52 patients, taken at time of diagnosis (n=20), progression (n=26) and transformation (n=34), and the t(14;18) positive lymphoma cell lines SCI-1, DoHH2 and RL 2261. Analysis was performed using the genome oriented laboratory file system (GOLF), a software package designed to interpret SNP data. In the absence of available germline DNA from the majority of these patients; an algorithm was devised to define significant regions of homozygosity. The criteria of > 96% homozygosity in at least 50 contiguous SNPs was found to detect no abnormalities in 23 normal remission bone marrows and was therefore adopted. *Results.* Abnormalities were detected in 63/80 patient specimens; these were non-random with recurring sites of aUPD on several chromosomes. In the patient samples aUPD was observed most frequently at 6p (n=12; 23%) and 12q (n=10; 19%); all 3 cell lines had aUPD 12q and aUPD 6p was seen in SCI-1. Additionally, loss of heterozygosity at 1p was seen in 7 patients (13%); this was copy neutral in 4/7 cases with reduced copy number in the other 3 cases. Rearrangements of the distal end of chromosome 1p have been described in >10% of cases of Non-Hodgkin's Lymphoma and in all 7 cases the overlapping region included 1p36 the location of the known tumour suppressor genes CHD5, ID3 and p73. Twelve out of 52 patients studied (23%) have either loss of the whole of chromosome 6 (n=2) or aUPD of chromosome 6p (n=10). This appears an early event in lymphomagenesis given that it was identified at disease presentation, progression and transformation. The sites of mitotic recombination cluster in a region immediately proximal to the MHC complex at 6p21-12 in 9/12 cases. Mutations in CCND3, CDKN1A, HLA-DQB1 and two translocation partners of BCL6; SRP20 and HIST1H4I, which are all located within or just distal of this cluster, were excluded by direct sequencing. Ten patients had aUPD of 12q, which ran from varying points on the long arm to the telomere in 9/10 cases; 1 case had an interstitial UPD of 17 Mb (95-112 Mb). *Conclusions.* This study highlights a high frequency of novel areas of aUPD in FL and the selective basis of aUPD at these locations in the pathogenesis of lymphoma continues to be investigated.

0173

INCREASED LEVEL OF B-CATENIN MRNA AND MUTATIONAL ALTERATIONS IN APC GENE ARE PRESENT IN ACUTE LEUKEMIA

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*Background.*WNT proteins initiate B-catenin-regulated canonical signaling cascade by binding to seven-transmembrane spanning receptors of the Frizzled (FZ) family,leading to phosphorylation of the disheveled protein forming a complex with the axin and APC (adenomatous polyposis coli) proteins that blocks the kinase activity of GSK3B. A functional APC protein is necessary for the proper regulation of the Wnt signaling pathway. Loss of APC expression interferes with the phosphorylation and degradation of B-catenin, allowing a B-catenin/TCF/LEF transcription factor complex to act in the nucleus, where it stimulates cell growth by targeting genes such as cyclin D1 and c-myc. *Aims.*Our aim was to determine the role of the WNT pathways in the development of acute leukemias. *Method*. We studied the gene expression levels of several components of WNT signaling pathway; WNT5A, WNT10B, FZ5, B-catenin, APC, TCF1 (T-cell factor), LEF1 (lymphoid enhancer factor) and their important targets c-MYC and CCND1 (cyclin D1), in 34 AML patient and 118 ALL patient (T-ALL, n=42, B-ALL, n=46 and preB-ALL, n=30) bone marrow and/or blood samples and normal hematopoietic cells by quantitative real time polymerase chain reaction. APC and B-catenin mutations were studied with dHPLC and sequencing methods. *Results.*Results were compared by peripheral blood samples from healthy donors by using Ct values. WNT5A, WNT10B, FZ5, B-catenin and LEF-1 showed high level of mRNA in B-ALL patients. APC expression levels in pre B-ALL samples were higher than B-ALL (p>0.001) and T-ALL (p>0.001). LEF-1 expression was found to be increased in B-cell and in preB-ALL samples and significantly different in T-ALL patients (p=0.01). Highest level of TCF-1 mRNA was observed in the T-ALL patients. c-MYC expression levels were found to be increased both in preB-ALL(p=0.02) and T-ALL (p=0.02) patients. B-ALL patients did not significantly express c-MYC gene compared to normal hematopoietic controls (p=0.3). CCND1 mRNA level was observed to be slightly increased in preB-ALL (p=0.04) but not in T-ALL and B-ALL groups . In AML FZ5 gene expression (10 fold) and LEF1gene expression (3 fold) showed a significant increase when compared to controls (p>0.01 and p=0.02 respectively). B -catenin was 5 fold higher in AML patients than normal peripheral blood samples. The WNT10b expression was slightly lower comparing to other genes. TCF1 expression level also seemed to be higher in AML patients (p=0.06). c-MYC gene expression in AML patients showed five fold increase compare to CCND1 gene expression (p>0.001). The decreased APC mRNA levels led us to investigate APC and B-catenin mutations with dHPLC and sequencing methods. Sequential alterations in APC gene were determined in 65% of AML patients and 47% of ALL patients. B-catenin mutation was detected in one ALL patients and no mutation was observed in AML patients. *Summary.*These results provide the evidence of WNT signal activation existing in acute leukemia patients and the different functions of WNT signaling through TCF/LEF between acute lymphoid and myeloid leukemias. We also believe that APC function could be the key to the pathogenesis of acute leukemias therefore we are currently studying its methylation status in our patient group .

0174

RELATION BETWEEN LOW PML-RARα LEVELS, FLT3-ITD, OVEREXPRESSION OF CXCR4 GENE AND UNFAVORABLE PROGNOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background. The quantification of PML-RARα fusion transcripts have become an important part of the routine clinical practice. Moreover, there is an increasing interest in gene expression analyses for identifying different subclasses of AML. However, few clinical studies have been carried out in this way, and there are discrepancies concerning their prognostic significance. *Aims.* 1) To determine the correlation of PML-RARα levels, FLT3 mutations and gene expression patterns with pre-treatment characteristics of 141 newly diagnosed APL patients. 2) To evaluate the prognostic significance of these alterations to identify subsets of patients at increased risk of relapse. Methods. Real-time quantitative RQ-PCR (TaqMan technology) was used. The normalized copy number (NCN) of fusion gene was reported as: number of PML-RARα copies per 1×10^4 ABL copies. Genes encoding multi drug resistance proteins (MDR1 and MRP1), the chemokine receptor CXCR4, the tyrosine kinase receptor FLT3 and growth factors upregulated in APL (HGF and FGF13) were studied. *Results.* 84 (59.6%), 5 (3.5%) and 52 (36.9%) patients showed the bcr1, bcr2 and bcr3 PML-RARα isoforms, respectively. The median NCN was 4100 with a wide range of expression (1100-35000). The low levels of transcripts (NCN<3500) were significantly associated with adverse features at diagnosis: higher WBC counts (26 vs 6×10^9/L, $p<0.0001$), higher blast cells percentage in bone marrow (86 vs 79, $p=0.006$) and in peripheral blood (55 vs 35, $p=0.002$) and elevated LDH levels (971 vs 614, $p<0.0001$). Moreover, patients with NCN<3500 showed worse overall (OS, $p=0.05$) and disease free survival (SLE, $p=0.031$), with higher relapse risk (23% vs 8%, $p=0.025$). When the cut off was established in 2500 copies, OS and DFS of patients with NCN<2500 were even worse ($p=0.01$ and $p=0.006$, respectively). Moreover, the study of FLT3 status showed an increased incidence of ITDs in the group with NCN<3500 (42% vs 5.4%, $p=0.001$), being the size of the ITD longer in them ($p=0.004$). However, no differences in expression of the FLT3 gene were observed. Interestingly, FGF13 gene was underexpressed when NCN<3500 (0.76 vs 1.17, $p=0.027$), this finding was associated with worse OS in the global series ($p=0.034$). The high expression of the CXCR4 gene was also related with shorter DFS in the overall group ($p=0.031$), but it did not explain the bad prognosis of patients with NCN<3500. The rest of genes did not show significant differences. Moreover, immunophenotypical analyses were performed: expression of CD34 and CD117 was more frequent in patients with NCN<3500 although not significant (29.6% vs 15.1%, $p=0.124$, and 100% vs 87.5%, $p=0.083$, respectively). In adition, 70% of these patients did not express CD15 ($p=0.033$). Multivariate analysis indicated that poor DFS in the overall group was related to lower expression of PML-RARα ($p=0.005$) and higher expression of CXCR4 ($p=0.033$). *Conclusions.* Our data demonstrate the bad outcome of APL patients with low levels of fusion transcript, which could be explained by the presence of adverse clinical characteristics, FLT3 mutations and different gene expression patterns. Moreover, a relationship with a more immature phenotype was found, suggesting that these leukemias have a lower ability to produce transcripts.

Hodgkin Lymphoma

0175

THE VALUE OF IMMUNOHISTOCHIMICAL EXPRESSION OF P53 AND K167 AND AGNOR IN HODGKIN'S LYMPHOMA

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Introduction. P53 tumor suppressor gene functional inactivation is a key step in carcinogenesis or progression of many human malignancies. This is due to either gene mutations, binding and inactivation, or degradation by viral or cellular proteins. Ki67 is a monoclonal antibody that detects a nuclear antigen that is strictly associated with cell proliferation. Nucleolar Organizer Regions represents loops of DNA which have the genes for ribosomal RNA, together with a number of acidic protein that have a high affinity for silver. AgNOR is used for rapid identification of NORs in light microscope sections using a simple one-step silver technique. The AgNOR proteins are considered to be a marker both for rRNA transcriptional activity and/or the DNA transcription potential. *Aim of the study.* Evaluate the P53 & Ki67 and AgNOR in Hodgkin's lymphoma cells in relation to histological subtypes to determine their role in disease prognosis. *Material and Methods.* Sixty-five cases of Hodgkin's lymphoma were diagnosed, at the Dept. of Histopathology in Medical City Teaching Hospital-Baghdad, during the period between January 1990 to December 2001. Informations regarding the clinical features, including age and sex, also the site of lymph node biopsies and histological subtype were recovered from the routine histopathological files. Lymph node biopsies stained with H & E, P53, Ki67, and Ag NOR. *Results.* Cervical lymph nodes were the most common sites of involvement in this study (67.70%) followed by axillary lymph node and generalized lymphadenopathy (9.23% each). Gender distribution according to subtypes of Hodgkin's lymphoma studied. It showed a statistically significant difference ($p<0.01$). The mean ages showed significant statistical differences among the subtypes of Hodgkin's lymphoma. Positive P53 score show statistically significant difference from negative P53 score in relation with Hodgkin's lymphoma subtypes, while intensity of P53 staining reveals no statistically significant relationship with the histological subtypes of Hodgkin's lymphoma. The study of Ki67 stain positivity in relation to gender, age, and site of lymph node involvement show no statistical significance. Ki67 stain percentage differs according to Hodgkin's lymphoma subtype. LDHL subtype have the higher mean±SD followed by NSHL (we excluded one case of stage II), and MCHL for classical HL, while NLPHL have the lowest mean±SD for all subtypes. Mean number of dots of AgNOR staining according to Hodgkin's lymphoma subtypes, showing significant differences within Hodgkin's lymphoma subtypes. We found that there is a strong direct correlation between P53 scoring & Ki67 stain percentage and a strong direct correlation between AgNOR dot numbers & Ki67 stain percentage both at $p<0.05$. *Conclusions.* P53 and Ki67 immunohistochemistry are valuable parameters in the evaluation of clinical outcome of HL subtypes in that they can be used as prognostic markers in this particular tumor for Iraqi patients. P53 and Ki67 results are going side by side almost in all cases studied and thus can be regarded as analogous as well as mutual indicators of the final clinical outcome. Accordingly, each one of them can be used alone or alternatively they can be used together as prognostic factors in the context of HL. AgNOR as a prolifrative index gives figures similar to that of Ki67. Thus, AgNOR may substitute Ki67 as a prognostic factor, especially when one takes in consideration the new methods used for AgNOR counting. The latter has helped bringing the figures of both prolifrative indices (Ki67 and AgNOR) closer together.

0176

TREATMENT APPROACH OVERPOWERS IPI PROGNOSTIC SIGNIFICANCE IN HODGKIN'S DISEASE

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Our Department has been engaged in managing more than 500 HD patients over a 25-year period. We have studied the prognostic impact that disease onset manifestations, mostly included in the IPI scoring system, but also different treatment approaches, a parameter not evaluated by the IPI concept, have on the disease course and its outcome. Of a total of 473 HD evaluable patients, 119 were with clinical stages 1-2A,

and were analyzed regardless of the disease mass (bulk). Evaluable advanced stage patients, with clinical stages 2B-4, also irrespective of tumor mass size, were 348 of them. Multivariate statistical analyses show that of the seven IPI parameters, the scoring system backbone still retains its prognostic significance: Hb and Alb levels. Patient's gender, and a slightly modified range for age (<40 vs >50) and clinical stage (eqally 3B and 4) of the disease, also remain important factors. Selective statistics reveal that this importance is valid mostly due to factor's impact on advanced stages of HD, whereas the evidently better prognosis of early stage HD patients does not seem to be affected by these parameters. The values for the chi-square tests are 45.9023 and 24.5866 for 3 degrees of freedom (gender, Hb, Alb) for the whole population and the advanced disease subset respectively, and the p-values are highly significant (0.00000 and 0.00002), when the analysis is performed on standard IPI values. Modified values achieve even higher significance and incorporate more parameters. Historical and timely stratified analysis, reveals that the IPI concept is dominantly applicable to the patients treated in the MOPP-like era. Early stage patients are also incomparable, since by default their IPI consist of a maximum of only 6 prognostic factors. Patients treated in the ABVD-like period are not influenced by all of the IPI parameters. Realizing that onset manifestations did not have statistical influence on the outcome of early stage disease patients, we extended the analysis to the post-diagnostic period. Analyzing different types of treatment engaged through the historical observation period, as well as similar approaches, it is clearly evident that these patients benefit from treatment modalities containing the gold standard and the increasingly competitive newer chemotherapy regimen: ABVD and BEACOPP. Interestingly enough, and possibly due to the low number of entries, combined modality treatment did not show significant advantage over chemotherapy alone in these early stage patients. On the other hand, advanced stage disease course with a poor IPI could not be significantly altered by employing even more aggressive treatment approaches. Rough grouping of the standardly and contemporarily treated subsets, show an evident difference of 25-40% in overall survival ($p<0.001$). Both observations clearly imply that rapid diagnosis, diminishing the possibility of disease advancement, and utilization of aggressive treatment options in the first instance, speculating that the appreciated effect is mostly attributable to the potential of doxorubicin and very possibly etoposide, are the milestones on which the successful story of HD management has been created and installed.

0177

ADDING RADIOTHERAPY TO CHEMOTHERAPY DOES NOT IMPROVE OUTCOME OF PATIENTS WITH STAGE I-II HODGKINS LYMPHOMA: RESULTS OF A RETROSPECTIVE ANALYSIS OF A REGIONAL ITALIAN EXPERIENCE

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Background. Six-eight courses of ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) represent the standard treatment for patients with advanced stage Hodgkin's Lymphoma (HL). Radiotherapy, on the other hand, forms the basis of conventional therapy for early stage HL patients. The observation of late risks of developing second tumours and coronary heart disease after radiotherapy has led to a reduced utilization of involved or extended field radiotherapy in the front line treatment. There is increasing evidence that early stage HL patients are likely to be cured by 3-6 courses of ABVD, with radiotherapy being utilized only on limited residual disease. *Methods and patients.* With the aim of comparing outcome of early stage HL patients treated with the association of chemotherapy and radiotherapy or with chemotherapy only, we retrospectively reviewed clinical features, therapy and long term outcome of 120 stage I-II HL patients diagnosed and followed in oncohaematologic divisions of Liguria (Italy) from 1995 to 2005. Fifty patients (41,7%) were treated with 3-6 courses of ABVD (CT group) and 70 patients (58,3%) were treated with chemotherapy (a median of 3 courses of ABVD or Stanford V regimen) plus involved field or extendend field-radiotherapy (CT+RT group). The two therapeutic groups were statistically comparable for median age (32 years and 31 years, for CT and CT+RT, respectively), male/female ratio, histology (nodular sclerosis in 80% and 84%; lymphocyte predominance in 12% and 9%, mixed cellularity in 8% and 7%, respectively), stage distribution (86% and 87% had stage II disease, respectively), B symptoms (42% and 38%) , bulky disease (18% and 16%). The two therapeutic groups had a different median follow up (38 and 77 months for CT and CT+RT groups, respectively). *Results.* In the CT group 44 patients achieved CR (88%) and 5 obtained PR (10%) after first line therapy. Two out of the 5 partial responders achieved CR after high dose therapy (HDT), so that 46 patients overall (96%) achieved CR. In the CT+RT group 69 patients achieved CR (98,6%) and 1 obtained CR after high dose therapy (HDT), so that the final CR rate was 100%. All relapses occurred in the first 48 months after the completion of therapy, 6 in the CT group (12%) and 8 in the CT+RT group (11,4%). At 48 months relapse free survivals are 88% and 89% in patients receiving chemotherapy only and in those treated with chemotherapy and radiotherapy, respectively. All patients of both groups are alive at 48 months. A second neoplasia has been diagnosed in 5/70 patients treated with chemotherapy and radiotherapy. *Conclusions.* The retrospective analysis of our series shows that in the vast majority of stage I-II HL patients long term control of disease may be achieved with a limited utilization of radiotherapy. The addition of radiotherapy might furthermore increase the risk of developing a second neoplasia.

0178

REFRACTORY AND RELAPSED HODGKINS LYMPHOMA: PRELIMINARY RESULTS OF THE BEACOPP REGIMEN

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Background. 25-30% of Hodgkin's lymphoma (HL) patients relapse or do not respond to first line chemotherapy. Durable responses after a second line treatment followed by peripheral blood stem cells transplantation (PBSCT) can be achieved in about 50%. Patients relapsing after PBSCT have a poor prognosis and a low probability of obtaining a subsequent durable complete remission (CR). A large and well-known experience demonstrates the efficacy of the BEACOPP regimen (doxorubicin, cyclophosphamide, etoposide, vincristine, bleomycin, procarbazine and prednisone) as first-line treatment for advaced stage HL, while no data are available for relapsed or refractory HL patients. *Aims.* To retrospectively evaluate the efficacy of the BEACOPP regimen in refractory or relapsed HL patients after first-line therapy or after PBSCT. Response rate, overall survival (OS), disease-free survival (DFS) and toxicity were analyzed. *Methods.* Eighteen HL patients, admitted between December 2005 and May 2006, were studied. Nine patients (group A) were refractory or relapsed after first-line therapies and 9 patients (group B) were refractory or relapsed after PBSCT. All patients received salvage chemotherapy with BEACOPP (4-8 cycles) at standard or escalated dose on the basis of previous treatment, medical history, disease status and the general conditions of patients. *Results.* Of the 9 group A patients, 7 were treated in first relapse and 2 were refractory to first-line treatment. Of these, 8 (89%) achieved CR, 1 patient was refractory and underwent salvage chemotherapy and PBSCT. After a median follow-up of 11 months, 7 patients (67%) are in continuous CR and 2 patients (22%) have relapsed after 18 and 11 months, respectively. One patient died in CR 12 months later due to acute leukaemia. Of the 9 patients treated after PBSCT (group B), 7 were treated after relapse and 2 were refractory. Eight patients (89%) achieved CR and 1 proved refractory: in this patient, a transformation into non-Hodgkin lymphoma (NHL) was demonstrated. After a median follow-up of 12 months (range 3-41 months), 5 patients (55%) are in CR, 3 patients have relapsed 1, 9 and 12 months off-therapy, respectively, and the patient with NHL died due to disease progression. OS and DFS are 88% and 60% for group A and 70% and 58% for group B, respectively. Summarizing, 16 of the 18 patients (89%) achieved a CR, while 2 resulted refractory. Considering the 16 responding patients, after a median follow-up of 11 months, 11 patients (68%) are in CCR, 3 patients (18%) have relapsed after 9, 12 and 18 months, respectively; 2 patients have died. OS and DFS are 80% and 60%, respectively. All patients had hematologic toxicity (WHO 3-4); one patient presented an aspergillary pneumonia during severe neutropenia and 1 patient suffered from an acute pericarditis. One patient had a congestive heart failure 10 months off-therapy. Two patients had an aseptic osteonecrosis caput femoris. *Conclusions.* Our retrospective study indicates that the BEACOPP regimen is efficacy also for relapsed and refractory HL patients, allowing to achieve a high CR rate (89%), although a longer follow-up is necessary. The BEACOPP regimen should be considered in these poor prognosis patients, especially after a PBSCT.

0179

ESHAP VS GIN AS SALVAGE AND MOBILIZING REGIMENS IN RELAPSED OR REFRACTORY HL

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Background. Patients with relapsed or refractory HL are treated with salvage chemotherapy and subsequent high dose therapy and autologous stem cell transplantation (HDT/ASCT). Salvage chemotherapy aims to disease debulking, testing of chemosensitivity , as well as mobilization of peripheral blood stem cells. Platinum-based regimens (DHAP, ESHAP, ICE) are frequently used for this purpose. However, the optimal salvage chemotherapy regimen is still not known. Recently the combination of gemcitabine, ifosphamide, vinorelbine and methylprednisolone (GIN) has been shown to be an effective salvage and mobilizing regimen in HL. *Aims.* To compare the efficacy of ESHAP (etoposide, methylprednisolone, high dose cytarabine and cis-platinum) vs GIN chemotherapy as 2nd line treatment for relapsed or refractory HL patients eligible for HDT/ASCT. *Methods.* Between 2001 and 2006 most patients scheduled for ASCT received ESHAP as first salvage (n=37), while GIN was introduced as first salvage during the last year (n=10). We retrospectively compared these two regimens regarding mobilization parameters, disease control (overall response rate) and a combined endpoint, including both successful mobilization and disease control prior to ASCT. *Results.* Patients' characteristics did not differ between ESHAP and GIN groups, except of bulky disease at relapse/progression, which was more frequent in the latter (3 vs 1 patient, p=0.047). GIN was more effective as a mobilizing regimen: peak circulating $CD34^+$ cell count was higher (median 206.2 vs 75.2, p=0.003), the number of total $CD34^+$ collected cells was higher (median 14.71×10^6/kg vs 4.32×10^6/kg, p=0.002), while all patients were successfully mobilized with GIN vs 90% in the ESHAP group. In addition, time to neutrophil engraftment following ASCT was faster with GIN (median 9 vs 10 days, p=0.02). The median time to apheresis was also shorter with GIN (12 vs 16 days, p<0.001). Response rates were similar with both regimens (60% vs 53% with GIN vs ESHAP, p=0.69). The combined endpoint of successful mobilization and disease control prior to ASCT was achieved in a similar percentage of patients with both regimens (60% vs 51%, p=0.63). *Conclusions.* GIN appears to be a more effective mobilizing regimen compared to ESHAP in relapsed/refractory HL. More patients are needed for a meaningful comparison of efficacy.

0180

PROGNOSTIC ROLE OF MUM1/IRF4 IN CLASSICAL HODGKIN LYMPHOMA

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Background. Although most cases of Classical Hodgkin lymphoma (CHL) are cured, a significant minority experiences refractoriness to treatment. The investigation of biological markers could ameliorate the predictive capacity of clinical staging systems. The aim of our study was the detection of MUM1/IRF4 expression in CHL and the correlation of its expression with different clinical characteristics and prognosis. *Methods.* Clinical data and biopsy samples from 107 patients with CHL diagnosed from 1992-2003 were enrolled in the study. 55 were males and 52 females. The median age at diagnosis was 37 years (range 13 to 79 years). Immunohistochemical staining was performed using the monoclonal antibody MUM1/IRF4 (clone MUM1p, DAKO, dilution 1:20) in paraffin embedded tissue. Fisher's-exact test, the Kaplan-Meier method, the Mann-Whitney test and the log-rank test were used for statistical analysis. Univariate and multivariate analyses were also performed. *Results.* A positive MUM1/IRF4 nuclear staining was observed in 96 cases (92.3%). H/RS cells showed moderate or strong nuclear positivity in the majority of positive cases (92.7%). MUM1/IRF4 expressing patients were less prompt to progressive disease (p<0.001), they had better time to disease progression (TTP) (p<0.001), and overall survival (OS) (p=0.03). No significant association was found between MUM1/IRF4 expression and the rest of clinical and laboratory characteristics of the patients. Univariate analysis for TTP revealed that lack of MUM1/IRF4 expression, age>45 and advanced stage (III/IV) disease were associated with significantly worse TTP (p<0.001, p=0.009, p=0.017 respectively). Univariate analysis for OS revealed that lack of MUM1/IRF4 expression, age >45, advanced stage (III/IV) disease, B symptoms and extralymphatic sites of involvement were associated with significantly worse OS (p=0.043, p=0.009, p=0.017, p=0.049, p=0.01 respectively). MUM1/IRF4 expression was an independent predictor factor for reduced TTP (p<0.001) on multivariate regression analyses. *Conclusions.* In conclusion our study has shown that MUM1/IRF4 is expressed in the majority of CHL cases and the lack of its expression in a minority of cases is associated with significantly shorter TTP and OS suggesting an association with a more aggressive clinical course. The above findings need to be confirmed in larger number of cases to demonstrate the prognostic role of this marker in CHL.

0181

CHOP21 FOR HODGKIN'S LYMPHOMA. AN EXPLORATORY EVALUATION IN COMPARISON TO ABVD AT A SINGLE INSTITUTION

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Background. ABVD remains a standard chemotherapy for Hodgkin's Lymphoma (HL) despite many efforts to prove a superiority of other regimens. Bleomycin was proven marginally active in this combination (J Clin Oncol 2004; 22:1532) but adding significant toxicity. Response to ABVD is often slow and relapse rate of 20-30% is a concern. ABVD has never been directly compared to CHOP, the other global standard for other lymphomas that is composed of agents certainly active in HL. *Aims.* Current study is an update on initial report of 2004 (Blood 2004; 104: Abstract 1311). *Methods.* In addition to extending the follow up, we compared outcome after CHOP in a pilot series of previously untreated patients with a retrospective results of ABVD therapy at our institution. *Results.* CR/CRu rates were 88% and 62% for CHOP and ABVD, respectively. In CHOP CS III-IV group, more patients had at least 3 risk factors (80%) than in ABVD CS III-IV group (40%). In contrast to ABVD, there were no deaths in CHOP group but EFS was inferior. This might result from a higher risk level in CHOP patients. A median follow up for CHOP and ABVD patients was 47 and 63 months. In a multivariate analysis of EFS the hazard ratio [95% C.I.] for CHOP vs. ABVD in CS I-II and CS III-IV patients was 2.4 [0.99, 5.8] (p=0.054) and 3.63 [1.14, 11.5] (p=0.029). Toxicity: grade 3/4 leukopenia in 9%, grade 1/2/3 peripheral neuropathy in 6% of ABVD pts., no grade 3/4 toxicity in CHOP pts. *Conclusions.* CHOP-21 is an active and low toxic regimen in HL with risk factors. A prospective comparison of CHOP with a standard chemotherapy in a randomized study will be justified.

Table 1.

0182

EXPRESSION OF ANGIOGENESIS RELATED FACTORS IN HODGKIN'S LYMPHOMA

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Background. Angiogenesis is a prerequisite for solid tumour growth and dissemination but there is relatively few data related to its significance in Hodgkin lymphoma. *Aims*. The purpose of the study was to examine the immunohistochemical expression and distribution of angiogenic and proliferation markers in Hodgkin biopsies and their relationship to clinical parameters. *Materials and Methods.* Slides from paraffin embedded lymph nodes from 62 patients with Hodgkin lymphoma were obtained from the Pathology Departments of two tertiary hospitals. Immunohistochemical staining was performed on tissues after dewaxing and rehydration with Vascular Endothelial Growth Factor (VEGF) (Santa Cruz), Hypoxia Inducible Factor 1 α (HIF1a) (Santa Cruz), Platelet Derived Growth Factor Receptor alpha (PDGFRa) and MIB-1 (Neomarker). Alkaline phosphatase polymer was used for detection of all antibodies. CD31 staining was performed and the microvessel density (MVD) was defined by identifying three hot spots at 100X magnification and counting at 400X with a graded graticule corresponding to a 0,0625 mm^2 surface area. Appropriate negative and positive controls were used. In all cases neoplastic cells, reactive background cells and endothelial cells were evaluated. A case had a score of 0 when less than 10% of neoplastic cells reacted with the antibody, 1 for staining of 10-30% of cells, 2 for 30-50% and 3 when more than >50% of cells were stained. *Results*. Immunohistochemistry showed that VEGF was negative in the neoplastic population in 53% of cases whereas 30% of cases showed strong staining (score 3). The reactive population in the lymph node biopsy was positive in over 50% of cases. HIF1a was positive in the neoplastic compartment in 31% of cases whereas PDGFRa in 95% of cases. MIB-1 was positive in 70% of cases and in the range of 20-50% of Reed Stenberg and alike cells. The MVD had a median of 2.6 which was not different from reactive lymph nodes. VEGF in the non-neoplastic compartment correlated significantly with Ann Arbor stage with increased staining in I-II versus stages III and IV (spearman rho: -0.329, *p*:0.017). Also higher VEGF score in reactive cells correlated with increased incidence of complete response (*p*:0.03). Increased MVD was associated with the presence of necrotic lesions in the material (*p*:0.05). *Conclusion.* Microvessel formation is not increased in Hodgkin in comparison to reactive lymph nodes although there is expression of angiogenic molecules by neoplastic and surrounding cells. VEGF shows a higher level of expression in earlier stages of disease.

0183

EARLY INTERIM FDG-PET SCAN IN LOCALISED HODGKIN LYMPHOMA: EVALUATION IN 5 FRENCH CENTERS

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Long-term survival from Hodgkin lymphoma (HL) in early-stage (I-II) patients is more than 85%. However, certain patients have a primary refractory disease with a worse evolution. Early interim FDG-PET scan performed after 2 courses of chemotherapy (PET-2) provides an early and accurate assessment of response and a correlation has been demonstrated between normalization of PET-2 and patient outcome. *Aims.* To evaluate the percentage of negative PET-2 in early-stage patients, and to seek clinical or biological factors predictive of positive PET-2. *Methods.* Forty-seven patients from five French centers with early-stage Hodgkin lymphoma received ABVD as first-line chemotherapy. PET-2 was performed 3 weeks after the second course of ABVD. Radiotherapy and changes in management according to FDG-PET scan result could be decided by the clinician. Evaluation was retrospective. *Results.* The median age was 33 years (range17-72). Thirty patients were male. Seventy percent of patients were in unfavorable group according to EORTC criteria (one or more of the following criteria: age > 50, systemic symptoms, elevated ESR >50 mm, bulk disease and more than three lymph node areas involved). Thirty-nine patients had a pre-treatment FDG-PET scan with a modification of staging in 6 cases. Initial staging according to CT scan or FDG-PET scan was as follows: IA: 5 patients, IB: no patient, IIA: 26 patients and IIB: 16 patients. Thirty nine patients (83%) had a negative PET-2 and 2 had minimal residual uptake whereas 6 patients (13%) had a clearly positive PET-2. Among the 39 patients with negative PET-2, 31 patients undergo radiation therapy after completion of four courses of ABVD. Among the 6 patients with positive PET-2, treatment intensification (BEACOPP) occurred for 3 patients with a negative FDG-PET scan after two courses. For patients having ABVD, FDG-PET scan results after four cycles were as follows: one remained with minimal disease, one had a negative FDG-PET scan and one had a positive FDG-PET scan. At a median follow-up of nine months, one patient with negative PET-2 relapsed early after the end of chemotherapy. The 46 other patients are in failure-free survival. Unfortunately, no clinical or biological factor (from EORTC criteria) was significantly predictive for PET-2 result. *Conclusions.* We showed in this series that negative PET-2 is obtained in 83% of patients with early stage disease. These results are similar to those expected in the EORTC H10 trial which evaluates PET-2 guided treatment adaptation and expect about 85-90% of negative PET-2. No clinical or biological factor was significantly predictive for PET-2 result. Prospective studies, like H10 EORTC trial are warranted to confirm these results and find predictive factors for a positive PET-2.

Immunology and gene therapy

0184

EARLY RECOVERY OF THYMUS-DERIVED NAÏVE T CELLS AND OF NK CELLS IN PEDIATRICS PATIENS AFTER T-CELL DEPLETED HLA-HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOR THALASSEMIA

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Background. Delayed immune recovery post transplant remains a significant obstacle and results in increased risk of infections. T cells are regenerated via 2 pathway, thymus-derived and peripheral expansion, processes for which IL-7 is critical. *Methods.* To analyse the mechanisms involved in immunological reconstitution, we studied six thalassemia patients after 20 and 60 days post T-cell-depleted HLA-haploidentical stem cell transplantation. The mean age ranged from 14 to 5 years. As controls, 6 healthy donors matched by sex and age with the patients were included. We analysed T cell subsets by flow cytometry. Stromal cells, obtained from long term culture of bone marrow mononuclear cells were analysed by immunohystochemistry and the stromal IL-7 production was analysed by ELISA. *Results.* Day + 20 post transplant, the patients had significantly lower $CD4^+$ T cells in comparison to the controls (1.9±1.4% vs. 47.5±6% respectively), and this reduced number was mainly observed in $CD45RA^+CD62L^+$ (naïve phenotype) subset (1.3±2% in patients vs. 52±12% in controls). A significant decrease of peripheral $CD45RA^+CD31^+$ Th cells (thymic naïve Th cells) (on average 0.5±0.3% in patients vs. 37±10% in controls) was observed, whereas $CD8^+$ T cells numbers did not statistically differ between patients and controls (24.2±33.7% vs. 20±7%). NK cells were among the first lymphocytes to repopulate the peripheral blood, and up to 70% of these cells were CD56 bright whereas CD56dim $CD16^+$ NK cells were reduced. Day + 60 post transplant an increase in the percentages of $CD4^+$ T cells, naïve $CD4^+$ cells and in thymic naïve Th cells were observed (3±1.2%, 2.9±2.1%, 2.7±1%, respectively). $CD8^+$ T cells were also increased (in mean 35±27.5%). Compared with normal subjects, thalassemia patients showed a significant increase of $CD4^+$ cell activation markers (CD95, HLA-DR and CCR5) and this was observed after 60 days post transplant, in parallel with the increase of the $CD56^{dim}$ $CD16^+$ NK cells especially in the patients with full engraftment. Stromal cells secreted lower IL-7 levels (0.3+0.1 pg/mL vs. 0.8+0.1 pg/mL, in controls) and displayed by immunohistochemistry an altered phenotype (macrophage-like morphology). *Discussions*. A significant decrease in total lymphocyte counts and depletion of $CD4^+$ T cells expressing predominantly the $CD45RA^+CD62L^+$ phenotype were observed after 60 days post transplant. Also the $CD4^+CD45RA^+CD31^+$ T cell subset was initially reduced but an increase has been observed at day + 60 post transplant, suggesting a thymus involvement in these patients. An IL7/IL7R pathway dysregulation has been also observed, possibly involving bone marrow stromal cells. NK cells were among the earliest lymphocytes to repopulate the peripheral blood, but. $CD56^{dim}$ $CD16^+$ NK cells were increased after 60 days post transplant, especially in the patients with full engraftment, suggesting a role of donor NK cells on bone marrow engraftment. We hypothesize that the recovery of T cell compartment may be due to an altered production of new T cells starting from the haematopoietic stem cells under the influence of stromal cytokines production.

0185

VACCINATION WITH DENDRITIC CELLS: CELLULAR IMMUNE MODULATION BY LEUKOCYTAPHERESIS IN PATIENTS WITH METASTATIC MALIGNANT MELANOMA

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Background. Monocyte-derived Dendritic Cells (DC) are promising tools for the cellular immunotherapy of cancer (Banchereau et al, Nature Rev Immunol 2005; 5:296-306). Monocytes are, however, immune competent cells and consist of different subpopulations (Gordon et al, Nature Rev Immunol 2005; 5:953-964) with different physiological roles (Clancy *et al.,* J Leukoc Biol 2006; 79:757-766). However, no data is available yet considering the impact of leukocytapheresis on monocyte subpopulations in patients with metastatic malignant melanoma. *Aims.* Leukocytapheresis is a stimulus for recruitment of distinct monocyte subpopulations in patients and the enrichment of monocytes in apheresis products. The focus is on the main population of $CD14^{++}$ monocytes and the subpopulations of $CD14^+CD16^+$ monocytes, CD33dim$CD16^+$ monocytes, and CD33bright monocytes. The impact of the apheresis procedure on these important monocyte subpopulations was demonstrated. *Methods.* 18 patients with metastatic malignant melanoma and 22 healthy blood donors were investigated before and after leukocytapheresis procedures. Additionally, monocyte apheresis products (MAP) of patients were compared with the results of healthy blood donors. The percentage of monocyte subpopulations were investigated by flow cytometry (FACS Calibur, BD). The impact of the apheresis procedure was demonstrated by calculation of the recruitment factor (RF). Calculation formula: RF = (postdonation cell count + cell yield) / predonation cell count. *Results.* Monocyte subpopulations showed different enrichment in patients, in healthy blood donors and in MAP. In patients (n=18) with metastatic malignant melanoma the $CD14^+CD16^+$ monocytes increased by 57% (p=0.002) in the postdonation count. Additionally, the CD33dimCD16+ monocytes increased by 40.3% (p=0.002) whereas the same cell population in healthy blood donors decreased by 12% (p=0.079) in blood counts after apheresis procedure. $CD14^+CD16^+$ monocytes were enriched in MAP by factor 2 (4.99±2.53% vs. 10.14±6.71%, p<0.0001). The pre- and postdonation counts of CD14+CD16+ monocytes in donors (n=22) differed significantly (4.8% vs. 5.3%, p=0.001). Significant differences between the RF of $CD14^{++}$ monocytes and $CD14^+CD16^+$ monocytes could be demonstrated (p=0.003). The calculated RF of CD33bright monocytes differed significantly from the RF of $CD14^+CD16^+$ monocytes (p=0.003) and CD33dim$CD16^+$ monocytes (p=0.016). Between the RF of $CD14^+CD16^+$ monocytes and the RF of CD33dim$CD16^+$ monocytes no significant difference was detectable. *Summary and Conclusions.* These results suggest a different cell recruitment of monocyte subpopulations in patients with metastatic malignant melanoma compared to healthy blood donors. $CD14^+CD16^+$ monocytes and $CD33^{dim}CD16^+$ monocytes were increased in patients with metastatic malignant melanoma after leukocytapheresis. The leukocytapheresis procedure itself is a cellular immune modulation because of both, the removal and the different mobilization of distinct monocyte subpopulations. This immune modulation may have implications for the development of cellular therapies with DC and may impact on the results of vaccination with DC.

Figure 1.

0186

PROGNOSIS AFTER UNMANIPULATED HLA HAPLOIDENTICAL BLOOD AND MARROW TRANSPLANTATION IS CORRELATED TO THE NUMBERS OF KIR LIGANDS IN RECIPIENTS

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Background and Aims. The goal of this study was to explore the role of NK cell alloreaction in predicting prognosis under unmanipulated HLA-haploidentical blood and marrow transplantation and examine whether the presence of any individual donor-activating KIR gene had an influence on the clinical outcome. *Methods.* We studied the HLA and KIR genotype of 64 donor-recipient pairs, who underwent transplantation. *Results.* In contrast to Perugia's KIR ligand-ligand mismatch model or Handgretinger's KIR receptor-ligand mismatch model or Bignon's KIR gene-gene mismatch model between donor-recipient pairs, we found that the cumulative incidence of 3-year disease-free survival (DFS), overall survival (OS), and transplantation-related mortality (TRM) were best predicted by the number of KIR ligands carried by patients (HR 0.355, 95%CI, 0.186-0.678, p=0.002 for DFS; HR 0.445, 95%CI, 0.233-0.848, p=0.014 for OS; HR 0.450, 95%CI, 0.219-0.926, p=0.030 for TRM). Moreover, an analysis of KIR ligand numbers was found to be correlative in patients with lymphoid malignancy. The KIR ligand-ligand mismatch model is a good predictor of acute graft versus host disease (aGVHD, HR 3.812, 95%CI, 1.667-8.720, p=0.002). Meanwhile, the presence of donor-activating KIR2DS3 also contributed significantly to acute (HR 2.967, 95%CI, 1.265-6.958, p=0.012) and chronic GVHD (HR 2.541, 95%CI, 1.127-5.730, p=0.025). *Conclusions.* These data indicate that prognosis after transplantation is associated with the numbers of KIR ligands in recipients and T cell alloreaction may play a predominant role in this model.

0187

DENDRITIC-LEUKEMIA CELL HYBRIDS GENERATE SPECIFIC ANTI LEUKEMIA CTLS *IN VITRO*

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Background. Allogeneic stem cell transplantation (alloSCT) contributes significantly to better disease control in patients diagnosed with high risk AML. . Nevertheless, a significant proportion of patients suffer from recurrence of the disease. For those high risk patients, novel therapeutic strategies based on cellular immunotherapy have been explored to improve the clinical outcome of alloSCT. However, in most cases the leukemia specific antigens are unknown, and hence the cellular or non-antigen specific immunotherapy is based preliminary on the administration of allogeneic, donor derived T lymphocytes (DLI), aiming to induce a clinically significant graft-versus-leukemia responses. *Aims.* The aim of our study was to induce a potent and specific anti-leukemia cytotoxic T lymphocyte (CTL) response, utilizing dendritic-leukemia cell hybrids, to treat leukemic relapse in patients after alloSCT. Such fusion cell vaccine has the advantage of presenting both known and unidentified leukemic antigens, in the context of co-stimulatory signals. *Methods and Results.* Purified human monocyte-derived dendritic cells (DCs) were isolated from peripheral blood mononuclear cells of 5 healthy HLA-identical stem cell donors. Immature DC were successfully fused with recipients irradiated leukemic cells utilizing polyethylene glycol (PEG), and underwent further maturation in the presence of TNF-α , IL-6 , IL-1β , and PGE2. Fused population of DC-leukemia was estimated by flow-cytometry using two membrane incorporated fluorescent dyes, and DAPI stain was utilized to confirm the true presence of DC - leukemia cell hybrids. Generation of leukemia specific donor CTLs was performed by co-culture of donor mononuclear cells with irradiated DCs-leukemia hybrids under IL-2 deprivation. T cells were then further expanded in culture, and tested for their specific in-vitro cytotoxic activity against the leukemia cells that was utilized as fusion partner by LDH cytotoxicity colorimetric assay. In 4 out of 5 cases we were able to demonstrate a significant and specific cytotoxic activity of the hybrids-primed CTLs against the patients leukemic cells. *Conclusions.* Our results clearly demonstrate that the hybrid vaccination approach in AML is technically feasible. Such specific anti-leukemic donor CTLs may be utilized to maximize the anti-tumor effects of DLI in patients relapsing after allogeneic transplantation.

0188

TOLL-LIKE RECEPTOR EXPRESSION ON TRANSPLANTED T-CELL SUBSETS INFLUENCES OUTCOME AFTER ALLOGENEIC UNRELATED STEM CELL TRANSPLANTATION

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Introduction. Recently, Toll-like receptor (TLR) 2 and 4 have been identified as the most important receptors for LPS, which is contained in the cell wall of gam-negative bacteria and is known to be a main inducer of graft versus host disease (GVHD). The role of TLR expressing T-cells within the graft for the induction of GVHD in patients after unrelated peripheral blood stem cell transplantation (PBSCT) is unknown. *Methods and patients.* We therefore determined by flow cytometry TLR expression on T-cells within the graft of 63 patients receiving unrelated PBSCT after intensive conditioning followed by cyclosporine A and methotrexate as GVHD prophylaxis. Additionally, donor specific single nucleotide polymorphisms (SNP) for TLR2-R753Q, TLR4-D299G and TLR4-Y135A were determined. The data were finally correlated with clinical endpoints. *Results.* As expected, TLRs were not significantly expressed on T-cells in peripheral blood of healthy donors (TLR 2: <1.0%, TLR4 <0.5% of T-cells, n=6). In contrast we detected a distinct up-regulation of these receptors on T-cells within the grafts. TLR2 and TLR4 expression on $CD4^+$ T-cells ranged from 1.2%-12.3% (median 3.1%) for TLR2 and from 1.2%-12.0% (median 3.7%) for TLR4. Among the $CD8^+$ T-cells 0.9%-16.1% (median 3.3%) expressed TLR2 and 0.7%-13.3% (median 3.5%) expressed TLR4. The SNP for TLR2-R753Q and TLR4-D299G was found in 8.6% and 10.3% of the allogeneic donors, respectively but did neither correlate with the expression levels of TLR on T-cells nor with clinical endpoints. Treatment-related mortality from infections was observed in 10 patients (16%). Interestingly, higher expression of TLR2 and 4 on $CD4^+$ but not $CD8^+$ T-cells was significantly associated with an increased cumulative incidence of fatal infections (38% vs. 8% p=0.03 for TLR2 and 37% vs. 9% p=0.007 for TLR4). Neither the overall $CD3^+$, $CD4^+$ and $CD8^+$ cell dose nor the expression of TLR2 and 4 on $CD4^+$ and $CD8^+$ T-cells showed a significant association with the incidence of acute or chronic GVHD or relapse. *Conclusions.* These data suggest a previously unrecognised up-regulation of TLR on $CD4^+$ and $CD8^+$ T-cells contained in G-CSF mobilized apheresis products. Whether these phenotypic changes impact on T cell function or patient outcome warrants further investigation.

0189

GENETIC LABELING OF HUMAN CD34-POSITIVE HEMATOPOIETIC PROGENITOR CELLS AND MESENCHYMAL STEM CELLS BY HIGHLY EFFICIENT MRNA-BASED GENE TRANSFER

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Transfection is an essential tool for numerous *in vitro* applications including studies of gene expression, promoter analysis, intracellular signaling pathways and therapeutic strategies such as tissue engineering and gene therapy. Furthermore, genetic labeling of HPC and their consecutive fate-mapping *in vivo* is an approach to answer intriguing questions in stem cell biology. We recently reported an efficient transient genetic labeling of human $CD34^+$ HPC by nucleofection with the truncated low affinity nerve growth factor receptor (deltaLNGFR) that is nowadays accepted for human *in vivo* application. In this study we optimized this method regarding transfection efficiency and viability loss by transfering *in vitro* transcribed mRNA in human $CD34^+$ HPC and MSC and compared the data with samples transfected with plasmid DNA. The marker gene deltaLNGFR was *in vitro* transcribed to mRNA or was cloned in the pVAX1 plasmid. Cells were transfected with LNGFR-encoded mRNA or plasmid DNA using nucleofector technology (amaxa system). Marker gene expression was assessed using antiLNGFR antibody by flow cytometry over a time periode of 10 days. Nucleofection of $CD34^+$ HPC and MSC with mRNA of the marker genes deltaLNGFR and EGFP resulted in transfection efficiencies up to 85% or 91% one day after nucleofection, respectively, without a significant viability loss (72% or 68% viable cells, respectively). In contrast, introduction of plasmid DNA caused a decrease in viability with a gene expression of only 50%. Transgene expression declined background levels 6 days after transfection, showing no differences between mRNA and plasmid transfection.

Cell viability was not affected by mRNA-transfection. Moreover, differentiation assays of deltaLNGFR-selected MSC after transfection, showed that differentiation of MSC into mesenchymal cells like chondrocytes, adipocytes and osteoblasts was not affected by mRNA nucleofection. Taken together, nucleofection is a powerful, highly efficient and non-toxic approach for transient labelling of human progenitor cells. This transfection method is significantly more efficient using mRNA instead of plasmid DNA. Nucleofection with mRNA might be a useful tool to transiently manipulate stem and progenitor cells without inducing cell toxicity.

0190

SELECTIVE DEPLETION OF ALLOANTIGEN-REACTIVE T CELLS BY TRYTOPHAN CATABOLITE 3-HYDROXYANTHRANILIC ACID

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Background. Indoleamine 2,3-dioxygenase (IDO) is a potent immunoregulatory enzyme that converts the essential amino acid tryptophan to catabolic products collectively known as kynurenines. Generation of tryptophan-derived catabolites by IDO is an important mechanism in IDO-induced T cell tolerance. *Aims.* We show the downstream molecular mechanism of the selective depletion of alloantigen-reactive T cells by tryptophan catabolite 3-hydroxyanthranilic acid (3-HAA). *Methods.* Peripheral blood mononuclear cells (PBMCs) were labeled with CFSE to distinguish between alloantigen-reactive T cells and resting T cells. CFSE-labeled PBMCs were cocultured with allogeneic cells in the presence or absence of 3-HAA. FACS analysis was performed to measure the cell cytotoxicity, intracellular ROS generation, and GSH levels. Lethally irradiated BDF1 mice were transplanted with T cell-depleted bone marrow plus splenocytes form B6 donors. Recipients were intraperitoneally treated with 3-HAA or with control solvent. *Results.* 3-HAA selectively depleted alloantigen-reactive T cells by inducing apoptosis *in vitro*. Treatment with 3-HAA markedly increased intracellular reactive oxygen species (ROS) generation by depleting intracellular glutathione (GSH) in alloantigen-reactive T cells. Replenishment of GSH with 2-mercaptoethanol (2-ME) and N-acetylcysteine (NAC) completely protected against selective T cell depletion by 3-HAA. Administration of 3-HAA in mouse prevented GVHD by selectively depleting alloantigen-reactive donor T cells through apoptosis. *Conclusions.* 3-HAA enhances intracellular ROS generation of depleting GSH in activated T cells, thus resulting in selective depletion of alloantigen-reactive T cells *in vitro* and *in vivo*. Our data suggest that tryptophan catabolites, especially 3-HAA, could represent a novel target for the development of new drugs for the treatment of transplantation rejection.

0191

IMPROVING THE CLINICAL APPLICABILITY OF CHIMERIC-RECEPTOR TRANSDUCED T CELLS: CD20 AS A MARKER FOR SELECTION, TRACKING AND ANTIBODY-MEDIATED KILLING OF TRANSDUCED CELLS

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Background. an emerging new tool for cancer immunotherapy is the infusion of T lymphocytes transduced with chimeric-receptors that redirect them against molecules expressed on the surface of cancer cells. The clinical applicability of this approach could be further augmented by effective methods to enrich, track and eliminate genetically modified cells. *Aims.* we tested whether a chimeric-receptor directed against CD19, a molecule widely expressed in B-lineage acute lymphoblastic leukemia (ALL) and B-cell non-Hodgkin lymphoma, could be co-expressed with CD20. This would offer a means to select transduced cells *ex vivo* and eliminate them *in vivo* using anti-CD20 antibodies. *Methods.* a MSCV retroviral vector containing the anti-CD19-CD28-zeta-IRES-CD20 insert (M-CD19-CD28-zeta-I-CD20) was generated by substituting the GFP gene with the human CD20 gene in the anti-CD19-CD28-ζ-IRES GFP vector. The CEM-C7 and Jurkat cells were then transduced by incubation with retroviral supernatant on retronectin-coated tube. Three days after transduction the expression of chimeric receptor and CD20 has been evaluated by flow cytometry and $CD20^+$ cells were then immunomagnetically purified. *Results.* CEM-C7 and Jurkat cells were efficiently transduced with the M-CD19-CD28-zeta-I-CD20 vector. After transduction, the anti-CD19 receptor was expressed by 11% of CEM-C7 and by 32% of Jurkat cells. Although levels of expression were weaker than that achieved with constructs lacking CD20, the receptor was functional, as shown by the induction of IL-2 transcripts after exposure of transduced Jurkat cells to the CD19+ALL cell line OP-1. Expression of CD20 was detected on 35% of CEM-C7 and on 63% of Jurkat cells; after immunomagnetic cell-sorting with an anti-CD20 antibody over 95% of sorted cells expressed the anti-CD19 receptor. Experiments to determine whether exposure to the clinical anti-CD20 antibody Rituximab can eliminate the transduced cells, and the relative potency of radioisotope-conjugated anti-CD20 antibodies are planned.

0192

DNA VACCINE AND ALL-TRANS RETINOIC ACID COMBINED TREATMENT ELICITS SPECIFIC AND PROTECTIVE CELLULAR IMMUNE RESPONSES IN A MOUSE MODEL OF PRE-ESTABLISHED ACUTE PROMYELOCYTIC LEUKAEMIA

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Background. DNA vaccines can be effective in the acquisition of humoral and cell-mediated immune responses. Our studies on an acute promyelocytic leukemia (APL) mouse model show that DNA vaccination combined with all-trans retinoic acid (ATRA) results in a survival advantage with a significant increase in the Th1 cytokine IFNg (Padua *et al.,* Nat. Med. 9:1413, 2003). ATRA alone can act as an adjuvant to induce immune responses as measured by an increase in anti-RARa antibody production, which correlated with improved survival in mice. Similar increases in antibody production have been observed in our patients after maintenance therapy (Robin *et al.,* Blood 108:1972,2006). *Aim.* 1) To use immunomonitoring and functional assays to evaluate the presence of activated T-cells and to demonstrate APL-specific killing. 2) To determine if the protective effect of DNA vaccination is $CD4^+$ mediated. *Methods.* Cytokine release was measured using a cytokine bead array kit. Using an APL transplant model in FVB/N mice, CD107a, expressed on the surface of lytic granules of activated T-cells, was measured by flow cytometry. A flow based CFSE assay was used to measure APL specific cell killing by cytotoxic T-cells (CTLs). As FVB/N mice have H2q haplotyes, blocking anti-H2q antibodies were used to determine if the cytotoxic activity was MHC restricted. Immunophenotyping by measuring $CD4^+$ and $CD8^+$ absolute counts were conducted. Mice injected with APL cells were depleted of $CD4^+$ cells with anti-CD4 antibody treatment and assayed for efficacy of the DNA+ATRA combined therapy. *Results*. In long-term survivors, Th1 cytokines TNFa and IFNg were increased and specific activated CD3/CD8 T cells were detected and observed to release cytotoxic granules in the presence of APL cells. A dose dependent decrease in CFSE positive cells was observed assaying effectors from spleens of ATRA alone, ATRA+DNA treated mice and CD107a+ sorted cells from the latter using APL cells as targets. This effect was MHC restricted as anti-H2q antibodies reduced the specific cytotoxic activity. $CD4^+$ absolute numbers of non-responders (NR) (died before 80 days) and responders (R) (survived more than 80 days) showed a significantly higher number of $CD4^+$ cells in the latter compared with the former on day38 (p=0.007). The DNA +ATRA treated mice died earlier in the CD4+ depleted mice compared with the undepleted animals. *Summary and conclusions.* In the long-term survivors, the presence of activated T-cells and MHC restricted APL specific cell killing was detected. An increase of Th1 cytokines is indicative of DNA effects. DNA vaccination requires CD4+ cells for efficacy as there was no extension of lifespan in DNA treated mice in the $CD4^+$ depleted FVB/N. This correlates with the observation that on days 38 of the protocol following transplant of APL there is an increase in $CD4^+$ absolute numbers in the Rs compared to NRs. These data are consistent with an increase in anti-RARa antibody production previously measured in other protocols. Therefore we have been able to detect protective cellular and humoral responses in mice with the combined treatment of DNA+ATRA, which correlates with outcome.

0193

IMMUNE RECONSTITUTION OF THE T CELL COMPARTMENT IN HEMATOLOGICAL MALIGNANCIES FOLLOWING ALLOGENEIC NON MYELOABLATIVE HEMATOPOIETIC CELL TRANSPLANTATION

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Background and Aims. Allografting is a potentially curative therapy for a variety of hematological malignancies. However, relapse and treatment-related toxicity are major obstacles to cure. Reduced-intensity/non-myeloablative conditionings 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 medically unfit or elderly patients (up to 70 years). However, the risk of post-transplant infections and the efficacy of graft-versus-tumor effects also rely on the thymic function, potentially reduced with age. For this reason it is mandatory to evaluate the residual thymic function. *Material and methods.* The immune recovery of the T cell compartment was evaluated by flow cytometry in 66 patients with hematological malignancies, median age 54 years (range 34-64), conditioned with low dose TBI (200 cGy), with/without fludarabine (90 mg/m^2 total), followed by G-CSF mobilised peripheral blood stem cell infusion from HLA identical siblings. The analyses were performed at different timepoints: 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 red cell depleted and stained with four-colour combinations with 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 timepoints. *Results*. Peripheral CD4+ T cells to >200/ul promptly recovered by day +28 with median values of 274/uL, gradually increasing to 474/ul, 682/uL, and 964/uL, at 1, 3 and 6 years, respectively. Naïve CD4+CD45RA+bright T cells increased to 49/uL, 66/uL, 122/uL, at day +28, and at 2, 6 years, respectively. Memory CD4+CD45R0+bright remained stable with median values of 153/ul and 128/uL from day +28 through month +3, respectively; then increased to 234/uL, 332/uL, 529/uL, at 1, 2, 6 years. The evaluation of the coexpression of the CD45 isoforms showed that the number of CD4+CD45RA+CD45R0+ T cells reached median values of 63/ ul by day +28 and 73/ul at 6 months; then increased to 128/ul, 191/ul, 237/ul at 2, 5, 6 years, respectively. CD8+ T cells reached median values of 156/ ul by day +28, increasing to 445/uL, 880/ulL, at 6 months, and at 4 years, respectively. CD4/CD8 ratio was 1.8 by day +28, decreased to 0.57 at 1 years, and then increasing at 0.9 at 4 years. In a subset of 35 patients the presence of naïve 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 4 years with a median value of 836/ul, and 433/ul, respectively, while they remained stable at 5 and 6 years. TREC copies/100 ng DNA from peripheral mononuclear cells and sorted CD4 cells were measured in 52 and 46 patients, respectively, at the same timepoints: median baseline value from PBMC was 0.5, then it gradually increased to 2.6 at 1 years, reached 53.7 at 5 years, and remained stable at 6 years. A significant correlation was demonstrated between TREC values from PBMC and CD4+CD62L+CD45RA+bright T cells (p<0.00003). *Conclusions.* Our findings suggest a slow T immune reconstitution during the first two years post-transplant that differs from normal T lymphocyte ontogenesis. Preliminary results show a significant correlation between the quantitative analysis of TRECs and the analysis of very naïve T cells by CD62L expression and allow to quantify the residual thymic function in this group of elderly patients.

0194

USING HSV-TK SUICIDE GENE TRANSFER TO IMPROVE THE SAFETY OF DLI

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Background. The possibility to manipulate the graft, e. g. to select CD34 positive cells or to deplete CD3 positive cells, has lowered the incidence of acute graft versus host disease (aGvHD) occurring after hematopoetic stem cell transplantation (HSCT). But this beneficial effect has its drawback in high relapse rates, increased infection rates, and a higher risk of graft failure due to the lack of T-cells. To circumvent these problems, donor lymphocyte transfusions (DLI) are given to the patients. *Aims.* Transfusion of unmanipulated donor T-cells can lead to aGvHD even after 100% donor chimerism is achieved. In order to have the benefit of DLI and lower the risk of aGvHD, the DLI can be transduced with a suicide gene (e.g. the herpes simplex virus thymidine kinase HSV-TK) in order investigate the possibility of eliminating severe aGvHD by induction of the suicide mechanism using ganciclovir (5 μg/kg BW). Safety and feasibility of this technique were investigated in a set of 9 patients in our institute. *Methods.* 9 patients, 7 with AML and 2 with CML, were enrolled in the study. They were transplanted from HLA-identical sibling donors with CD34-enriched stem cells without further immunosuppression. Donor-T-cells were transduced with the replication-deficient retrovirus SFCMM-3, which expresses HSV-TK as a suicide gene and the truncated version of the low affinity nerve growth factor receptor ('LNGFR) for selection purposes. In the follow up blood samples of the patients were collected at certain intervals and the transduced cells were tracked by flow cytometry and PCR. In addition, reconstitution of the TCR repertoire was checked by spectratyping. *Results.* After transfusion, SFCMM-3 transduced T-cells were detectable in all patients by PCR and FACS-analyses immediately after transfusion and during the follow up period (range: 1.1-3.8 years). One of 9 patients developed aGvHD of the skin, grade 1, at 56 days after the transfusion of the transduced cells. In a patient positive for bcr-abl, bcr-abl gene expression was not detectable any more after an expansion of transduced cells. Donor chimerism was stabilized after transfusion of the transduced cells in all patients treated. Spectratyping showed a normalisation of the TCR repertoire over time, additionally the transduction apparently did not cause severe skewing in transduced donor cells. To date, 8 of 9 patients are alive and well. *Conclusions.* The administration of DLIs transduced with a suicide gene showed no negative side effects so far. Therefore, a phase II clinical trial in an haplo-identical setting using this technique has been started at our institution.

0195

MINOR HISTOCOMPATIBILITY ANTIGEN ALLOREACTIVITY: CLINICAL RELEVANCE OF FUNCTIONAL TESTS

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Background. Minor Histocompatibility (mH) antigens alloreactivity play a determinant role in Graft versus Host Disease (GvHD) and Graft versus Leukemia effect (GvL) after HLA-identical hemapoietic stem cell transplantation. *Aims.* We investigated the impact of alloreactivity against minor antigens on GVHD and relapse from donor versus recipient or versus current identified mH epitopes measured in blood samples until 3 months or later after allogeneic transplantation. *Methods.* 27 patients transplanted with an HLA compatible donor were studied for mismatch in un mismatch en mHA parmi HA-1 HA-2 HA-3 HA-8 HB-1 ACC-1 UGT2B17 H-Y HwA-9 HwA-10. Function against recipient of T lymphocytes after transplant was studied by ELISPOT (IFN γ) and the detection of T lymphocyte performed by flow cytometry using multimere HLA-minor antigen derived peptides. Biological findings were correlated with clinical data : GVHD or risk of relapse (Graft versus leukemia). *Results.* Alloreactivity, during the 3 first months is associated with acute GvHD and GvL (p″0.015). Functional response measured by ELISpot IFN-γ against mH epitopes is also relevant to acute GvHD and GvL (p″0.046) but the presence of specific T-cells measured by multimers HLA/peptides is not. Correlation of early alloreactivity and risk of relapse appears strong since the median post transplant follow-up for patients reach 24 month. Donor versus recipient reactivity observed next to the third month is associated with chronic GvHD and has a positive predictive value of 91% on GvL (p″0.01). Nevertheless the response observed individually against mH epitopes by ELISpot assay beyond 3

month is not relevant to chronic GvHD or GvL. The presence of mH epitope specific T-cells is also not linked to the GvHD but very interestingly is associated to the GvL effect (GvL $p''0.01$). *Conclusions.* Our results suggest that monitoring of mH responses on a restricted panel of antigens could have interest very early following hemapoietic stem cell transplantation. This a could give arguments for early therapeutic intervention for patients with a high risk of relapse and when no detectable alloreactivity could be observed during the first 3 months post transplant.

0196

HIGH ALLOREACTIVE POTENTIAL OF SUICIDE GENE EXPRESSING CENTRAL MEMORY T LYMPHOCYTES CULTURED WITH HOMEOSTATIC Γ-CHAIN CYTOKINES FOR THE CURE OF HEMATOLOGICAL MALIGNANCIES

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Background. Alloantigen targeting adoptive immunotherapy is a powerful therapeutic approach to increase the graft-versus-leukemia (GvL) effect mediated by allogeneic hematopoietic stem cell transplantation, for the cure of hematological malignancies. Although alloreactivity mediated by donor lymphocytes plays a crucial role in treating and preventing disease relapse, its extensive exploitation is limited by the risk of a life-threatening complication: Graft-versus-host disease (GvHD). To solve this double bind, we've investigated the therapeutic potential of donor lymphocytes retrovirally transduced to express the suicide gene thymidine kinase of Herpes Simplex virus (TK cells) in patients affected by leukemia, and showed that the infusion of TK cells induces GvL and allows to control severe GvHD by ganciclovir (GCV). Initial clinical experience showed that infused TK cells have a reduced alloreactivity and are mainly $CD45RA^-/CD62L^-$ effector memory (EM) T cells, known to have limited survival potential. This functional phenotype is possibly due to the *ex vivo* manipulation based on the enforced expansion of lymphocytes by polyclonal stimulation (soluble anti-CD3 antibodies) and high concentration of IL-2. *Aims.* The aim of the study is to maximize the alloreactive potential of TK cells for the cure of leukemia, while controlling severe GvHD. *Methods.* We hypothesized that culture with anti-CD3/CD28Ab coated magnetic beads (3/28b) and homeostatic γ-chain cytokines may allow to fully maintain alloreactivity on TK cells, while permitting retroviral transduction. We tested IL-7, a central regulator of the survival and maintenance of naïve and memory T lymphocytes, and IL-15, a regulator of the initiation, clonal expansion, contraction, and maintenance of memory cells. We used the SFCMM3 vector for T cells transduction. Phenotypes, antigen reactivity, and survival of TK cells were analysed *in vitro* and *in vivo*; using a murine model based on TK cells infusions in NOD/Scid mice, previously transplanted with allogeneic human skin. *Results.* The combination of 3/28b, IL-7 and IL-15 generated high numbers of $CD45RA^-/CD62L^+$ central memory (CM) TK cells with preserved CD4/CD8 ratio, a g-IFN/IL-2 secretion profile, and persistent expression of high levels of IL-7R-α, a molecule associated to memory lymphocytes survival. In mixed lymphocytes cultures, CM TK cells showed higher alloreactivity than EM TK cells, and maintained a CM phenotype after multiple allogeneic stimulations. Moreover, CM TK cells were eliminated by GCV as efficiently as EM TK cells. *in vivo,* infused CM TK cells were engrafted and expanded more than EM TK cells, showing preserved CD4/CD8 ratio and persistent expression of high levels of IL-7R-α. Most importantly, CM TK cells were more potent than EM TK cells in inducing both xenogeneic and allogeneic GvHD, as documented by higher engraftment and a more extensive infiltration of TK lymphocytes in the allogeneic human skin (grade 3 vs grade 1 allo-GvHD). *Summary.* This study shows that CM TK cells, generated by CD3/CD28 activation and culture in the presence of homeostatic cytokines combine a high alloreactive potential with the selective sensitivity to GCV-mediated cell death, providing a tool for maximal anti-leukemia activity and controlled GvHD.

0197

SELECTIVE DEPLETION OF ALLOREACTIVITY AND PRESERVING OF ANTI-TUMOR ACTIVITY OF SPECIFIC T CELL CLONES IN PATIENS WITH LEUKEMIA AND RENAL CARCINOMA

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Background. A major challenge in the field of hematopoietic stem cell transplantation (HSCT) is to prevent the alloreactivity of donor T-cells which leads to acute graft-versus-host disease (GVHD) while preserving graft-versus-tumor (GVT) effect. GVHD is leading cause of morbidity and mortality after HSCT. *Aims.* Selective depletion using anti-CD25 immunotoxin (IT) can eliminate harmful alloreactive T-cells while preserving other donor T-cells with antileukemic/antitumor reactivity. *Methods.* We have used irradiated peripheral blood mononuclear cells (PBMC) from cancer patients and healthy donor PBMC as responder cells in primary mixed leukocyte reaction (MLR).To prepare GVL/GVT-specific T-cells, alloreactive T-cells in primary MLR were depleted with anti-CD25 IT. The remaining T-cells had insignificant alloreactivity in secondary MLR. Allodepleted donor cells were then repeatedly stimulated using purified leukemia/tumor cells from the same cancer patient. Leukemia/tumor-reactive donor T-cells were purified immunomagnetically on the basis of INF-γ production. *Results.* 17 MLRs (10 with leukemic and 7 with renal carcinoma cells) were performed. Selective depletion of alloreactive donor T-cells with anti-CD25 IT led to more than 2log depletion. Graft-versus-leukemia (GVL) effect of donor T-cells was well preserved while the graft-versus-host (GVH) reactivation of donor cells was negligible ever after repeated stimulation with patient's PBMC. In the case of renal carcinoma GVT-effect was less dominant and GVH-reactivation of donor cells led to significant amount. *Summary.* In conclusion, it is possible to selectively deplete donor alloreactive T-cells with anti-CD25 IT. In the case of patients with leukemia, the GVL-effect can be separated from GVHD, but in case of renal carcinoma severe GVHD-effect re-appeared.

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0198

WT1 FULL LENGTH PROTEIN VACCINATION SHOWS HIGH IMMUNOGENICITY AND SIGNIFICANT ANTI-TUMOUR ACTIVITY IN THE MOUSE MODEL

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Background. The Wilms' tumor gene (WT1) is overexpressed in many types of haematological malignancies including Acute Myeloid Leukaemia (AML), Acute Lymphoblastic Leukaemia (ALL), Chronic Myeloid Leukaemia (CML) and Ph negative myeloproliferative disorders. WT1 holds great promise for immunotherapy of leukaemia since it is expressed at high levels in blast cells but not in normal tissues, it is involved in the maintenance of malignant phenotype and it is spontaneously immunogenic. Many clinical trials using MHC class I-restricted WT1 peptide, have been recently performed in patients affected by AML, MDS, lung and breast cancer with satisfactory clinical results and without relevant toxicity. *Aims.* the aim of the study was to set up a vaccination approach in the mouse model using the WT1 full length protein and to test the safety and efficacy of the vaccine. *Methods.* We purified the WT1 full length protein. Complete coding sequence was cloned in a pGEX bacterial expression vector for the production of the fusion protein GST-WT1 that was subsequently purified by glutathione conjugated beads. The murine leukemic cell line C1498 was transduced with lentiviral vector PKG WT1 (kindly provided by Dr. L. Naldini) in order to obtain a syngeneic cell line expressing WT1. 40 C57BL7/6 mice were immunized with 50 μg of purified WT1 protein and 50 μL of Freund adjuvant every 15 days, for a total of 3 immunizations; 40 mice were injected with GST and adjuvant and 40 with PBS alone as control. 2 weeks after the last administration, 10 vaccinated mice and 10 controls have been injected with 200.000 TRAMP-C2 cells and 10 of each group with C1498. 10 mice from for each group were sacrificed and lymphocytes, cultured in presence of interleukin-2 to perform a cytotoxicity assay. Antibodies against WT1 have been evaluated. Long term toxicity has been evaluated. Blood values have been constantly tested. *Results.* Toxicity on normal tissues has been ruled out using the histochemical analysis of different tissues. After mice vaccination, no organ toxicity has been observed. White blood cell, Hb and platelets counts did not change

significantly in the different groups. Iimmunized mice showed high levels of both, IgM and IgG antibodies with a mean value of luminescence of 690000 in WT1 injected mice vs 7500 in control mice and high levels of CTLs against WT1 as compared to mice injected with PBS or GST alone. Importantly, in control mice injected with TRAMP-C the mean size of the tumor was 1,6±0,4 cm and 2±0,5 cm in control mice injected with C1498. By contrast in vaccinated mice the tumour mass was significantly smaller (0,3±0,1 cm) after 8 weeks of follow-up. *Conclusions.* The full length protein vaccination approach is able to elicit both, umoral and cytotoxic response and it results in a significant antitumor effect *in vivo*. Although further studies are required to compare the efficacy of this approach to the peptide based vaccine, WT1 full length protein could represent a valid vaccination strategy allowing to overcome the HLA restriction limit of the peptide approach

0199

DETECTION OF HUMORAL IMMUNE RESPONSES AGAINST WILMS TUMOR GENE (WT1) PRODUCT IN PATIENTS AFFECTED BY HAEMATOLOGICAL MALIGNANCIES

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Introduction. 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 Myelodysplastic Syndromes (MDS). Moreover WT1 gene is overexpressed 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. *Aims.* 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 elicit an immune response against WT1 with vaccination using WT1 peptides or protein. *Methods.* After informed consent sera and Peripheral Blood samples were collected from 98 patients: 30 Myelodysplastic syndromes (MDS), 11 Acute Myeloid Leukaemia (AML), 4 Acute Lymphoblastic leukaemia (ALL), 23 Multiple Myeloma (MM), 20 Chronic Myeloid Leukemia (CML), 6 Idiopatic 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. *Results.* 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 RA, 30% 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). *Conclusions.* 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.

0200

γ-GLOBIN GENE TRANS-ACTIVATION IN K562 CELLS BY A SYNTHETIC ZINC-FINGER ACTIVATOR GENE TRANSFER

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The activation of the γ-globin gene pharmacologically or by γ-globin gene transfer has been considered a valid alternative strategy for treatment of diseases arising from mutations in this locus, such as of sickle-cell anemia and thalassaemia versus the transfer of the normal β-globin gene. Lately (Graslund et al 2005) the development of a selective, synthetic activator of γ-globin gene, Zif-VP64, based on zinc-finger DNA binding proteins was presented, producing potentially therapeutic levels of Haemoglobin F. K562 cells express the γ-globin gene and are trisomic for chromosome 11, the aim, therefore, is to establish an increase in the γ-globin in the transfected cell lines, as compared to the untransfected ones, using a non-viral episomal vector. We constructed an episomal vector containing: the activator Zif-VP64; the reporter gene eGFP, driven by the CMV promoter; the S/MAR element, which enables the plasmid to establish itself in the nucleus of the host cell. We transferred this new, episomal vector of γ-globin activator, Zif-VP64-Ep1, into human hematopoietic K562 cells, by electroporation. Routinely 1x107 K562 cells were used for every experiment and transfection efficiencies were around 55%. Our results show that Zif-VP64-Ep1 is maintained as a stable episome in K562 cells without integrations in the genome as shown with Southern Blot and plasmid rescue experiments, running into the tenth month of continuous culture with and without selection pressure and without loss in cell viability or in expression of eGFP, as observed under Fluorescent Microscope and documented by Flow Cytometry. RT-PCR, Western blotting and Intracellular Flow Cytometry were employed to investigate γ-globin mRNA and Haemoglobin F protein levels in transfected K562 cells. Our data indicate a significant increase in Haemoglobin F protein of up to 350% of its level in the untransfected K562 cells, at list 200 generations post-transfection. This is the first time that an episomal vector for gene transfer is applied for the trans-activation of specific gene expression.

Infection and supportive care I

0201

COMBINATION ANTIFUNGAL THERAPY WITH ECHINOCANDIN AND POLYENE IN IMMUNOSUPPRESSED PATIENTS WITH INVASIVE FUNGAL INFECTIONS FAILING FIRST LINE TREATMENT

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Background. Invasive fungal infections (IFI) are strongly associated to illness and death in immunosuppressed or high risk patients (pts), especially in those experiencing failure to primary therapy. *Aims.* At evaluating second line combination antifungal therapy (CAT) with echinocandin and polyene in critically ill pts with IFI. *Methods.* From November 2003 to April 2005, 18 pts affected by IFI (8 with candidemia due to C. albicans, 3 due to C. glabrata, 2 due to C. krusei, and 5 with pulmonary aspergillosis), who had failed primary antifungal therapy, were treated with Caspofungin (CSP) 70 mg on 1st day, and 50 mg thereafter, plus low dose (LD) AmphotericinB deoxycholate (dAmB) 0.5 mg/kg/d. All pts had high risk underlying conditions (5 acute myelogenous leukemias, 6 solid tumors, 5 prolonged intensive care unit (ICU) stays, and 2 major abdominal surgical interventions). Failure to prior therapy was determined by: 1) fever and worsening of clinical conditions, and 2) persistent candidemia, or 3) worsening of lung CT scan together with increase of Aspergillus galactomannan antigenemia (AGA), after 96 hours (hrs) from the start of antifungal therapy. *Results.* All 18 pts were clinically unstable and critically ill, and 13 out of 18 had been admitted to ICU at the time of switching therapy; 5 patients never required admission to ICU. Within 72-96 hrs from the beginning of CAT, clinical stability and fever clearance, together with negative blood culture, or negative AGA were observed, and confirmed thereafter. LD dAmB did not require any premedication, but none of the pts suffered from side effects, nor treatment discontinuation was needed. All patients survived. Mean CAT duration was 26 days, and mean ICU stay was 9 days, before pts transfer to either medical or surgical wards. None of the pts relapsed within a follow up period of at least 60 days from the end of treatment. *Conclusions.* In heavily immunosuppressed pts, antifungal therapy remain a major challenge. CAT with echinocandin, such as CSP, and polyene, is an appealing option supported by promising data. In our experience, this treatment schedule with CSP and LD dAmB appeared effective in critically ill pts with IFI failing primary treatment. The synergistic activity of dAmB, even at LD, plus CSP seems to be clinically relevant; LD dAmB allows not only a lower incidence of side effects, but also a remarkable cost sparing in comparison with lipid formulations. Moreover, compared to the available clinical data in similar situations, both time to clinical stability, and to discharge from ICU appear shortened in patients under CAT. Wider clinical studies in these selected settings are needed to clarify the impact on survival of this salvage treatment schedule.

0202

HIGH INCIDENCE OF INVASIVE FUNGAL SINUSITIS IN PATIENTS UNDERGOING UNDERGOING INDUCTION THERAPY FOR ACUTE MYELOID LEUKAEMIA OR MYELODYSPLASIA WITH FLUDARABINE AND CYTARABINE BASED REGIMENS: A RETROSPECTIVE ANALYSIS

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Background. Invasive fungal infections have been relatively well documented in stem cell transplantation4 (SCT) but not in the setting of intensive chemotherapy (ICT) alone. Aspergillus species are the commonest causative organism. Optimal treatment involves systemic antifungal therapy, combined with surgical debridement 1,6. Reported mortality is 40-70%5,7 and long term morbidity approaches 70%. *Aims*. We evaluated the impact, and differential incidence, of IFS in patients with de novo, relapsed or refractory acute myeloid leukemia (AML) or myelodysplasia (MDS) who received either fludarabine plus cytarabine (FLA) based induction chemotherapy or alternative ICT. *Methods.* All 121 patients from 2 centres with a primary diagnosis of AML or MDS who received ICT between 1 January 2003 and 1st October 2006 were retrospectively included in the study. Over that time 39/121 patients received FLA based regimens. Records of hospital maintenance/ construction activity, climatic data and microbiological surveillance were also reviewed. IFS was classified according to current EORTC/ BAMSG criteria. *Results.* 5 proven, 1 probable and 1 possible cases of IFS were identified. The latter was excluded due to subsequent identification of a possible dental abscess. By univariate analysis there was no significant difference between the FLA and other ICT group in median age, sex, duration of neutropenia and incidence of positive blood cultures (table 1). The FLA group contained significantly more patients with relapsed/ refractory AML. All patients received prophylactic fluconazole except during a brief period of minor construction work on one unit when itraconazole was used. IFS occurred only in the FLA group (Chi2 analysis $p<0.001$) and in all but 1 patient developed within 21 days of receiving FLA. No other possible contributory factors were identified. During the study period 260 patients underwent haemopoietic SCT (179 autologous, 81 allogeneic). None developed IFS. 129 patients received ICT for acute lymphoblastic leukaemia with one episode of IFS and one patient with aplastic anaemia developed Mucor spp IFS whilst on iron chelation. Treatment included surgical debridement (4 cases) and liposomal amphotericin B (L-AmB). Treatment commenced at 1-3 mg/kg/day but all cases clinically progressed and ultimately required at least 5mg/kg/day, however the total cumulative per kilogram dose varied considerably. Two patients also received caspofungin and clinically improved within 48 hours but this coincided with L-AmB dose escalation and neutrophil recovery. 2 patients received intravenous voriconazole in addition to L-AmB for 72 hours. Two patients had significant long term morbidity. No patient died due to IFS. Of 5 patients scheduled for further ICT, 4 had subsequent treatment delayed (35 to >50 days) and 1 had treatment abandoned. Two of this group died of relapsed haematological disease. *Summary and conclusions.* A major change in AML/ MDS remission induction therapy over the last decade has been the introduction of FLA based regimen. Recent studies have questioned both their superiority compared to other regimens especially in view of profound immunosuppression due to fludarabine. Our study highlights a high incidence of IFS as a previously unrecognised consequence of FLA chemotherapy in patients with previously treated or de novo myeloid malignancy.

Table 1. Characteristics of the patient cohorts.

0203

ERYTHROCYTE POPULATIONS WITH CD55 AND/OR CD59 DEFICIENCY IN PATIENTS WITH HIV INFECTION AND HEMOPHILIA

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Background. Anemia is present in almost 35% of patients with HIV infection. Its pathogenesis is multifactorial and includes chronic inflammation, anti-viral agents, hemolysis, etc. Myelodysplastic syndrome (MDS)-like features have also been described in HIV. CD55 and CD59 are complement regulatory proteins that are linked to the cell membrane via a glycosyl-phosphatidylinositol anchor. They are reduced mainly in paroxysmal nocturnal hemoglobinuria (PNH) and other hematological

disorders, such as MDS. An increased sensitivity of lymphocytes from HIV-infected patients to lysis by complement has been directly correlated with a decreased expression of CD55 and CD59. The aim of this study was to evaluate the presence of CD55 and/or CD59 deficient erythrocytes in HIV patients and explore possible correlations with clinical parameters. *Patients and Methods.* CD55 and CD59 expression was evaluated in erythrocytes surface of 37 patients (30M/7F; median age 39 years) with HIV infection. Twenty five of them were hemophilia patients. At the time of evaluation, all patients were under antiretroviral therapy. The detection of CD55- and CD59-deficient red cells was performed using the sephacryl gel microtyping system (DiaMed-ID MicroTyping System PNH test, Cressier-sur-Morat, Switzerland). The presence of the deficient red cell populations was blindly scored by two independent observers and expressed semiquantitatively as 100%, 75%, 50%, 25% and 10%. In all samples with CD55- or CD59-negative populations Ham and sucrose lysis tests were also performed. Eight patients with PNH and 121 healthy subjects were also studied as control populations. *Results.* Anemia was present in 14/37 (37.8%) HIV patients. Interestingly, we found that all HIV patients had erythrocyte populations with CD55 and/or CD59 deficiency. More specifically, deficient red cell populations for both CD55 and CD59 antigens were detected in 8 patients (21.6%): in seven of them erythrocytes were deficient for both antigens at a proportion of 10%, while one patient had erythrocytes with 25% of CD55 deficiency and 10% of CD59 deficiency. Isolated CD55 negativity was observed in 29/37 patients (78.3%): 26 had red cells with 10% CD55 deficiency and only 3 had erythrocytes with 25% CD55 deficiency. Isolated CD59 deficiency was not detected. Among 121 normal subjects, two of them (1.6%) had red cells with double negativity for CD55 and CD59, while 3 others (2.4%) had erythrocytes with an isolated CD55 or CD59 deficiency; these red cells were counted for not more than 10% of the total. All patients with PNH had a simultaneous CD55 and CD59 deficiency. Positive Ham and sucrose tests were found only in patients with PNH. There was no correlation between the percentage of red cell population with CD55 and/or CD59 deficiency and the type or length of antiretroviral therapy, the $CD4^+$ counts, the plasma viral load or the concomitant hepatitis C infection. *Summary and conclusions.* Our study provides evidence supporting the presence of erythrocytes with CD55 and/or CD59 deficiency in patients with HIV. Further studies using molecular techniques will be required for clarifying the exact role of this deficiency to the increased susceptibility of these populations in complement lysis and the subsequent development of anemia in these patients.

0204

PREVENTION OF OVARIAN DAMAGE DURING CHEMOTHERAPY BY GONADOLIBERINE ANALOGUES ADMINISTRATION

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Background. Frequent negative consequence of chemotherapy is ovarian damage and premature ovarian failure (POF). The risk of POF onset depends mainly on women's age and foliculogenesis status in ovary, chemotherapy regimen used and cumulative dose of single cytotoxic agents. *Aims.* Aim of this prospective case-control study is evaluation of gonadoliberine analogues (GnRH-a) administration to patients with Hodgkin´s lymphoma (HL) during chemotherapy and prevention of ovarian damage depending upon chemotherapy dose and regimen.*Methods.* Study group consists of 72 patients in fertile age (28.4±4.1 years) with HL diagnosis treated in 2004-2005 by curative chemotherapy together with GnRH-a administration according standardized protocol. Patients were divided to 3 groups according clinical stage of disease and risk factors and treated by three types of chemotherapy regimens with increased cytotoxicity (German Hodgkin Study Group protocols): group A - ABVD regimen, group B - baseline BEACOPP and ABVD regimen in combination, group C - dose-escalated BEACOPP regimen. Ovarian function of all patients was assessed by gonadotrophins levels (FSH, LH) analysis from peripheral blood before treatment and also 6 and 12 month after it. Number of women with POF after chemotherapy in study groups was compared with control group (n=45, age 26.8±4.6) of patients treated in 2002-2003 according the same protocol, but without protective GnRH-a application. In statistical evaluation two sample binomial test with α/α=0.05 was adopted together with adjustment of level of statistical significance by Bonferroni correction for multiple tests. *Results.* In study group with GnRH-a administration during chemotherapy there was statistical significantly (p<0.001) less cases with POF (38.2%) in 6 month after end of chemotherapy than in control group (73.4%). After 12 month POF was detected in 48.8% of cases versus 69.3% in control group (p<0.001). Comparative analysis depending on cytotoxicity of chemotherapy regimen used showed statistically significant differences in percentage of patient with acquired POF between study and control group only in less aggressive chemotherapy protocols (group A and B). Difference in number of cases with POF in patients treated with chemotherapy regimen C was not statistically significant (74.1% vs. 63.5%) in both observation periods. *Summary and Conclusions.* Study proved significant reduction of ovarian failure risk in women with HL treated with less aggressive chemotherapy regimens. Reproductive functions protection in fertile women requires early and close cooperation between oncology department and assisted reproduction center.

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0205

PEGYLATED G-CSF AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. It has been claimed that, in patients receiving aggressive chemotherapy, pegylated G-CSF has advantages over conventional G-CSF in terms of PMN recovery, infectious complications and the duration of hospitalisation. *Aims*. The aim of this study was to compare the efficacy of pegylated and conventional G-CSF in autografted patients receiving the same supportive therapy. Methods Over the last 12 months, 38 consecutive autografted patients received pegylated G-CSF on day +1 to shorten the duration of neutropenia. The baseline diagnoses were AML (1), HD (3), multiple myeloma (18) and NHL (16); the conditioning regimens were Mel-200 in 22, BCNU-containing in seven, TBI-containing in three, and melphalan/mitoxantrone in six. The patients were comparable in age, gender and the number of infused CD34 cells with a historic group of 86 patients autografted in the previous three years, who had received daily G-CSF 5 mcg/kg from day +5. Their baseline diagnoses were AML (5), HD (7), multiple myeloma (36) and NHL (38), and the conditioning regimens were Mel-200 in 46, BCNU-containing in 23, TBI-containing in six, and melphalan/mitoxantrone in eleven. All of the patients received oral levofloxacin 500 mg and fluconazole 400 mg as prophylaxis for febrile neutropenia. Febrile episodes were treated with an empiric antibiotic combination including piperacillin/tazobactam and amikacin; empirical antifungal therapy was started after five days of ineffective antibiotic therapy. The selected study endpoints were time to PMN (>500/mcL) and platelet recovery (>50.000/mcL), the number of febrile episodes, the rate of empiric antifungal therapy, the duration of hospitalisation, and the number of G-CSF administrations in the control group. *Results*. There was no significant difference between the patients receving or not receiving pegylated G-CSF in terms of PMN recovery (median 10 vs 11 days), platelet recovery (14 vs 14 days), hospitalisation (17 vs 17 days) or empiric antifungal therapy; the median duration of treatment in the G-CSF group was eight days (range 6-30). There was a significant between-group difference in the number of febrile episodes (p<0.05); considering the patients receiving Mel-200, 11/22 remained afebrile in the pegylated group and 10/45 in the control group (p=0.02). Conclusions In our two groups of autografted patients receiving the same supportive therapies but different types of G-CSF, the use of pegylated G-CSF led to no advantage in terms of most of the considered endpoints, and the number of G-CSF administrations in the control group does not suggest any significant cost reduction with the pegylated form; PMN recovered earlier after pegylated G-CSF, but larger series are required to show a possible significant difference. Nevertheless, the patients receiving Mel 200 (accounting for about half of both groups) form a subset that may benefit from pegylated G-CSF, which proved to be effective in reducing the number of febrile episodes, thus improving their quality of life and avoiding the possible toxicity of antimicrobial agents.

0206

POTENTIAL ROLE OF CMV IN THE ONTOGENY OF MONOCLONAL TCRαβ+/CD4+ T-LARGE GRANULAR LYMPHOCYTE [LGL] LYMPHOCYTOSIS

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Background. Patients with TCR-Vβ13.1+/CD4+ T-LGL lymphocytosis display a common HLA-DRB1*0701 genotype and express identical motifs in the CDR3-TCR-Vβ sequence, suggesting an antigen-driven chronic T-cell stimulation origin for this particular disease.[1] Interestingly, peripheral blood [PB] CMV-specific CD4+ T-cells from healthy adults are mostly antigen-experienced cytotoxic T-cells, which show a marked bias of the TCRVβ repertoire associated with certain HLA genotypes.[2] Of note, the expansion of CMV-specific TCR-Vβ13.1+ CD4+ T-cells was associated with the HLA-DRB1*0701 allele. Thus, CMV would be a good candidate to play a role in the ontogeny of monoclonal TCRαβ+/CD4+ T-LGL lymphocytosis. *Aims.* To identify and characterize the CMV-specific clonal CD4+ T-cells from patients with different T-cell chronic lymphoproliferative disorders [CLPD]. *Methods.* PB samples from patients with monoclonal TCRαβ+/CD4+ T-LGL lymphocytosis [n=12], TCRαβ+/CD8+ LGL leukemias [n=2], TCRγδ+ LGL leukemias [n=4] and other CD4+ T-CLPD than LGL [n=9], were stimulated for 6h with both CMV and EBV whole lysates. Identification of virus-specific CD4+ T-cells was assessed by flow cytometry, through the detection of surface TNFα on secreting cells. Soluble levels of IFNγ, TNFα, LTα, GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 and IL-13 in culture supernatants were quantified using the Cytometric Bead Array(TM) system [BDB]. Surface detection of CD69 and CD25 as well as intracytoplasmic detection of IFNγ in CD4+ T-cells were both performed in TCRαβ+/CD4+ T-LGL patients, to evaluate CMV and EBV-specific responses after longer periods of stimulation [24-48h]. *Results.* Most patients [92%] were found to be CMV- and EBV-seropositive. However, the percentage of patients with CMV-specific CD4+/TNFα+ T-cells was significantly higher in TCRαβ+/CD4+ T-LGL lymphocytosis [83%] than in either non-CD4+/T-LGL leukemias [17%] or non-LGL/CD4+ T-CLPD [22%]. Furthermore, the former cases produced larger amounts of both IFNγ [$p<0.001$] and TNFα [$p=0.002$] Th1 cytokines in response to CMV. Interestingly, TCRαβ+/CD4+ T-LGL patients showed a percentage of clonal CMV-specific TNFα+/CD4+ T-cells significantly higher than that of normal residual CMV-specific TNFα+/CD4+ T-cells [median 4% vs 0.9%; $p=0.02$]. In contrast, the percentages of EBV-specific TNFα+/CD4+ T-cells were similar within clonal and normal CD4+ T-cells [median 0.1%]. The response of clonal CD4+ T-cells to CMV maintained stable after long periods of stimulation, as shown by the high percentage of CD69+ and CD25+ CMV-specific T-cells [~40%] observed after 48h stimulation. Likewise, the percentage of IFNγ+/CD4+ T-cells was significantly higher [$p=0.04$] in response to CMV [median 10%] than to EBV [median 1.2%]. *Summary and conclusions.* The specific response of clonal TCRαβ+/CD4+ T-LGL cells to CMV suggests that the T-cell expansion could originate in a CMV-driven chronic T-cell stimulation.

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0207

THE IMPACT OF ORAL CANDIDIASIS AND HERPES SIMPLEX VIRUS INFECTION ON CHEMOTHERAPY-INDUCED ORAL MUCOSITIS

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Background and Aims. Oral mucositis is an important problem in patients receiving chemotherapy. Multiple factors influence the severity of mucositis. We evaluated the role of oral candidiasis and herpes simplex virus (HSV) infection in chemotherapy induced oral mucositis. *Methods.* We retrospectively reviewed charts of patients receiving chemotherapy for hematological malignancies at National Taiwan University Hospital from January 2005 to November 2006. 136 episodes (in 81 patients) of chemotherapy-induced oral mucositis that had been evaluated by cultures for both fungus and HSV were analyzed. The results were correlated with associated clinical features. *Results.* Among the 81 patients, 38 were males and 43 were females and the median age was 47 years. The hematological malignancies these patients had included AML (n=26), NHL (n=24), ALL (n=15), MM (n=11), HD (n=1), acute biphenotypic leukemia (n=1), CML (n=1), CLL (n=1) and myelofibrosis (n=1). 76% (n=103) of the mucositis were detected at the time when absolute neutrophil counts were below 1000/μL. Overall, 104 episodes (76%) of the oral mucositis were related to either fungal or HSV-1 infections, including 63 episodes (46%) of fungal infection alone, 23 episodes (17%) of HSV-1 infection alone and 20 episodes (15%) of both fungal and HSV-1 infections. Among the 83 episodes of fungal mucositis, C. albicans was the most common pathogen isolated (69 episodes), followed by C. tropicalis (8 episodes). Thirty-three of the 84 patients (39%) who had received anti-fungal agents before the occurrence of mucositis had HSV-1 infection, compared with 10 of the 52 (19%) who had not ($p=0.0221$). Those patients with HSV-1 infection suffered from grade 3~4 mucositis more frequently (13/43 versus 12/93, $p=0.0302$) and tended to depend on longer TPN ($p=0.0447$) for nutritional support and topical lidocaine for symptomatic relief (17/43 versus 17/93, $p=0.0107$). *Conclusions.* These results suggest that Candida albicans and HSV-1 play an important role in oral mucositis. It is essential to perform fungal culture and HSV isolation promptly once mucositis occurs after chemotherapy and to start empirical treatments immediately. HSV infection should be considered in the differential diagnosis of oral mucositis particularly in those patients who have prior antifungal therapy.

0208

CONTRIBUTION OF GALACTOMANNAN DETECTION FOR EARLY DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN DIFFERENT GROUPS OF HEMATOONCOLOGICAL PATIENTS

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Background. Invasive aspergillosis (IA) has been a significant cause of life-threatening opportunistic infections in hematooncological patients (pts.). Serum galactomannan (GM) detection is considered to be a useful test for early diagnosis and follow 'up of invasive aspergillosis. *Aims.* To assess the role of galactomannan detection for diagnosis of IA in various groups of pts. with hematological malignancy. *Methods.* From 5/2003 to 10/2006 patients at risk for IA, with hematological disorders and/or bone marrow transplant, were monitored for GM using a double sandwich ELISA assay (Platelia Aspergillus, BioRad, France). Test positivity was defined in accordance with the manufacturer's recommendations (index of positivity - IP>0.5). Patients were considered to have confirmed or probable invasive aspergillosis, based on clinical and radiological data.

Table 1. PPV of GM ELISA in different treatment groups.

Results. 11 360 of blood samples from 911 pts. have been tested for GM (mean no. of samples/pt. - 12.5). 42 pts. in this period had proven or probable IA. 891 samples (8%) had IP > 0.5 and 128 pts (14%) fulfilled criteria to be considered as GM positive (2 x IP >0.5). Sensitivity, specificity, PPV and NPV of the test in the entire group of pts. were 97.6%, 90.1%, 32% and 99.9%. False positivities were significantly more frequent in groups of pts where prevalence of IA is low - 48.8% of false positive results in high risk group (allogeneic HSCT, treatment for AML) vs. 77.6% in low risk group (autologous HSCT and treatment for other hematological malignancies). PPV of the test (very low in the entire group of pts. - 32%) markedly differ in various pts groups: allogeneic HSCT -

75%, induction for acute leukemia - 50%, consolidation for acute leukemia - 33%, autologous HSCT - 10%, other treatment for miscellaneous hematological malignancies - 33%. *Summary and conclusions.* GM detection by Platelia Aspergillus kit is very useful for diagnosis of IA in hematooncological pts. The test has very high sensitivity and especially very high NPV and so negative test result can exclude IA. The main problem of the test is false positivities, especially common in group of hematological pts. with low prevalence of IA. Therefore the regular screening for GM should be performed only in pts. groups were prevalence of IA is high - pts undergoing allogeneic HSCT or treatment for acute leukemia. In low risk group the test should be performed only when there is clinical suspicion of invasive fungal infection and positive result should be interpreted very carefully to avoid overtreatment with expensive new antifungals.

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0209

INCIDENCE OF INVASIVE ASPERGILLOSIS IN ALLOGENEIC STEM CELL TRANSPLANTATION RECIPIENTS: AN ITALIAN PROSPECTIVE MULTICENTER STUDY

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Background. So far only retrospective studies have been published focusing on the incidence of Invasive Aspergillosis (IA) in allogeneic hematopoietic stem cell transplantation (alloSCT). *Patients and Methods.* Between December 2003 and October 2005, 206 patients admitted to four Piedmont Hematological institutions, on behalf of *vita vitae* project. Patients, accounting for 217 alloSCT, were consecutive enrolled in the trial on the role of combined intensive screening for circulating galactomannan (GM) and early high resolution chest CT (HRCT) for IA diagnosis. GM sampling were collected twice in a week from admittance until day +365; HRCT and/or bronchoscopy with bronco-alveolar lavage (BAL) were performed when IA was suspected. AlloSCT were performed for: Multiple Myeloma (61), Lymphoma (39), Acute Leukemia (64), Myeloprolipherative Disorders (15), Severe Aplastic anemia (2), solid tumor (14) and Chronic Lymphocytic Leukemia (8). Donors were HLA-Identical siblings (179) or alternative (38). Stem cell source was peripheral (190), cord blood (2) and bone marrow(15). Conditioning was myeloablative (104) or Reduced-intensity (113). Previous autologous SCT was done in 82 patients. One-hundred-fifty-five patients underwent alloSCT in PR and 72 in CR. Acute and chronic Graft versus Host Disease (GvHD) affected 157 alloSCTs. Only proven and probable IA, diagnosed according to the EORTC/MSG criteria were considered for the analysis of IA incidence. *Results.* With a follow-up range of 12 to 34 months, 115 patient were alive and 91 had died. Relapse accounted for 57 deaths, transplant related mortality (TRM) for 58, including 10 due to IA Twenty-five cases of IA were diagnosed: 2 proven and 23 probable. Six cases were classified as early IA (within day +40 from SCT) and 19 as late IA (beyond day +40 to one year), with an overall incidence of 11,5%. Neutropenia <500/mm^3) was observed in 7/25, persistent fever in 10 and GvHD in 17. Diagnosis of IA was made thanks to high GM serum values in most patients (23/25), in one for Aspergillus spp. detection on BAL and in one for high GM values on CSF fluid. Chest CT scan contributed to IA diagnosis in 23/25 patient; in more than half of them(14/23), major clinical criteria were present. The remaining 2 cases were CNS aspergillosis. In 15/23 CT positive patients GM elevation occurred before CT scan, and in 13 of them GM was the trigger for further evaluation, in absence of clinical suspect of IA. No deaths due to aspergillosis were observed among the early IA. Among 19 late IA, only 10 patients had died for overt IA. *Conclusions.* The IA incidence is 11.5% in our experience, with a lethality rate for IA of 40%. This figure compares favourably with previous published mortality rates up to 60-90%. Such combined GM/ HRCT diagnostic approach allows an early IA diagnosis, a prompt preemptive antifungal treatment, with a significant lowering of IA attributable death.

0210

CMV SPECIFIC TETRAMERS MONITORING PATIENTS AFTER STEM CELL TRANSPLANTATION

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Background. The reactivation of latent viruses like Cytomegalovirus (CMV) contributes significantly to morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Risk factors for the reactivation of CMV are the conditioning regimen, prophylaxis of graft versus host disease (GvHD), like T-cell depletion of the graft, the development and treatment of acute or chronic GvHD. Moreover, transplantation of a CMV-seropositive recipient with cells from a CMV-seronegative donor is a risk factor for recurrent reactivation of CMV. To control viral reactivation, the existence of virus-reactive T-cells is crucial. *Aims.* In order to improve the understanding of restoration of CMV-immunity, we monitored absolute numbers of CMV-reactive cytotoxic T-cells using MHC-I-peptide-tetramers available for the major HLA class I groups. In addition we checked the percentage of patients that can be monitored with one or more currently commercially available tetramers to answer the question, whether this technique is suitable as a standard test. In some patients reactivity of tetramer-positive T-cells has been compared with the number of CD8$^+$ IFN-γ secreting cells after stimulation with pp65. *Methods.* Screening for CMV-epitope specific CD8+ T-cells was started prior to HSCT and continued on days +50, +80, +100, + 180 and +365 or weekly in case of CMV reactivation or increased immunosuppressive treatment due to development of GvHD. *Results.* To date we included 53 patients, 26 are currently monitored prior and after Tx, 17 were included past day +100, another 10 are either pre Tx or deceased. The cut off for protection against recurrent CMV-reactivations seems to be 10 CMV-specific T-cells/µL blood. Interestingly, in 2 patients transplanted from seronegative donors CMV-reactive Tcells (total of 13 to 59 cells/µL) were detected around day +50 and seem to provide protection against CMV-reactivation. Taking all patients undergoing HSCT in a certain time period from July 2006 until February 2007 in consideration, 67% of our patients could be monitored after stem cell transplantation with the commercially available set of tetramers. The setting seropositive recipient and seronegative donor contributes 18% of this cohort. Of 36 patients monitored before and after Tx, including those still prior to Tx or deceased, 17 can be monitored with 1 tetramer, 16 patients match 2 and 3 patients match 3 of the tetramers. *Conclusions.* Our results indicate that under certain conditions screening of patients with CMV-specific tetramers is suitable to predict the risk of CMV-reactivation and/or protection against reactivation due to recipient/donor CMV-specific T-cells. Thus, for patients at risk of reactivation suitable therapies, like the infusion of CMV-specific T-cells, can be considered early on. Yet the question how many T-cells binding the tetramers are functionally active has still to be answered. Lack of a compatible HLA-I-tetramer complexes and epitopes, respectively, can be an additional problem. Therefore an intracellular staining test will be used in parallel to answer this questions, while tetramer screening is ongoing.

0211

HUMORAL AND CELL MEDIATED RESPONSE TO THE PNEUMOCOCCAL CONJUGATE VACCINE (PREVENAR) IN PATIENTS WITH MYELOMA AND CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Infection with Streptococcus pneumoniae is responsible for significant mortality in patients with a defective humoral response. Immunisation with pneumococcal polysaccharide vaccine (PsV) is not always effective in these individuals. Conjugation of polysaccharides renders vaccines more immunogenic through a T-cell dependent mechanism. Impressive results in children lend themselves to evaluation in other high risk groups. *Aims.* To assess the humoral and cell mediated responses to Prevenar in patients with myeloma and CLL. *Methods.* The schedule followed UK Department of Health guidance for vaccination of high risk children; two doses of Prevenar followed by a dose of PsV (Group 1). Patients who had previously received PsV were only offered Prevenar (Group 2). For assessment of humoral immunity we utilised a serotype-specific IgG Bioplex assay for all 7 serotypes in Prevenar, levels of ≥0.35 ∝g/mL are protective. Cellular responses were evaluated using

3H-thymidine uptake in cell cultures. *Results.* Data are available on 30 individuals. Twenty four patients had a diagnosis of myeloma and 6 CLL. The median age was 64 years (range 51-75). Six patients had never required treatment. The median number of prior chemotherapeutic treatments was 2 (range 0-5). Seventeen patients had undergone a transplant procedure. The median time from last treatment was 14.5 months (range 6-69). Eight patients with myeloma were receiving maintenance thalidomide. Two patients had low baseline total IgG levels, 11 low IgM and 8 low IgA. Prior to vaccination, the median number of serotypes per patient with protective antibody levels was 1 in Group 1 [n=20], (range 0-6) and 4 in Group 2 [n=10], (range 0-7). Following vaccination the median number of serotypes with protective antibody levels increased to 5 in Group 1 (range 1-7). 45% of patients achieved an adequate response. In Group 2 the median number of serotypes with protective levels remained at 4 (range 0-7). The proportion of patients with an adequate response did not increase. In preliminary experiments, 5 individuals had cellular responses evaluated. Proliferative responses to the protein conjugate were observed only in those who subsequently developed a protective antibody response (3 individuals). *Conclusions.* The pneumococcal conjugate vaccine Prevenar appears to be immunogenic in patients naïve to PsV; however it does not benefit those previously immunised with PsV.

0212

DIAGNOSING LATENT TUBERCULOSIS INFECTION IN PATIENTS WITH HAEMATHOLOGICAL MALIGNANCIES: USE OF THE NEW T-CELL INTERFERON- RELEASE ASSAYS

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Background. patients with haematological malignancies have an increased risk of progression to active tuberculosis (TB), and TB mortality. The standard diagnostic test for latent tubercolosis infection (LTBI), the tuberculin skin test (TST), may be unreliable in immunosuppressed patients. T-cell interferon-γ (IFN-γ) release assays (TIGRAs) QuantiFERON-TB Gold In tube (QFT-IT) and T-SPOT.TB (TS.TB) hold promise to provide a more accurate diagnosis of LTBI. Blood tests are based on detection of IFN-γ produced by T cells in response to antigen specific M. tuberculosis antigens encoded by the RD1 region. *Methods.* between February 1st 2006 and February 1st 2007 96 patients (mean age 61.3±14.5 years) have been blindly tested with TST, QFT-IT and TS.TB at the time of first diagnosis, about at half of the chemotherapy course and 1 month at least after treatment completion. *Results.* patients were affected by non Hodgkin's lymphoma (n=46), chronic lymphocytic leukemia (n=31), Hodgkin's disease (n=8), multiple myeloma (n=7), idiopathic myelofibrosis (n=1), myelodysplastic syndrome (n=1), hairy cell leukaemia (n=1) and systemic amyloidosis (n=1). Patients were treated according to current institutional protocol. Two patients were excluded due to high background levels with QFT-IT and 1 with TS.TB. At enrolment, 10/96 (10.4%), 21/94 (22.3%, $p<0.001$ vs TST), 24/95 (25.3%, $p<0.001$ vs TST) patients tested positive with TST, QFT-IT and TS.TB, respectively. TIGRAs had moderate agreement with the TST (QFT-IT vs TST k=0.50, TS.TB vs TST k=0.45). QFT-IT had more indeterminate tests (n=4, 4.3%) compared to TS.TB (0%). All TST-positive patients were also positive with both blood tests. Evaluation during treatment of 49 patients showed high concordance between TIGRAs (QFT-G IT vs TS.TB k=0.68) and a lower concordance with the skin test (TST vs TS.TB k=0.52, TST vs QFT-IT k=0.53). In patients after treatment completion (n=20) concordance between TIGRAs was still good (QFT-IT vs TS.TB k=0.64), while concordance with the skin test further dropped (QFT-IT vs TST k=-0.05, TS.TB vs TST k=-0.07). *Conclusions.* TIGRAs provided more positive results compared to the skin test in patients with haematological malignancies, thus suggesting that the prevalence of LTBI in these group of patients may be higher than previously estimated. Results of blood tests seem not to be affected by chemotherapy, while TST efficacy may be more susceptible to immunosuppressive treatment.

0213

POLYMORPHISMS WITHIN CHEMOKINE (CCL5, CXCL12) AND CHEMOKINE RECEPTOR (CCR5) GENES AND THEIR ASSOCIATION WITH THE RISK OF EBV REACTIVATION IN PATIENTS AFTER ALLOGENEIC HSCT

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Backround. Our former studies showed that the risk of EBV reactivation after allogeneic HSCT is associated with the presence of the low producer IFNG3/3 genotype in the recipient (Bogunia-Kubik et al. Br J Haematol 2006). *Aims.* In the present study the CXCL12 (3'UTR 801 G/A), CCL5 (-28 C/G; -403 G/A) single nucleotide polymorphisms and CCR5D32 deletion were analyzed in 76 HSCT donor-recipient pairs and related with EBV load. The control group for the polymorphism study constituted 99 healthy individuals. *Methods.* DNA was extracted from peripheral blood taken onto EDTA using silica membranes. Viral load were assessed 2-3 months after HSCT in blood cells by qPCR. The detection threshold for viral reactivation equaled 10 EBV-DNA copies/105 peripheral blood cells that were apparent in 27 patients. The CCR5d32 mutation was detected by PCR while SDF-1 and CCL5 alleles by PCR-RFLP. *Results.* Distribution of CCR5, CCL5 -28 and CXCL12 genotypes was similar in patients, donors and controls while CCL5 -403 AA homozygosity was more frequent in donors than controls (0.61 vs 0.47, $p=0.036$). The higher number of EBV copies were detected in patients lacking CCR5>32 deletion (0.42 vs 0.13, $p=0.026$). No significant correlation was found between EBV reactivation and CCL5 -28 C/G or CXCL12 801 G/A polymorphisms. Patients transplanted with donors homozygous for the CCL5 A allele (genotype associated with an increased expression of the CCL5 gene) more frequently presented with EBV reactivation (0.85 vs 0.49, $p=0.002$). In conclusion, the lack/lower expression of functional CCR5 or the presence of CCL5 low producer genotype decreases the susceptibility for EBV reactivation. Lack of the relationship with CXCL12 might be explain by the results of the recent study on the SDF-1 gene expression in EBV-transformed lymphoblastoid cells that did not show any evidence for the contribution of the SDF-1 3'UTR 801 A/G polymorphism to the amount of the SDF-1 mRNA.

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0214

PULMONARY DISPOSITION OF AMPHOTERICIN B LIPID FORMULATIONS

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Background. Invasive fungal infections of the lung are a major cause of morbidity and mortality in patients suffering from haematological malignancies. Lipid formulations of amphotericin B (AmB) are a cornerstone in their treatment. The three licensed preparations liposomal AmB (LAMB), AmB colloidal dispersion (ABCD) and AmB lipid complex (ABLC) display considerable differences in their pharmacokinetic behaviour. *Aims.* AmB concentrations in epithelial lining fluid (ELF) and whole lung tissue were determined after administration of AmB lipid formulations in order to investigate their target-site penetration. *Methods.* AmB levels in ELF were measured in bronchoalveolar lavage specimens of 30 critically ill patients, who were on treatment with lipid formulated AmB (LAMB: n=6, ABCD: n=18, ABLC: n=6). The penetration ratio was calculated by comparing the concentrations in ELF with simultaneous levels of lipid formulated AmB in plasma. Whole lunge tissue concentrations of AmB were determined in autopsy material of 29 patients (LAMB: n=7, ABCD: n=13, ABLC: n=9). All samples were concentrated and purified by solid phase extraction. Subsequently AmB was quantified by high-performance liquid chromatography. *Results.* Remarkable differences in the lung penetration of the three AmB lipid formulations were found. After treatment with ABCD and ABLC, mean concentrations in lung tissue were higher than after LAMB (ABCD: 32.62 μg/ml, ABLC: 31.96 μg/mlL, LAMB: 11.63 μg/mL). Mean concentrations in ELF were much lower compared to whole lung tissue. The highest concentrations in ELF, were observed after treatment with LAMB (1.86 μg/ml) and ABLC (1.86 μg/mL) in contrast to ABCD (0.33 μg/mL). The penetration ratio in ABLC- and ABCD treatment was higher than in LAMB therapy. *Conclusions.* The levels of AmB at target-site may depend on the administered formulation. Further studies are required for clarifying the impact of these differences on clinical outcome.

0215

TUBERCULOSIS (TBC) FOLLOWING SINGLE-UNIT MYELOABLATIVE UMBILICAL CORD-BLOOD TRANSPLANTATION (UCBT) FOR ADULTS WITH HEMATOLOGICAL MALIGNANCIES

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Introduction. Allogeneic hematopoietic stem cell transplantation (allo-SCT) recipients, especially those receiving UCBT, are prone to serious infections, including TBC. Incidence rates of TBC after allo-SCT in several case series vary from less than 0.1 to 5.5%. However, only one study has been published on TBC after UCBT. *Patients and Methods.* Medical records of 106 adult patients with hematological malignancies who underwent single-unit UCBT with a myeloablative conditioning at our institution from May 1997 to December 2006 were retrospectively reviewed for the diagnosis of TBC. Conditioning regimen included thiotepa, busulfan (oral, 43; iv, 63' iv single daily dose, 34), cyclophosphamide (72) or fludarabine (34), and antithymocyte globulin (Lymphoglobulin, 32; Thymoglobulin, 74). All received cyclosporine plus prednisone for graft-versus-host disease (GVHD) prophylaxis and filgrastim to fasten engraftment. Diagnoses were acute lymphoblastic leukemia (ALL) in 37, acute myeloblastic leukemia (AML) in 30, chronic myelogenous leukemia (CML) in 22, myelodysplastic syndrome (MDS) in 9 and other lymphoid malignancies in 8. No patient had personal or family history of TBC prior to UCBT. The pretransplant evaluation of the respiratory system included chest radiographs and pulmonary function tests that were normal in all patients. Sputum smears and cultures for acid-fast bacilli were not routinely obtained. A diagnosis of TBC was based on the identification of M. tuberculosis in at least one fluid or tissue specimen (sputum, bronchoalveolar lavage, pleural fluid, lymph node). *Results.* Median age and weight were 31 yr (range, 15-49) and 71 kg (range, 41-112). HLA match (HLA-A and -B at antigen and -DRB1 at allelic level) was 6/6 in 6 (6%), 5/6 in 39 (37%), and 4/6 in 61 cases (57%). The median number of nucleated cells and $CD34^+$ cells infused was 2.1×10^7/kg and 1×10^5/kg respectively. Median time to neutrophils > 0.5×10^9/L was 22 days (range, 11-57). Mycobacterium tuberculosis infections were diagnosed in six of the 106 patients (5%). They had no different risk factors for TBC compared with the other 100 patients. Their detailed clinical characteristics are shown in Table 1. Patient #1 had a Mycobacterium kansasii infection 6 months before TBC. Patients were scheduled to receive antituberculous treatment according to drug susceptibility patterns for at least 6 months. Patients #2, #5, and #6 died of disseminated TBC. *Conclusions.* TBC is an infrequent but severe infection after allo-SCT, and is associated with high mortality, especially when occurring within 3 months of transplantation. It can also complicate post-transplant management as antituberculosis drugs frequently interfere with immunosuppressive therapy and they may narrow therapeutic ranges. T-cell recovery is one of the most important factors for curing TBC infection. Shortening the diagnostic delay could also have a pivotal impact on survival. Transplantation centers should maintain a high level of suspicion for TBC in patients receiving UCBT. Screening before UCBT with PPD skin test or other tests should be performed in these patients.

Table 1.

Patient	1	2	3	4	5	6
Age/Sex	33/M	34/M	42/M	44/F	38/M	44/F
Disease/ Status	MDS	ALL/CR1	AML/CR1	ALL/CR1	AML/CR1	MDS
GVHD	No	Acute 3	Chronic	Chronic	No	No
CD4 ($\times10^6$/L)	>500	<10	>500	<10	<10	<10
Diagnostic specimen	Sputum	Pleural fluid	BAL	Lymph node	BAL	Sputum
Onset of TBC	Month +18	Day +78	Day +160	Day +26	Day +69	Day +75
Delay of treatment (days)	43	2	33	78	2	22
Outcome	Alive, +44 mo	Dead, +99 d	Alive, +386 d	Alive, +270 d	Dead, +250 d	Dead, +106 d

0216

PALIVIZUMAB TREATMENT OF RESPIRATORY SYNCYTIAL VIRUS INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Respiratory syncytial virus (RSV) infections after HSCT can lead to severe respiratory failure and is associated with a fatal issue in a substantial number of patients (pts). Since palivizumab (P) has been shown to be efficient to lower RSV infection related hospitalization in high risk infant, P has been used in an uncontrolled manner to treat RSV infections in HSCT recipients, including in our transplant unit, with no clear knowledge of its impact on outcome. This study was conducted to determine outcome in HSCT recipients diagnosed with RSV infection and to estimate P impact, if any, on mortality. From January 1999 to March 2006, all pts with RSV infections after HSCT at Saint- Louis Hospital were retrospectively reviewed in order to determine cumulative incidence of RSV-related death, risk factors for 4-month transplant-related-mortality post RSV diagnosis (early TRM), and eventual impact of P. Forty pts with RSV infections were identified (median age : 16 years - range 3 to 65), at a median interval from transplant to RSV diagnosis of 90 days (d) (range: -15 d to 987 d). 24 pts had received an unrelated stem cell graft. Characteristics at diagnosis were: pneumonia in 16, bronchitis or bronchiolitis in 8 and upper respiratory disease in 16 pts; 16 pts had hypoxemia. Among them, 18 received P at diagnosis (15 mg/kg - intravenous course - 1 to 3 monthly injections. Pts treated with P were significantly younger, have received more cord blood transplant, had lower neutrophil and lymphocyte counts, and a shorter interval between transplantation and infection. One-year overall survival was 75% (95% CI: 63-91), corresponding to 77% (95% CI: 60-99) and 75% (95% CI: 59-97) for palivizumab-treated and -untreated patients, respectively. P did not shorten RSV excretion or significantly prevent progression from upper to lower respiratory tract infection Only one patient (treated with P) died from RSV pneumonia alone, giving a 2.6% cumulative incidence of RSV-related death (95% confidence interval (CI): 0-7.7). Seven other pts died during the 4 months following RSV diagnosis. All of them died from respiratory failure of multiple causes (RSV +: n = 4; RSV-: n = 3). Four-month non-relapse mortality was 16% (95% CI: 17-27): 23% (95% CI: 0-50) and 9% (95% CI: 0-22) of patients treated with or without P, respectively. In a multivariate Cox model, the only risk factors for 4-month non-relapse mortality were stem cell source (unrelated cord blood, hazards ratio (HR): 4.9, 95% CI: 1.1-22, p=0.039; and oxygen required at RSV diagnosis (HR: 7.31, 95% CI: 1.8-49, p=0.012). In conclusion this study doesn't support the use of P as an RSV curative therapy after HSCT. Because P was predominantly used in pts with classical poorer prognosis, we cannot robustly test its impact; nevertheless classical poorer prognosis factor were not found to be significant in this study. Given the high cost of palivizumab therapy and the arrival of several new compounds, our findings do not support further use of this drug in an uncontrolled manner.

0217

TUBERCULOSIS AMONG A COHORT OF 35 PATIENTS WITH BLOOD DISEASES

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Tuberculosis (TBC) is still a common infection in Spain, especially in the North-Western region of Galicia, with Incidence/prevalence rates over 40 cases /100,000 inhabitants. Tuberculous infection in hematological patients has been scarcely studied. *Methods.*We describe the pattern of infection in 35 patients from 3 Hospitals in Galicia. Data were obtained from a questionnaire sent to Hematology Services, which gathered cases form their databases, necropsic reports and reports from Microbiology Services. The diagnostic criteria for TBC were those defined by WHO (010 to 018 in CIE-9 classification); infections needed to be documented with microbiological and/or pathological data. 'Blood diseases' included acute leukemias, myeloproliferative disorders (MPD), myelodysplastic syndromes (MDS), myeloma (MM), lymphoma and aplastic anemia. The diagnosis or TBC should have been made concomitant or subsequent to the hemopathy, not priorly. *Results.* 35 HIV (-) patients were included (median age, 73.7; range 53-86): 15 non-Hodgkin lymphoma; 6 MDS; 5 MPD (3 essential thrombocythemia; 2 CML); 3 MM; 3 acute leukaemia; 3 chronic lymphocytic leukaemia. The involved organs were: lymph nodes, 15 (41%); lung, 14 (35%); kidney, 1; skin, 1; peritoneum, 1; bone, 1; pericardium, 1; 3 cases presented with the miliary form. Diagnosis was obtained by: detection of acid-fast bacilli, 12 cases; culture, 24; examination of tissue, 18 (2 fine-needle aspiration, 16 biopsies). The majority of biopsies came from adenopathies (neck, 10; axilar, 2; mediastinum, 1; 2 unknown). 26 patients have died when data were collected. Post-mortem diagnosis was made in 5 cases (4 lung; 1 peritoneal); the infection could have contributed to the death of other 13. In 12 cases, the diagnosis of TBC and the hematologic disease was concomitant (lung, lymph nodes); in the other 23, TBC was found after the other disease (median interval, 31.7 months; range: 1-90 mo.). In 3 patients there was a history of probable TBC more than 30 yr. before. Only 12 patients had recorded data of PPD test: it was negative in 4; due to several reasons, positivity was followed by prophylaxis with isoniazid only in 1of the remaining 8 (who developed TBC anyway 9 yr. after). According to IDSA criteria, former risk procedures were: glucocorticoids, 19 cases; chemotherapy (including low dose araC), 15; in 11, no adscription to risk groups could be established; 4 received only hydroxyurea. 29 patients diagnosed while being alive received anti-TBC therapy. *Conclusions.* TBC may be extremely proteiform in hematologic patients (especially in the elderly), with a majority of extrapulmonary cases, affecting unusual areas (lymph nodes predominantly). The infection may occur concomitantly or after the start the hemopathy, in both cases posing a difficult diagnostic problem due to the coincidence of symptoms with blood diseases. In highly prevalent areas like ours, TBC must be always taken into account, even in patients not belonging to risk groups, since the delay of therapy may be lethal. PPD test should also routinely performed in hematologic patients, even though its negativity does not preclude latent infection and prophylaxis is not completely protective nor safe.

Myelodysplastic syndromes I

0218

PRION-LIKE DOPPEL GENE (PRND): A NEW MOLECULAR MARKER POTENTIALLY INVOLVED IN LEUKEMOGENESIS

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The PRND gene encodes Doppel (Dpl), a protein that is strongly expressed in testis and at much lower levels in other tissues. Despite the recent discovery of Dpl involvement in spermiogenesis and in apoptotic death of cerebellar neurons, the physiological role of this prion-like protein remains unknown. Recently, we observed a weak Dpl expression in normal CD34$^+$ bone marrow cells, whereas high levels of PRND transcripts were detected in leukemic cell lines as well as in bone marrow cells from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). In order to clarify the clinical and biological relevance of Dpl overexpression in these disorders, we searched for possible correlations among Dpl expression, some biological parameters and clinical-pathological features. In some MDS patients we sequentially studied Dpl levels, to evaluate their changes in the time as well as the influence of the disease progression and of therapy, whereas in AML patients treated with aggressive polychemotherapy Dpl levels were sequentially quantified to assess minimal residual disease. Moreover, we characterized PRND transcriptional and translational patterns. Immunocytochemistry, flow cytometry, biochemical and molecular (real-time quantitative PCR) studies were carried out on bone marrow cells from 64 AML patients at diagnosis, after induction therapy and at relapse, and from 98 MDS patients at diagnosis and during disease progression. Controls were 16 non-hemopathic subjects. Dpl, barely detectable in normal controls, was detected in almost all AML and MDS cases, with median percentages of positive cells of 11.5% (IQR 7-24%), and 17.5% (IQR 11-29%) respectively. In AML no significant relationship was observed between Dpl levels and clinical and laboratory features nor did Dpl levels predict response to therapy. In patients achieving complete remission (22/34) a significant reduction of both transcript and protein levels (p=0.02) was observed. In 5 relapsing patients Dpl was again overexpressed at levels similar to those observed at onset. Also in MDS, Dpl levels were unrelated to clinical and laboratory aspects nor did they predict disease progression. Their behaviour was variable during evolution towards acute leukemia. In pathological samples Dpl was abnormally localized in the cell cytoplasm, while normal CD34+ cells exhibited the expected membrane localization. The ectopic localization was probably dependent on abnormal cellular trafficking because of glycosylation pattern modifications of the protein. Moreover, in pathological samples an abnormal nuclear retention of the transcript was observed. In conclusion, our findings confirm the clinical usefulness of Dpl evaluation for AML or MDS diagnosis and for the assessment of minimal residual disease. Moreover, they suggest its possible physiopathological role at least in the early phases of cell transformation. Studies are in progress to better understand which factors may contribute to the modulation of PRND activity: identifying the promoter region and critical elements for the activation of the gene may provide new insights into the involvement of Dpl in leukemic transformation.

0219

BIOLOGICAL AND CLINICAL RELEVANCE OF MATRIX METALLOPROTEINASES 2 AND 9 IN ACUTE MYELOID LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES

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Matrix metalloproteinases (MMP) are a family of zinc-dependent endopeptidases which are able to degrade all the protein components of the extracellular matrix. MMP-2 (type IV collagenase, gelatinase A) and MMP-9 (type V collagenase, gelatinase B) have been implicated in tumor progression and metastasis and, recently, it was suggested that these enzymes may also contribute to leukemic dissemination. We analyzed the expression of MMP-2 and MMP-9 in bone marrow cells from 54 patients with acute myeloid leukemia (AML), 153 patients with myelodysplastic syndrome (MDS) (68 RA, 32 RARS, 31 RAEB, 5 RAEB-t, 17 CMML), not previously treated, and 52 non hemopathic subjects,

in order to evaluate whether abnormalities in their expression were associated with relevant laboratory and clinical findings. Moreover, a possible correlation was investigated between MMP positivity and altered apoptosis level, as measured by TUNEL technique, or altered proliferative activity, as evaluated by MIB-1 immunostaining. MMP-2 and MMP-9 were detected on bone marrow smears by an immunoalkaline phosphatase method (streptavidin-biotin complex) using primary murine monoclonal antibodies raised against human MMP-2 (clone A-Gel VC2, NeoMarkers) and human MMP-9 (clone IIA5, NeoMarkers). In normal samples MMP-2 was detected only in occasional myeloid cells, whereas MMP-9 was expressed in some 20-30% of maturing myeloid cells. Normal $CD34^+$ bone marrow cells did not express MMPs. MMPs were detected in almost all MDS and AML cases. In MDS the percentages of MMP-2 positive myeloid cells (median 34%, IQR 18-49%) were significantly higher than in normal controls ($p<0.0001$); in early forms also MMP-9 positive myeloid cells (median 41%, IQR 29-53%) tended to be more numerous than in normal controls ($p=0.06$). MMP-2 and MMP-9 were often coexpressed in MDS myeloid cells; moreover, many erythroblasts expressed MMP-2. There was a significant positive correlation between MMP-2 erythroblast expression and erythroid dysplasia ($p=0.009$) and a significant inverse correlation between either MMP-2 or MMP-9 myeloid expression and blast cell percentage ($p=0.05$ and $p=0.03$ respectively), whereas MMP expression was independent of the apoptosis levels or proliferative rate. In the whole MDS group a high MMP-2 or MMP-9 expression was associated with significantly longer overall survival ($p=0.008$ and 0.02 respectively) and evolution-free survival ($p=0.03$ and 0.05). In AML percentages of MMP-2 positive cells (median 15.5%, IQR 3-28%) lower than in MDS ($p<0.0001$) but higher than in controls and percentages of MMP-9 positive cells (median 8%, IQR 4-21%) lower than in both MDS and control cases ($p<0.0001$) were observed. MMP levels were unrelated to clinical and laboratory aspects nor did they predict response to therapy. In conclusion, we have demonstrated an abnormal MMP expression profile not only in AML but also in MDS. Our findings suggest that the production and release of these enzymes may influence hematopoietic cell behaviour, possibly by the processing of regulatory proteins in marrow. 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.

0220

PROPOSED MINIMAL DIAGNOSTIC CRITERIA FOR MYELODYSPLASTIC SYNDROMES

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Background. In most patients with MDS, the diagnosis based on the typical clinical presentation with macrocytic transfusion-dependent anemia and frank dysplasia in bone marrow smears is straightforward. However, not all patients with MDS have these clinical features at diagnosis, particularly at an early phase of disease. Aims and Methods. To assist in evaluation of these *early phase* patients, we propose minimal diagnostic criteria for MDS, sufficient to separate MDS from other diseases and from idiopathic cytopenia of uncertain significance (ICUS). Results. Our Consensus Working Group recommends that these minimal criteria include two required diagnostic prerequisites: constant cytopenia (for at least 6 months unless accompanied by a specific karyotype or bilineage dysplasia, in which case only 2 months of stable cytopenias are needed) and the exclusion of other potential disorders as a primary reason for dysplasia or/and cytopenia. In addition to these two diagnostic prerequisites, the diagnosis MDS requires at least one of three MDS-related (decisive) criteria: i) dysplasia (at least 10% in one or more of the three major bone marrow lineages), a blast cell count of 5-19%, and iii) a specific MDS-associated karyotype, e.g. del(5q), del(20q), +8, or -7/del(7q). Further, several co-criteria have been defined and can help reach the conclusion that the patient has a bone marrow disease resembling (highly suspicious for) MDS. These co-criteria include flow cytometry, bone marrow histology and immunohistochemistry (to detect or exclude fibrosis, dysplastic megakaryocytes, or an increase and atypical localization of immature progenitors, ALIP), and colony forming progenitor assays. These investigations help to distinguish MDS from patients with ICUS, a condition defined by unexplained constant cytopenia without the minimal diagnostic criteria of MDS. Summary. our newly proposed diagnostic criteria for MDS and for ICUS should assist in early and improved diagnosis and management of these patients.

0221

STEM CELL CULTURES AS A DIAGNOSTIC TOOL IN MYELODYSPLASTIC SYNDROMES

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The diagnosis of myelodysplastic syndrome (MDS) can be difficult as some of the hallmarks of the disease, cytopenias and dysplasia, can occur in other malignant and non-malignant conditions. Cytogenetic abnormalities are helpful in establishing the diagnosis, but are only seen in 40-70% of patients. The quantity and quality of specific hematopoietic progenitors can be assessed using stem cell cultures. Small studies have shown abnormal stem cell cultures in MDS patients, but no large study has evaluated stem cell cultures as a diagnostic tool in MDS. We investigated whether the different MDS subgroups can be distinguished from normal controls using stem cell cultures. We also evaluated whether stem cell cultures can be used in identifying MDS patients with normal cytogenetics and blast count <5% to discriminate this group from normal controls or patients with non-malignant cytopenias and dysplasia. Results of stem cell cultures performed on bone marrow (BM) and peripheral blood (PB) cells of MDS patients (BM and PB n=186, BM only n=43 and PB only n=20) and normal controls (BM n=50 and PB n=30) were examined. Cells were cultured in methylcellulose medium containing 50 ng/mL stemcell factor, 10 ng/mL GM-CSF, 10 ng/mL IL-3, 10 ng/mL IL-6 and 3 units/ml erythropoietin. Colonies were counted at day 16. Patients were subclassified based on the FAB classification. In the BM, an increase was seen in the total number of progenitors in patients with RAEBIT ($p=0.01$) and a decrease in patients with RARS ($p<0.01$), del5q syndrome ($p=0.05$) and MDS-NOS ($p=0.01$). A significant decrease in number of CFU-E (colony-forming unit-erythroid) and BFU-E (burst-forming unit-erythroid) was seen in all subgroups of MDS (p"0.01). CFU-GEMM (colony-forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte) were lower in RARS ($p<0.01$), RAEB ($p<0.01$), del5q syndrome ($p<0.01$) and MDS-NOS ($p<0.01$). CFU-GM (colony-forming unit'granulocyte-macrophage) was significantly higher in RAEBIT ($p<0.01$), del5q syndrome ($p=0.03$) and MDS/MPD ($p<0.01$). In the PB we observed an increase in total progenitors in RAEBIT ($p=0.01$) and MDS/MPD ($p<0.01$); a decrease in BFU-E in RARS ($p<0.01$), RAEB ($p<0.01$), RAEBIT ($p<0.01$) and del5q syndrome ($p=0.02$); a decrease in CFU-GEMM in RARS ($p<0.01$), RAEB ($p<0.01$), RAEBIT ($p<0.01$) and del5q syndrome ($p=0.02$); and an increase in CFU-GM in patients with RA ($p=0.04$), RAEBIT ($p<0.01$), del5q syndrome ($p<0.01$) and MDS/MPD ($p<0.01$). MDS patients with normal karyotype and blast count <5% (BM and PB n=54, BM only n=14 and PB only n=7) could be distinguished from normal controls and patients with non-malignant cytopenias and dysplasia by a decrease in total progenitors ($p<0.01$ and $p<0.01$ respectively) in the BM. Erythroid progenitors and CFU-GEMM were also significantly decreased compared to normal and the non-malignant group (CFU-E $p<0.01$ and $p=0.01$, BFU-E $p<0.01$ and $p<0.01$, CFU-GEMM $p<0.01$ and $p<0.01$). No significant differences were seen in the PB. In conclusion, stem cell cultures not only distinguish between normal controls and the subgroups of MDS, they are also helpful in discriminating between patients with MDS and patients with non-malignant cytopenias and dysplasia. More importantly, these findings can give insight into the pathogenesis of this set of diseases.

0222

COMPARISON OF IWG 2006 AND IWG 2000 RESPONSE CRITERIA FOR ANEMIA OF MDS IN 419 PATIENTS: THE GFM EXPERIENCE

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Backgground. Correction of anemia is an important goal in low risk MDS. Erythroid response criteria were recently modified (IW 2006) with elimination in particular of minor responses. *Aims*. In order to evaluate IW 2006, we compared responses according to IWG 2000 and 2006 criteria in a cohort of lower risk MDS. *Methods.* 419 MDS pts including 61 RA, 68 RCMD, 81 RARS, 57 RCMD-RS, 93 RAEB-1, 26 RAEB-2, 17 5q-syndromes and 16 CMML, median age 73.5 years, with Hb <10 g/dL and RBC transfusions in 55% patients, received 30,000 to 60,000 U/week of EPO or 300 mg DAR±G-CSF during at least 12 weeks in GFM centers(Park S et al, ASH 2006 #522). IPSS score was low, int-1, int-2 and high in 38%, 49%, 11% and 2%. *Results.* According to IWG 2000 criteria, the overall response rate was 63% (41% major and 22% minor) and median response duration 20 months, (24 and 14 months for major and minor responses). Predictive factors of response were endogenous EPO <200 UI/L, transfusion <2 RBC/month, IPSS low and int-1 but not WHO classification. With IWG 2006 criteria, 49% of the patients became responders and median response duration was 24 months. Prognostic factors of response were similar to those using IWG 2000 criteria except that IWG 2006 lowered response rates mainly in RARS, RCMD-RS and RAEB-1 (p=0.02 for responses in RARS+RCMD-RS vs. RA+RCMD, p=0.04 for RAEB-1 vs. MDS with <5% BM blasts) (Table 1).

Table 1.

Significantly shorter response was seen with IPSS>1 and presence of multilineage dysplasia, using both IWG 2000 and 2006. Of 88 minor responders (IWG 2000), 32/88 (36%) and 56/88 (63%) were reclassified as *responders* (R) and *non responders* (nR) with IWG 2006. R did not differ from nR for EPO level, transfusion requirements and WHO, but tended to differ for% of IPSS low and int-1 (100% in R, 88% in nR, p=0.06). Response duration was 16 months vs. 12.5 months in R vs. nR (p=NS). *Summary and Conclusions.* With switching from IWG 2000 to 2006 multilineage dysplasia and/or sideroblastic anemia tended to be poorer prognostic factors of response. On the other hand, among IWG 2000 minor responders, outcome of those who remained IWG 2006 *responders* could not be distinguished from those who became *non responders*, questioning the elimination of this category which, for instance, could benefit from combination therapy with EPO and other drugs.

0223

FUNCTIONAL CHARACTERIZATION OF ADULT MESENCHYMAL STROMAL CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Introduction. There is still some debate whether the *microenvironment* or *stroma* is part of the clone or rather has bystander functions in patients with MDS. Interestingly, some studies indicate that mesenchymal cells are cytogenetically and functionally normal, whereas others suggest that important quantitative and functional alterations are present in patients with MDS. Given the complex network of different cell types summarized within the term *stroma* including macrophages, known to be clonally involved, the aim of this study was to characterize mesenchymal stroma cells (MSC) of MDS patients in comparison to normal donors. *Methods.* Bone marrow samples were collected from healthy donors (n=12) and patients with MDS (n=19; 5q-syndrome n=5, RA(RS) n=5, RAEB n=9). After density centrifugation MSC were isolated according to the standard adhesion protocol and further characterized by flowcytometry, cytogenetic analysis, clonogenic (CFU-F) and osteogenic differentiation assays. Additionally, secreted SDF-1 levels and the ability of MSC to support long-term growth of hematopoietic cells (LTC-IC) were determined. *Results.* The efficiency of MSC generation and expansion was highly reduced in some patients. In fact, MSC from one third of the MDS patients seemed to grow slower than controls with a prolonged time to reach confluence. Additionally, preliminary data also suggest decreased levels of secreted SDF-1. Nevertheless, fibroblastic colony formation (CFU-F) was comparable to controls (control: 29.7±13.2; n=8, MDS: 25.±31.83; n=5). Long-term cultures (LTC-IC) showed a profound support and colony formation of healthy $CD34^+$ cells. Like controls, MSC from MDS patients also expressed the typical antigens for MSC being positive for CD 105, CD73, CD166, CD90 and negative for typical hematopoietic markers like CD45 and CD34. Patient specific chromosomal aberrations, being present in hematopoietic cells, could not be detected within the MSC pool by FISH analysis. Additionally, osteogenic differentiation assays resulted in the typical presence of Ca3(PO4)2 crystal deposits detected by von Kossa staining, and an increase in alkaline phosphatase activity. Interestingly, the latter could be inhibited by the incubation with thalidomide (100 ∝M). *Conclusions.* These data do not suggest that MSC are generally involved in the pathogenesis of patients with MDS. Nevertheless, there might be an altered response of MSC to the endogenous environment or exogenous signals e.g. drugs.

0224

CLINICAL RELEVANCE OF CYTOGENETIC EVOLUTION IN MYELODYSPLASTIC SYNDROMES: A STUDY ON 151 PATIENTS

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Our study wants to evaluate whether the cytogenetic pattern may change during MDS outcome, establishes how the newly developed chromosomal abnormalities effect overall survival (OS) and progression-free interval (PFI) and distinguishes chromosomal defects simply indicating karyotipic instability from those truly heralding MDS/AML progression. The 151 patients entered in the study were examined between January 1990 and December 2005 and were included in a larger series of 491 consecutive MDS patients. Seventy-four were females and 77 males; their median age was 60 years (range 18-89). According to FAB classification 30 patients were classified as RARS, 51 as RA, 59 as RAEB and 11 as RAEB-t. WHO classification was applied to 140 patients, 17 classified as RARS, 19 as RA, 12 as 5q- syndrome, 32 as RCMD-RCMDS, one as U-MDS, 28 as RAEB-1 and 31 as RAEB-2. Considering IPSS score, 34 patients were low-risk, 55 intermediate-1 risk, 38 intermediate-2 risk and 24 high-risk. At the time of the analysis 60 patients have experienced MDS/AML progression and 39 have died. Median cytogenetic follow-up was 22 months (range 2-168) and each patient was examined at least three times. On clinical diagnosis a normal karyotype was revealed in 56 patients, a 5q- syndrome in 12, -7 in eight, del(7)(q31q35) in six, +8 in nine, 12p- in seven, 20q- in seven, various defects in thirty-six, and a complex karyotype (with>3 defects) in five. Interestingly, five additional patients presented normal metaphases along with 1-3 metaphases showing defects not confirmed by FISH (non clonal defects). All reverted to a completely normal karyotype on subsequent analyses. Overall, a cytogenetic evolution occurred in a total of 48 patients (31.7%). It significantly predicted MDS/AML progression (p<0.001) and occurred in 35/60 (58.3%) patients who had progressed and in only 13/91 (14.2%) patients who did not. In the former patients the most common defects were +8 (22.4%), 5q- (14.2%), 7q- (8.1%), 17p-(8.1%), 3q-(6.1%), whereas in those with stable MDS various defects were observed in a limited number of cells (1-3%). Considering progressed patients only, cytogenetic evolution occurred in 11/16 normal karyotypes, in 2/3 5q- syndromes, in 3/4 del(7)(q31q35), in 3/8 +8, in 3/5 20q-, in 7/14 various defects and in 2/2 complex karyotypes; in contrast, it occurred at a lower frequency in -7 (2/8 patients). Intriguingly, a patient with the 5q- syndrome developed an additional clone with a single +8 which became predominant upon progression into RAEB-2 and a RA patient showed the disappear-

ance of the original clone marked by t(7;16)(p15;p13) and developed a new clone marked by 5q- along with +8 just before AML progression. In conclusion, i) in our series karyotipic evolution occurred in ablout 31% of patients; ii) some non clonal defects, which presence was not confirmed by FISH, simply signaled the instability of the dysplastic clone; iii) some clonal defects, especially +8, del(7)(q31q35) and 17p-, represented true steps in disease progression; iv) cytogenetic evolution significantly predicted MDS/AML progression and when occurred in patients of the IPSS good risk cytogenetic category completely changed their outcome.

0225

T-CELL RECEPTOR VB CDR3 OLIGOCLONALITY FREQUENTLY OCCURS IN CHILDHOOD REFRACTORY CYTOPENIA

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Background. Severe acquired aplastic anemia (SAA) and myelodysplastic syndrome (MDS) are rare diseases in childhood. SAA is a bone marrow failure syndrome charecterized by immune mediated destruction of hematopoietic progenitors. MDS is a malignant clonal stem cell disorder, in which the hypoplastic variant is, in case of absence of a cytogenetic clone, difficult to separate from SAA. Recently, studies provided a *molecular signature* of autoimmunity in adult SAA, by showing oligoclonality of TCR Vβ CDR3 region, which is refered to as TCR Vβ skewing. We investigated the value of TCR Vβ repertoire analysis in pediatric MDS-RC and (v)SAA patients. *Methods.* Peripheral blood and/or bone marrow mononuclear samples of patients with (v)SAA (n=38), MDS-RC (n=28) and 18 controls were analysed with Vβ heteroduplex analysis of extracted RNA. *Results.*Skewing was found in 21/38 (55%) of the SAA patients and in 17/28 (61%) of the RC patients. Seventeen patients with clinical SAA showed no oligoclonality. A significant difference in TCR skewing was found between the (v)SAA + MDS-RC patients as compared to the controls (χ^2 analysis, p=0.001), but not between MDS-RC and (v)SAA (χ^2 analysis, p=0.8). In this study paired samples (PB and BM) of 25 cases showed a high correlation between the skewing results in both compartments (Pearson correlation, rr 0.98). *Conclusions.* In this study TCR Vβ repertoire analysis did not discriminate between MDS-RC and (v)SAA. Prospective studies will be necessary to investigate whether there is a role for this molecular tool in pediatric MDS-RC for the identification of a subset of patients that is associated with autoimmunity and therfore could be treated with IST up-front, and whether it can be used for molecular response monitoring.

0226

INVOLVEMENT OF A T-CELL RECEPTOR REPERTOIRE IN THE PATHOGENESIS OF MYELODYSPLASTIC SYNDROMES

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Background and aims. The involvement of a T cell-mediated autoimmune process in the pathogenesis of the cytopenia in myelodysplastic syndromes is still under evaluation. To further investigate a T-cell involvement in the pathogenesis of MDS we have studied the complementarity-determining region 3 (CDR3) size distribution of the CD3, CD4 and CD8 T-cell receptor (TCR) V-β-chain subfamilies in the bone marrow and peripheral blood samples of MDS patients (n=63). *Methods.* We used the multiplex PCR based technique TCR spectratyping based on the size heterogeneity of the CDR3 region and compared the results with age-matched controls (n=16). *Results.* TCR V-β skewing of T-cell repertoire is more frequent in the bone marrow samples of MDS patients than in the whole blood samples. The most frequently skewing of TCR V-β fragments occurs in V-β short 1 (28%), V-β short 3 (20%) and V-β short 5.3 (25%). CD8 T-cells harbour the most pronounced deviations from a Gaussian distribution of TCR fragment length compared to CD4 T-cells. MDS subtype RA shows a different spectratyping pattern compared with MDS subtype RAEB. Five of the patients are now far enough after different types of therapy to evaluate the changes of the spectratyping pattern. *Summary.* We conclude that TCR V-β skewing is frequent in MDS especially an CD8 T-cells. Normalization of at least 1 initially skewed V-β profile after therapy occurred in 5 MDS patients. The analysis of a potential correlation between response to therapy and normalization of the TCR repertoire still requires a larger population.

0227

PRACTICAL RECOMMENDATIONS ON THE MANAGEMENT OF HAEMATOLOGICAL ADVERSE EVENTS IN MDS PATIENTS TREATED WITH LENALIDOMIDE

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Background. In January 2007, an international group of myelodysplastic syndromes (MDS) specialists reached a consensus on practical recommendations regarding the management of Lenalidomide (Len) treatment in transfusion-dependent intermediate-1/low-risk (IPSS) MDS patients with del5q. *Aims.* The presentation of practical recommendations on the management of haematological adverse events (AE) in MDS patients treated with Len. *Methods.* A moderated round table discussion. *Results.* In published studies, haematological AE have been the most common AE and the most frequent reason for dose adjustment. Transient neutropenia of CTC grade 3 or more occurred in 55%, mostly early in the treatment phase. Weekly monitoring of full blood count (FBC) is mandatory for the first 2 months of treatment, and may be continued biweekly up to 5 months. Biweekly monitoring may be considered thereafter, depending on haematological status. To prevent severe neutropenia, coadministration of G-CSF is recommended if the absolute neutrophil count (ANC) reaches <1000/∝L, or Len can be temporarily discontinued. As neutropenic sepsis is the only reported drug-related cause of death, patients should receive clear guidance how to react in the event of febrile neutropenia. This includes patient education, access to specialised haematological care at all times and application of antibiotics within 3 hours of fever onset. Transient thrombocytopenia of CTC grade 3 or more occurred in 44%. Len should be interrupted if platelets fall <25000/∝L without platelet support. Thrombocytopenia at baseline is not a contra-indication for Len; prophylactic thrombocyte transfusions may be considered until counts rise. Venous thromboembolism (VTE) occurred in 3% of MDS patients receiving Len monotherapy. Concurrent use of erythropoietin may increase the risk of VTE. Although VTE prophylaxis is not generally recommended in MDS patients because of the low incidence and an increased risk of bleeding, patients should be informed about and monitored for symptoms. In patients with VTE it is prudent to interrupt Len treatment and carefully re-introduce once stable anticoagulation has been established. In some patients, polycythaemia may occur. In those cases, Len should be continued and phlebotomy may be considered, depending on ferritin levels. Although usually transient, sometimes treatment interruption may be necessary. *Discussion.* A stringent strategy for the management of haematological AE due to treatment with Len is recommended.

0228

COMPARISON OF HYPOPLASTIC MYELODYSPLASTIC SYNDROME WITH NORMO-/HYPERCELLULAR MDS BY INTERNATIONAL PROGNOSTIC SCORING SYSTEM, CYTOGENETIC AND GENETIC STUDIES

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Background. Hypoplastic myelodysplastic syndrome (MDS) differs from aplastic anemia in several ways including bone marrow morphology, cytogenetic changes, and prognosis. The distinction between hypoplastic MDS (h-MDS) and normo-/hypercellular MDS (NH-MDS) remains unsettled. *Aims.* In this study, we aimed to compare h-MDS and NH-MDS patients in terms of clinical and laboratory features, chromosomal abnormalities, genetic and epigenetic markers, and prognosis. Meanwhile, the applicability of International Prognostic Scoring Sys-

tem (IPSS) to h-MDS was also investigated. *Methods*. We retrospectively reviewed medical records of 227 primary MDS patients diagnosed according to the criteria of French-American-British (FAB) classification at National Taiwan University Hospital. Secondary MDS due to antecedent hematological diseases or drugs was excluded. Every patient received bone marrow (BM) aspiration and trephine biopsy simultaneously. All patients were categorized by FAB classification and IPSS. MDS patients with BM cellularity less than 30% were considered as having h-MDS. Metaphase G-banding technique was utilized for chromosome study. Mutations of RAS, AML1, JAK2, PTPN11, KIT and FLT3 internal tandem duplication were analyzed by PCR with pertinent primers followed by direct sequencing. Hypermethylation of SOCS1 and SHP1 was detected by methylation-specific PCR. Demographics, complete blood counts, cytogenetics, genetic and epigenetic markers were all compared by Mann-Whitney U test or Pearson chi-sqaure test. Acute leukemic transformation rate and survival analysis were performed with Kaplan-Meier analysis. *Results*. Among the 37 patients (16.3%) diagnosed as having h-MDS, male-to-female ratio was 3.6 to 1. Mean white blood cell count, absolute neutrophil count and platelet count were significantly lower in h-MDS than in NH-MDS ($p<0.001$, $p=0.002$, and $p=0.014$ respectively). Based on FAB classification, refractory anemia (RA) was the predominant subtype (56.8%) in h-MDS whereas refractory anemia with excess of blast (RAEB) was the most common diagnosis (36.3%) in NH-MDS. Clonal cytogenetic abnormality was detected in 14 (42.4%) of 33 h-MDS patients and 76 (42.2%) of 180 NH-MDS patients who had the study. Monosomy 7 or 7q deletion was not found in h-MDS patients, compared with 16.1% in NH-MDS patients ($p=0.023$). In an array of genetic markers including mutations of RAS, AML1, JAK2, PTPN11, the internal tandem duplication of FLT3, and hypermethylation of SOCS1 and SHP1, there was a trend of lower incidence of RAS and AML1 mutations in h-MDS patients. But the distribution of other genetic alterations in these two groups of patients was not significantly different. Acute leukemic transformation occurred less frequently in h-MDS patients than in NH-MDS patients (8.1% vs. 27.1%, $p=0.005$)(Figure 1). Using Kaplan-Meier survival analysis, International Prognostic Scoring System (IPSS) was proved to be an ideal parameter to predict prognosis in h-MDS patients (median survival: 112 months in patients with low and Int-1 risk subgroups vs. 16 months in those with Int-2 and high risk subgroups, $p=0.002$)(Figure 2), similar to NH-MDS patients (Figure 3). Among patients of low and Int-1 risk subgroups, h-MDS patients had a significantly longer survival than NH-MDS patients (median survival: 112 months vs. 32 months, $p=0.002$) (Figure 4). *Conclusions*. Distinct from NH-MDS, h-MDS has different patterns of hemogram, FAB classification distribution, cytogenetic change and prognosis. IPSS is applicable to prognostic evaluation in h-MDS as in NH-MDS. In MDS patients with Low and Int-1 risk subgroups, h-MDS patients have better prognosis than NH-MDS patients.

Figure 1-2-3-4. Kaplan-Meier analysis of myelodysplastic syndrome.

0229

PRACTICAL RECOMMENDATIONS ON THE MANAGEMENT OF NON-HAEMATOLOGICAL ADVERSE EVENTS IN MDS PATIENTS TREATED WITH LENALIDOMIDE

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Background. In January 2007, an international group of myelodysplastic syndromes (MDS) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment in patients with transfusion-dependent MDS with del5q. *Aims.* To present practical recommendations on the management of non-haematological adverse events (NHAE) in intermediate-1 and low-risk (IPSS) MDS patients with del5q treated with lenalidomide. *Methods.* A moderated round table discussion. *Results.* The most common NHAE of any CTC grade were rash (36%), pruritus (42%) or dry skin (14%). Severe skin reactions were observed in 6%. Most resolve with time and do not necessitate discontinuation of lenalidomide treatment. A course with unselective antihistamines (e.g. clemastin) has proven effective in most patients. If unsuccessful, a short course (14 days) of 10 mg prednisone orally may be attempted. In case of persistent or severe rash, lenalidomide should be interrupted until it resolves. In the experience of the panel, lenalidomide can then be restarted without recurrence. Other NHAE include fatigue (grade 3/4: 3%), pruritus (3%), diarrhoea (3%), nausea (3%), and muscle cramps (2%). As these are mostly non-specific symptoms, other causes such as anaemia or auto-immune disorders should be ruled out. Generally, patients should be screened for thyroid-stimulating hormone (TSH) and baseline TSH levels should be compared to levels during the course of treatment. Diarrhoea is a common NHAE that should be treated symptomatically after other underlying causes have been ruled out. In some cases, diarrhoea has been attributed to the lactose in lenalidomide tablets and additional lactase supplementation has been helpful. Unlike thalidomide, lenalidomide does not lead to dose-dependent peripheral neuropathy, somnolence or constipation. In general, lenalidomide is well tolerated in patients with intermediate and low-risk MDS with del5q. *Discussions*. A concise strategy for the management of NHAE of lenalidomide is presented, which will aid safe administration and avoid unnecessary dose reduction and discontinuation, thus assuring best efficacy.

0230

CORRELATION BETWEEN OCCURRENCE OF CYTOPENIAS AND RESPONSE TO LENALIDOMIDE THERAPY IN DEL 5Q MDS PATIENTS

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Background. Lenalidomide is effective in myelodysplastic syndromes (MDS) patients with or without deletion (del) 5q cytogenetic abnormalities. The most common toxicities reported with lenalidomide therapy are neutropenia and thrombocytopenia. Both occurrence of cytopenias and response to lenalidomide therapy is more common in patients with the del 5q cytogenetic abnormality. *Aims.* This analysis was conducted to determine if the occurrence of cytopenias predicts response to lenalidomide treatment in del 5q MDS patients. *Methods.* This is a retrospective data analysis of patients enrolled in the MDS-003 study. Transfusion-dependent MDS patients with an associated del 5q abnormality were treated with 10 mg lenalidomide (daily or 21/28 days). Thrombocytopenia was defined as a platelet count <150,000/mm^3; neutropenia as an absolute neutrophil count (ANC) <2000/mm^3 (grade 1-4 using the CTC v2.0 or 3.0). Medication side effects of platelet and neutrophil compromise were assessed within the first 8 weeks of therapy. Response to lenalidomide therapy was assessed using the International Working Group criteria. *Results.* The 148 patients enrolled had a median age of 71 yrs, median duration of MDS of 2.5 yrs and median RBC transfusion rate of 6 units/8 weeks; 66% were female and 81% had a low/int-1 risk IPSS score. Of 147 evaluable patients, 59 (40%) had throm-

bocytopenia at baseline, 59 (40%) had neutropenia and 84 (57%) had either neutropenia or thrombocytopenia. As reported previously (List et al. NEJM 2006), 99 patients (67%) achieved RBC transfusion independence. Regardless of whether or not a patient was thrombocytopenic at baseline, a drop in platelet count by ≥50% within the first 8 weeks of therapy correlated with a higher chance of RBC transfusion independence (p=0.005). Comparing patients who had a ≥50% drop vs those who did not, transfusion independence was achieved in 76% vs 47% of patients without baseline thrombocytopenia and in 67% vs 38% of patients with baseline thrombocytopenia. Similar results held for patients without baseline neutropenia: 82% whose ANC fell ≥75% achieved RBC transfusion independence, compared to 56% whose ANC fell <75% (p=0.018). In patients with baseline neutropenia, however, ANC drop did not correlate with RBC response (p=0.75). In patients with at least one cytopenia at baseline, those whose ANCs fell by ≥75% and platelets fell by ≥50% were more likely to achieve RBC transfusion independence than those whose counts did not drop substantially, even controlling for baseline cytopenias (71% vs. 60%, p=0.024). *Summary and conclusions.* In MDS patients with del 5q, a drop in platelet count and, in those with normal baseline ANCs, a drop in ANC, correlates with response to lenalidomide, thus indicating an association between the induction of cytopenias and therapeutic response.

0231

ENDOPLASMIC RETICULUM GENE EXPRESSION PROFILE OF ERYTHROID PROGENITORS IN LOW RISK MYELODYSPLASTIC SYNDROMES

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Background. Ineffective erythropoiesis in low risk myelodysplastic syndromes (LR-MDS) is supported by morphological evidence and an impaired *ex vivo* differentiation of erythroid precursors with excessive apoptosis. We have previously shown that excessive apoptosis of eythroid precursors obtained by *ex vivo* culture of MDS bone marrow CD34+ cells involved a Fas-dependent amplification of caspase activation. We have observed also an accumulation of calcium in the endoplasmic reticulum (ER) of LR-MDS erythroid precursors and demonstrated a role for the ER in the spontaneous apoptosis of these cells. Whether these abnormalities, i.e. activation of a Fas-mediated pathway to death and abnormal accumulation of Ca^{2+} in the ER, are driven by intrinsic defects (such as genetic and epigenetic changes) or by extrinsic signals (from an altered bone marrow microenvironment or an auto-immune response), remains controversial. *Aim and Methods.* In the pilot phase of a study to provide some answers to these questions, we analyzed the gene expression signature of LR-MDS and normal control erythroid progenitors derived by *in vitro* liquid culture from $CD34^+$ bone marrow cells. Five LR-MDS (RA = 4, RAEB<10% blasts = 1) and 4 normal control samples were cultured for 7 days in the presence of Epo, IGF-1, SCF and dexamethasone. At this time point, the culture generated a majority of proerythroblasts and basophilic erythroblasts. In LR-MDS samples, apoptosis of erythroid precursors was observed 2 to 3 days later. For each sample, cRNA was processed for hybridization to GeneChip HG U133A gene chips (Affymetrix). *Results.* After normalization of signals using the GC-RMA method and significance analysis (ANOVA, p<0.01), we identified the differential expression of 134 genes between patients and controls. Based on this signature, hierarchical clustering using the GeneSpring software clearly separated LR-MDS from normal samples. Ten of these transcripts were confirmed to be differentially expressed in 8 LR-MDS and 6 control samples by RT-qPCR. Neighborhood analysis identified a pattern of 107 genes predictive of MDS (underexpressed: 66; overexpressed: 41). Ingenuity software classified these genes according to their biological theme (cell death & stress: 48, cell growth & signaling: 24, cell cycle: 18, DNA replication and repair: 17). In the group of cell death & stress genes, 27 (59%) play a role in ER stress response. Twenty-four of them (protein synthesis: 13; protein folding and maturation: 6; protein transport: 1; calcium homeostasis: 3; transcription: 1) were downregulated whereas three others (CALR, HSPA5, PDIA4) were overexpressed. In accordance with these changes, immunoblot analysis of LR-MDS erythroid cells identified an increased expression of calreticulin and Grp78/HspA5 proteins in MDS erythroid progenitors. *Conclusions.* Altogether, this preliminary study performed on a small number of samples suggests that a gene signature of LR-MDS erythroid precursors could be identified. The importance of changes in the expression of genes that regulate the ER metabolism enforces the previous demonstration of a role of this organelle in the disease pathophysiology.

0232

ALLOGENEIC STEM CELL TRANSPLANTATION FOR MDS AND SAML FOLLOWING FLAMSA-CHEMOTHERAPY, REDUCED INTENSITY CONDITIONING AND PREEMPTIVE DONOR LYMPHOCYTE TRANSFUSION

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Myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia (sAML) are diseases of the hematopoietic stem cell that are potentially curable by allogeneic stem cells transplantation (ASCT). MDS and sAML are most frequent in elderly patients with significant co-morbidity. Here we report our results with reduced intensity conditioning regimen and preemptive donor lymphocyte transfusions that have been successful in patients with advanced and refractory AML. Sixty seven patients were treated for MDS and 90 patients for sAML. The FLAMSA regimen consisted of a 4 day course of chemotherapy (Ara-C 2 g/m^2, Fludarabin 30 mg/m^2, and amsacrin 100 mg/m^2) followed by 3 days rest and reduced intensity conditioning with 4 Gy total body irradiation (TBI), cyclophosphamide (CY)±antithymocyte globulin (ATG). Overall survival at 10 years was 50% for MDS and 26% for sAML (p=0.014, log rank). In the MDS group most patients had preleukemic forms (IPSS 3 and 4). Survival at 5 years was 58% with FLAMSA, 44% with myeloablative conditioning with TBI and 67% with Busulfan (BUS). Non-relapse mortality at 5 years was 41% with FLAMSA, 20% for BUS and 50% for TBI. The relapse or progression rate was 0% for FLAMSA, 24% for BUS and TBI respectively. In sAML the 5 year survival was 17% for FLAMSA, 57% for BUS and 30% for TBI. Non-relapse mortality was 60% for FLAMSA, 36% for BUS and 66% for TBI, whereas the relapse rate was 62% for FLAMSA, 12.5% for BUS and 37% for TBI (p=0.04). In multivariate analysis adjusted for disease, conditioning treatment and source of stem cells ' marrow vs. mobilized blood - differences were not significant and the only significant prognostic factor was age less than 30 years (p=0.003). Therefore patients of the age of 60 years and older with AML were treated with FLAMSA containing BUS (8 mg/kg in two days) instead of 4 Gy TBI. The overall survival at 2 years was 64%, 61% with FLAMSA-TBI and 70% with FLAMSA-BUS. The non-relapse mortality was 9% for FLAMSA-BUS and 37% for FLAMSA-TBI; the relapse rate was 25% at 2 years. At day 120 patients received donor lymphocytes (DLT) in3 escalating doses from 1×10^6/ kg, 5×10^6/ kg on day 150 and 1×10^7/kg on day 180, if they had no GVHD, infection or relapse. Preemptive DLT appear to prevent relapse when compared to a historical control and contemporary patients not given DLT. We conclude from these data that MDS/sAML may have a different biology in young patients, and that in the elderly we can optimize the treatment with FLAMSA-BUS as a dose reduced regimen and preemptive immunotherapy for maintenance of remission.

0233

PREFERENTIAL CYTOGENETIC RESPONSE TO LOW-DOSE DECITABINE IN MDS WITH CHROMOSOME 7 ABNORMALITIES

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Background. Decitabine (5-aza-2'-deoxycytidine, Dacogen®, DAC) is a powerful demethylating agent that induces hematologic and cytogenetic remissions even in elderly and frail patients (pts) with MDS and AML. Cytogenetic remissions in pts with chromosomal aberrations are associated with improved overall survival (Lübbert et al., Br. J. Haematol. 114:349-57, 2001). The other zanucleoside very active in MDS, Vidaza® (5-azacytidine) has been suggested as effective in MDS patients with monosomy 7 (Raj *et al.,* ASH 2005, Blood 106 (Suppl. 1): 2530a). We now performed a retrospective evaluation of cytogenetic responses in MDS patients with aberrations of chromosome 7 upon treatment with low-dose DAC. *Methods.* 115 successfully karyotyped MDS pts (median age 70 years, range 38-89) treated with low-dose DAC (120-150 mg/mÇ) within three European phase II trials (PCH91-1, PCH95-11,

PCH97-19) were studied. Conventional cytogenetic analyses were done on bone marrow samples prior to therapy and during treatment (response evaluation prior course three and later). In selected cases, monosomy 7 was monitored by fluorescence *in situ* hybridization (FISH) during treatment. *Results.* Of 115 karyotyped pts, 51 (44%) had a normal karyotype and 64 (56%) had chromosomal aberrations. 34 (30%) pts showed single aberrations, including 6 pts with loss of chromosome 7 or del(7)(q22q34). In 20 (17%) pts, complex karyotypes were detected, 11 of these contained chromosome 7 abnormality. 4/6 (67%) pts with isolated chromosome 7 abnormality achieved complete (n=3) or major (n=1) cytogenetic responses. Median number of courses until best cytogenetic response was 2 (range 2-3), median time to (cytogenetic) relapse was 13.5 months (range 9-15). Median cytogenetic response duration in the 15 pts with other cytogenetic abnormalities was 8 months (range 3-12), this difference was statistically significant by t-test. 4/11 pts with complex karyotype including aberrations of chromosome 7 showed cytogenetic responses (median response duration 8 months, range 7-9) compared to 2 responders out of 9 pts with complex karyotype not containing chromosome 7 abnormality. *Conclusions.* A high cytogenetic response rate to low-dose DAC was seen in pts with sole chromosome 7 abnormalities, with a significantly longer response duration than for responding pts with aberrations of chromosome 7 in the context of a complex karyotype, or cytogenetic responders without initial chromosome 7 abnomalities. The reason for this preferential response is presently unclear, but might be associated with the more frequent methylated phenotype of these patients

References

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0234

DISEASE PROGRESSION IN DEL(5Q) MDS PATIENTS TREATED WITH LENALIDOMIDE: ANALYSIS OF RISK FACTORS AND LONG TERM OUTCOME IN 45 PATIENTS

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Lenalidomide is a novel immunomodulatory drug (IMiD?) that has successfully been used in patients with transfusion-dependent MDS with del(5q.31) chromosomal abnormality. The recently published Lenalidomide-MDS-003 study reported a 76% erythroid response rate with 67% transfusion independency in an intent-to-treat analysis of 148 patients. This result was unaffected by cytogenetic complexity or bone marrow blast percentage in the study population. During the first year of study, a number of disease progressions to higher FAB subtypes or to AML occurred that raised the question whether lenalidomide might promote disease progression in some patients. *Patients and Methods.* We retrospectively analysed data on 45 patients that were treated with lenalidomide at our institution to identify risk profiles that might account for progression. 28 female patients and 17 male patients (median age 71 years) were treated with initial lenalidomide doses of 10 mg po daily. Additional therapy used was G-CSF in case of neutropenia grade >2 and antibiotics. *Results.* 13 out of 45 patients (29%) experienced progression of disease to either higher FAB subtype or AML. Analysis of contributing factors showed that 7 of the 13 patients had RAEB at the point of first drug intake. 3 of those 7 patients had additional chromosomal aberrations (2, trisomy 21; 1, complex karyotype). Of the remaining 6 patients, 2 had a complex karyotype at time of lenalidomide therapy commencement, and 1 an additional inv(9)(p11q12). Of the remaining 3 patients, 1 patient had a hypocellular bone marrow at the start of lenalidomide therapy so that no FAB subtype could be assigned. *Conclusions.* Within the subgroup of del(5q) MDS, the best survival has been identified in patients with an isolated del(5q) chromosomal aberration and a bone marrow blast count of <5%. Patients with additional chromosomal abnormalities or a higher blast percentage have a much shorter overall survival and a higher risk for AML development. Our retrospective analysis confirms these data showing that progression to higher MDS subtypes or AML has nearly exclusively happened in patients with additional risk factors like >5% bone marrow blasts or additional chromosomal anomalies. Only 2 out of 45 patients (4.4%) with 5q-syndrome progressed to AML. Interestingly, both those patients developed acute erythroid leukaemia (FAB M6). Lenalidomide does not seem to increase the risk of transition of del(5q) MDS to higher stages of disease.

Myeloproliferative disorders - Biology

0235

FUSION OF FIP1L1 AND RARA AS A RESULT OF A NOVEL T(4;17)(Q12;Q21) IN A CASE OF JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile myelomonocytic leukemia (JMML) is a pediatric myeloproliferative disease (MPD), characterized by proliferation of granulocytic and monocytic lineages. 17q12 RARA rearrangements are the hallmark of acute promyelocytic leukemia (APL), characterized by a differentiation arrest of abnormal promyelocytes. Beside the frequent t(15;17) resulting in PML/RARA fusion, variant rearrangements involving 17q21 RARA in APL are PLZF/RARA t(11;17)(q23;q21), NPM1/RARA?t(5;17)(q35;q21), NUMA/RARA ?t(11;17)(q13;q21), STAT5b/RARA?der(17) and t(3;17)(p25;q21). In chronic eosinophilic leukemia (CEL) a FIP1L1/PDGFRA fusion gene has been identified due to a chromosome 4q12 interstitial deletion. FIP1L1 is an integral subunit of cleavage and polyadenylation specificity factor (CPSF). By FISH and RT-PCR analyses we identified FIP1L1/RARA fusions as a result of a t(4;17)(q12;q21) in a case of JMML. Sequencing analysis demonstrated an in-frame FIP1L1/RARA?fusion of exon 15 of FIP1L1, which is downstream of FIP1L1/PDGFRA breakpoints that are distributed in introns 7-13. The breakpoint fuses exon 3 of RARA, identical to all other RARA fusions. All known chimeric RARA fusion proteins provide additional homodimerization motifs, promoting formation of chimeric homodimers critical for leukemic transformation. Recently, it was shown that FIP1L1/PDGFRA mediated transformation, is FIP1L1 independent and results from disruption of the autoinhibitory JM domain of PDGFRA. Fusion of a strong homodimerization domain of ETV6 to PDGFRA could overcome the inhibitory function of the PDGFRA JM domain. These studies suggest that FIP1L1 does not seem to render direct homodimerization ability to FIP1L1/PDGFRA. Observations using retroviral transduced FIP1L1/PDGFRA?and FIP1L1/PDGFRA with an N-terminal deletion of the FIP1L1 moiety showed differences with respect to cytokine-independent colony formation and activation of multiple signaling pathways in human primary hematopoietic precursor cells (personal communication, Dr. Buitenhuis and Prof. Coffer, Dept. Immunology, University Medical Center Utrecht). These observations indicate that FIP1L1 does contribute to FIP1L1/PDGFRA resulting in a myeloproliferative phenotype. Therefore, our results, together with the data van Buitenhuis and Coffer, suggest a functional role of FIP1L1 in both chimeric proteins other than overt self-association. In conclusion, we report a t(4;17)(q12;q21) resulting in reciprocal FIP1L1/RARA fusion transcripts in a case of JMML. This is the second chromosomal aberration involving 4q12 FIP1L1 in MPD. Functional studies using FIP1L1/RARA and FIP1L1/PDGFRA might give new insights in the mechanistic contributions of chimeric FIP1L1 and RARA fusion proteins in MPD and APL. We will discuss this remarkable observation in relation to disease phenotype and molecular mechanism.

0236

BONE MARROW RENIN-ANGIOTENSIN SYSTEM EXPRESSION IN POLYCYTHAEMIA VERA AND ESSENTIAL THROMBOCYTHAEMIA DEPENDS ON JAK2 MUTATIONAL STATUS

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Background. Discovery of V617F mutation in JAK2 tyrosine kinase gene in myeloid progenitors provided new insight into the pathogenesis and clinical understanding of CMPD. There are several lines of evidence suggesting the existence of local hematopoietic bone marrow renin-angiotensin system (RAS) which contributes to the regulation of normal and disturbed hematopoiesis. Recently, it was shown that Jak2 kinase is important upstream component in the regulation of the AGT mRNA transcription: activated Jak2 kinase stimulates AGT gene transcription. These observations suggest possibility for constitutively active, mutated Jak2 to modulate transcriptional activation of AGT gene as well as the expression of other RAS genes in CMPD. *Aims.* We analyzed the expression of RAS genes (ACE, AGT, AT1R and REN) in normal BM and that of PV and ET patients with the respect to the presence of activating V617F JAK2 mutation. *Methods.* Fourteen PV-JAK2V617Fpos

patients (7 male, 7 female, median age 68), thirteen ET-JAKV617Fpos patients (6 male, 7 female, median age 72) and seven ET-JAK2V617Fneg patients (3 male, 4 female, median age 66) were included in the study. Samples of normal bone marrow were obtained from five healthy donors and one sample was from a non-Hodkgin's lymphoma patient with no lymphoma involvement of the marrow. Real-time PCR analysis for ACE, AGT, AT1R1, REN genes and internal housekeeping gene GAPDH was performed using Real Time PCR System. To compensate for inter-PCR variations, normalisation of target genes (ACE, AGT, AT1R1, REN) with an endogenous control (GAPDH) was performed. JAK2 V617F mutational status was determined by the allele-specific PCR. *Results.* ACE expression in BM of PV and ET patients was down-regulated to 2-20% of the donor BM values with no statistically significant difference in expression between patients. AGT expression was significantly higher in PV and in ET JAK2V617F pos comparing to ET JAK2V617F neg patients. Renin has shown similar pattern of expression as AGT: it was significantly higher in PV and in ET JAK2V617F pos patients comparing to ET JAK2V617F neg patients. AT1R gene was significantly higher expressed in PV patients in comparison to both ET subgroups, JAK2V617F positive or negative patients. ET patients did not differ for AT1R expression by their JAK2 mutational status. *Conclusions.* Our findings indicate up-regulation of AGT, AT1R and REN genes and down-regulation of ACE gene expression in clonal hematopoiesis of PV and ET. Different expression pattern of major RAS components in BM of PV and ET compared to normal BM is clearly related to the existence of JAK2V617F mutation and less to the PV or ET disease phenotype. However, interesting exception is excessive expression of AT1R in PV that was not observed in ET irrespective of JAK2 mutation. This latest observation provides ground for the future experimental and clinical studies for the use of AT1R blockers in improving clinical management of JAK2V617Fpos PV.

0237

THE MYELOPROLIFERATIVE DISEASE JAK2 V617F MUTANT CANNOT BE REGULATED BY SOCS PROTEINS

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Background. Many myeloproliferative disorders including Polycythaemia Vera (PV) are associated with a valine to phenylalanine (V617F) somatic mutation in the pseudokinase domain of JAK2, which leads to constitutive kinase activity. This JAK2 mutant requires a cognate receptor such as the Epo receptor (EpoR) for transformation to occur. Suppressor of cytokine signalling (SOCS) proteins are known to strongly negative regulate erythropoietin (Epo) signalling through interaction with both the EpoR and JAK2. *Aims*. The aim of this study was to determine to role of SOCS3 in the regulation of JAK2 V617F. *Methods*. Expressing the EpoR and JAK2 WT or JAK2 V617F in Ba/F3 cells that also express SOCS under the control of the Tet-off system as well as transient expression of the above proteins in 293T we also investigated the expression of SOCS-3 in leukocytes derived from PV patients and healthy donors. *Results.* We found that SOCS3 could not negatively regulate the mutant JAK2. Furthermore we found that the activation of JAK2 V617F can be enhanced in the presence of SOCS3 and that normal SOCS turnover is inhibited, causing an accumulation of both proteins. *Conclusions.* The presence of SOCS3 strongly inhibits cell proliferation in the presence of wild-type JAK2, but SOCS could not inhibit proliferation when co-expressed with JAK2 V617F. Moreover, leukocytes derived from PV patients express SOCS3 at higher levels than those derived from healthy donors. These findings suggest that the JAK2 V617F mutant can counteract normal SOCS3 regulation and may exploit this to enhance the myeloproliferative disease.

0238

DETECTION OF ACTIVATED STAT5 IN THE CYTOPLASM OF NEOPLASTIC CELLS IN VARIOUS MYELOID NEOPLASMS

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Background. The signal transducer and activator of transcription 5 (STAT5) has recently been implicated as essential pro-oncogenic factor in the pathogenesis of myeloid leukemias in mice (Cancer Cell 2005;7:87-99). More recently, STAT5 activation has also been described to occur in human leukemias. However, so far, little is known about the expression of activated/tyrosine phosphorylated STAT5 (pSTAT5) in various myeloid neoplasms and about the distribution of pSTAT5 in the cellular compartments of the normal and leukemic bone marrow (bm). *Methods.* We have examined the expression of pSTAT5 in the bm in patients with acute myeloid leukemia (AML, FAB M0, n=3, M1, n=6, M2, n=4, M3, n=5, M4, n=5, M5, n=4, M6, n=5, M7, n=4), chronic myeloid leukemia (CML, chronic phase, n=4, accelerated phase, n=5, blast phase, n=5), and systemic mastocytosis (SM, n=30), as well as in the normal bm (n=5). Expression of pSTAT5 was determined on paraffin-embedded bm sections by immunohistochemistry using the pSTAT5-specific antibody AX1. *Results.* In the normal bm, the antibody AX1 was found to react with megakaryocytes and immature myeloid progenitor cells, whereas erythroid cells and mature granulocytic cells did not stain positive for AX1. In patients with AML and CML, the distribution of pSTAT5 showed a similar pattern. In fact, pSTAT5 was found to be expressed in leukemic blast cells without differences among FAB types as well as megakaryocytic cells, but not in erythroid cells. In patients with SM, neoplastic mast cells were found to be immunoreactive for pSTAT5. Interestingly, in all patients and all cells examined, pSTAT5 was found to be localized in the cytoplasm rather than in the nucleus. The cytoplasmic distribution of pSTAT5 in neoplastic cells was confirmed by immunocytochemical staining experiments performed on primary isolated neoplastic cells (AML, CML, mastocytosis) and respective cell lines (U937, KG1, K562, KU812, HMC-1). In each case, the reactivity of neoplastic cells with the AX1 antibody was abrogated by preincubation of the antibody with a pSTAT5-specific blocking peptide. Moreover, the expression of cytoplasmic pSTAT5 in the leukemic cell lines was demonstrable by flow cytometry. To study the molecular mechanisms underlying STAT5-activation in neoplastic cells, Ba/F3 cells with doxycycline-inducible expression of disease-specific oncoproteins, namely BCR/ABL (CML) and KIT-D816V (SM) were employed. Induction of these oncoproteins in Ba/F3 cells resulted in massive activation of pSTAT5 and DNA binding activity as shown by EMSA and supershift assays. *Summary.* Our data show that neoplastic cells in AML, CML, and SM express cytoplasmic pSTAT5, and that disease-related oncoproteins contribute to STAT5-activation. The particular cytoplasmic localization of pSTAT5 in neoplastic cells suggests that apart from its function as a transcription factor, pSTAT5 may have an additional role as a cytoplasmic regulator in these malignancies.

0239

NF-E2 OVEREXPRESSION DELAYS ERYTHROID DIFFERENTIATION AND INCREASES ERYTHROCYTE PRODUCTION

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Background. A point mutation in the Jak2 kinase, Jak2V617F, is found in up to 95% of patients with Polycythemia vera (PV). Jak2V617F leads to constitutive kinase activation and in a mouse model recapitulates many disease features including erythrocytosis. However, the molecular mechanism by which the mutation exerts its effect has not been delineated. The transcription factor Nuclear Factor-Erythroid 2 (NF-E2) is overexpressed in the vast majority of PV patients. In murine cells, NF-E2 overexpression leads to EPO-independent growth, a hallmark of PV. Moreover, ectopic NF-E2 expression can reprogram myeloid cells towards erythroid maturation. *Aims*. We therefore hypothesized that Jak2V617F-induced erythrocytosis is mediated by NF-E2 overexpression. Consequently, we investigated the effect of NF-E2 overexpression in healthy peripheral blood $CD34^+$ cells. *Methods*. Peripheral blood $CD34^+$ progenitor cells were purified from healthy donors using antibody-based magnetic bead separation. Cells were retrovirally transduced with a NF-E2 cDNA in conjunction with a GFP marker. GFP-expressing cells were sorted and assayed for colony formation in methyl cellulose, or maintained in liquid culture medium promoting erythroid differentiation. Erythroid maturation was assessed by CD36 and CD235a (Glycophorin A) staining and FACS analysis as well as Wright-Giemsa staining of cytospins. *Results.* NF-E2 overexpression drastically altered erythroid colony formation. While empty vector transduced cells mainly formed CFU-E (70%±20%) and to a small degree BFU-E (30% ±20%), NF-E2 overexpressing cells formed almost exclusively BFU-E (80%±5%). In addition, NF-E2 overexpressing BFU-E were larger and more dispersed than control BFU-E. Concurrently, the absolute number

of erythroid colonies was reduced in NF-E2 overexpressing cells. Xu et al. have previously reported a similar reduction in cloning efficiency of Idiopathic Myelofibrosis CD34+ cells, which have been shown to overexpress NF-E2. NF-E2 overexpression lead to a delay in erythroid differentiation, manifested by a belated appearance of CD235a (Glycophorin A) positive mature erythroid cells. Morphological evaluation similarly revealed more immature cells in NF-E2 overexpressing cultures. Protracted differentiation lead to a significant increase in the accumulated number of mature erythroid cells per progenitor cell. *Summary and conclusions.* NF-E2 overexpression delays the early phase of erythroid differentiation. This results in an expansion of early erythroid progenitors, thereby increasing the total number of mature erythrocytes derived from one CD34+ cell. These data propose a role for NF-E2 in mediating the erythrocytosis of PV.

0240

STEPWISE TRANSFORMATION OF PRIMARY BONE MARROW PROGENITOR CELLS *EX VIVO*

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Background. Leukemogenesis is considered a multistep process of accumulating genetic alterations in hematopoietic stem or progenitor cells. Current experimental models using established cell lines or murine stem cell transplantation assays do not sufficiently allow investigation of the cooperative effects of defined genetic alterations. *Aims.* We established immortalized cell lines from murine bone marrow to study their stepwise progression into more transformed phenotypes. *Methods and Results.*Primary murine lineage negative bone marrow cells were retrovirally transduced to express either AML1-ETO or HoxB4 along with EGFP as a quantitative reporter under serum free conditions in the presence of SCF+IL-3+IL-6. Immortalized EGFP+ cell lines could be established in both conditions. The resulting cell lines are dependent on expression of the respective transgene but show strikingly different phenotypes. First, long-term cultures for AML1-ETO remain strictly SCF-dependent, whereas HoxB4 cultures only require IL-3 for cell survival and proliferation. Secondly, AML1-ETO but not HoxB4 cells express c-Kit and Sca-1 on more than 90% of the cells with a high proportion of side population (SP) cells. In addition, AML1-ETO cells form only blast like colonies with very low plating efficiency ($<10^{-4}$), whereas HoxB4 cultures give rise to large colonies of mature granulocytes and monocytes with a plating efficiency of more than 10%. Subsequent retrovirus-mediated expression of the V559D c-Kit mutant resulted in generation of cytokine-independent subclones for HoxB4 but not AML-1/ETO lines. These HoxB4/Kit cells maintain full differentiation and colony formation potential but do not induce hematopoeitic malignancies in murine stem cell transplantation assays. Interestingly, additional lentivirus-mediated RNA interference to silence p53 expression results in fully transformed cell lines, which induce lethal myeloproliferation in transplanted mice with very short latency. In contrast, AML1-ETO cells could only be transformed to factor independent growth *ex vivo* by silencing of p53 expression and simultaneous overexpression of V559D c-Kit. *Conclusions.* Primary murine bone marrow cells can be transformed to malignant phenotypes by specific genetic alterations in a defined order *ex vivo*. The cooperation of genetic alterations, their impact on gene expression, and the efficacy of therapeutic intervention can be studied in such models.

0241

A NOVEL ARG371HIS MUTATION IN THE HIF PROLYL HYDROXYLASE PHD2 IS ASSOCIATED WITH ERYTHROCYTOSIS

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Background. Disorders of erythrocytosis are characterised by an elevated red cell mass and variable erythropoietin (Epo) levels. Inappropriately normal or elevated Epo values suggest that the underlying defect lies in the oxygen-sensing pathway. This process controls Epo synthesis and is regulated by the hypoxia-inducible factor (HIF) transcription complex, which consists of an α and a β subunit. In normoxia the α subunit of HIF is proteasomally regulated by the prolyl hydroxylase domain (PHD) family of enzymes, whose activity is abolished in the absence of oxygen. The PHD2 family member specifically hydroxylates two prolines in the oxygen dependent degradation (ODD) domain of HIFα and promotes the association of the von Hippel Lindau (VHL) protein. Ubiquitinylation of HIF-α follows and degradation of the subunit by the proteasome occurs. Mutations in the VHL gene account for a high proportion of the inherited erythrocytoses identified thus far. Recently a novel mutation of PHD2, Pro317Arg, has been described in 3 members of the same family with erythrocytosis identifying this prolyl hydroxylase as a critical modifier of HIF in the oxygen sensing pathway. *Aims.* To identify molecular defects in the PHD2 gene in erythrocytosis individuals who are negative for mutations in the VHL gene and to investigate the effect of any mutations on the oxygen sensing pathway. *Methods.* DNA was isolated from peripheral blood and PCR-direct sequencing of the PHD2 gene was performed. Mutant and wild type PHD2 protein was prepared by *in vitro* translation and *in vitro* binding and enzymatic functional assays were performed. *Results.* Sequencing the PHD2 gene detected a heterozygous change of G to A at base 1112 in PHD2 in an individual with erythrocytosis, who presented in his early thirties with a raised haematocrit and an Epo level at the upper end of the normal reference range. The absence of the G1112A base change in 200 normal control DNA samples was confirmed. This mutation causes exchange of arginine with histidine at amino acid 371, which is located three residues away from an active site iron-chelating residue in PHD2, His-374. *In vitro* binding studies detected substantially less association of the Arg371His variant with the HIF substrate. Enzyme assays indicated the G1112A mutation significantly reduced the ability of PHD2 to hydroxylate HIF, and the mutant was also less active than wild type PHD2 in downregulating HIF-induced Hypoxia Response Element reporter gene activity.

0242

CHRONIC IDIOPATHIC MYELOFIBROSIS COMPLICATED BY PULMONARY ARTERIAL HYPERTENSION IS DISTINCTIVELY CHARACTERIZED BY ENDOTHELIAL PROGENITOR CELL INSUFFICIENCY AND INCREASED ANGIOGENESIS

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Background. Emerging evidence underlines the primary role of angiogenesis in Pulmonary Arterial Hypertension (PAH) development. Chronic Idiopathic Myelofibrosis (CIMF) is associated with an increased angiogenic status and PAH is a common complication of this disease. *Aims.* To investigate the prevalence of PAH in a cohort of CIMF patients and its association with angiogenic status. *Patients and Methods.* Systolic pulmonary arterial pressure (sPAP) was assessed by Doppler echocardiography in 36 CIMF patients (M/F: 20/16; median age 64.5 years [36-83]). Patients with PAH (sPAP ≥35 mmHg) were evaluated by high-resolution lung CT, lung perfusion scintigraphy, arterial blood gas analyses and pulmonary function tests with Dlco. Circulating Endothelial Cells (CECs: $CD45^-$, $CD146^+$, $CD31^+$) and Endothelial Progenitor Cells (EPCs: $CD45^-$, $CD133^+$, $CD34^+$) were evaluated by flow cytometry. Age-matched controls: 10 healthy (named Controls) and 10 affected by pulmonary hypertension due to cardio-pulmonary diseases (named S-PH). Serum VEGF, ET-1, TGFβ, PDGF-AB and sE-selectin were determined by ELISA. Bone marrow biopsy MicroVessel Density (MVD) were evaluated on 33 CIMF and 16 normal specimens using the *hot spots* method. *Results.* PAH, never due to cardio-pulmonary or thromboembolic diseases, was documented in 14 patients (36%), being mild (45≤sPAp<55 mmHg) in six of them. Three of these patients were symptomatic. EPCs were higher in CIMF (0.77×10^6/l [0-4.31], p″0.02) than both Controls and S-PH (0.29×10^6/L [0.13-1.71] and 0.41×10^6/L [0-0.56]). However EPCs were significantly lower in CIMF with PAH than in CIMF without (0.43×10^6/L [0-2.46] and 0.94×10^6/L [0.13-4.31], p=0.009). Additionally, EPCs negatively correlated with sPAP (p=.01). CECs number was significantly higher in CIMF in comparison to Controls (16.1×10^6/L [0.3-165.7] and 7.12×10^6/L[1.4-18.1], p=0.01). S-PH group's CECs were also higher than Controls (44.3×10^6/L[5-59.8], p=0.009), but not significantly different in comparison to CIMF, both with and without PAH. VEGF levels were significantly higher in CIMF in comparison to both Controls and S-PH (1484 pg/mL [267-2369], 260 pg/mL [69-303] p=.006 and 399 pg/mL [117-840] p<0.001). VEGF was also higher in CIMF with coexistent PAH than in PAH negative (1850 pg/mL [1151-2306] and 1037 pg/mL [267-2369],

p=0.03). There were no significant differences in the other assessed cytokine levels between CIMF and Controls, or among the subsets of CIMF. ET-1 was significantly higher only in S-PH. An increased MVD was present in CIMF group in comparison to normal (56.6 [23.2-90] and 15.2 [2.4-34.4], p<0.0001). Additionally, CIMF with PAH had higher MVD than PAH negative (66.6 [39.6-90] and 51.8 [23.2-88], p=0.006). *Conclusions.* PAH is frequent in CIMF without any known pulmonary or heart complications, being moderate in a subset, in whom close follow-up is advisable. CIMF complicated by PAH have markedly high VEGF levels and increased MVD together with relatively low EPCs levels. Our findings suggest that development of PAH in this setting is associated with an abnormally increased angiogenic status associated to EPCs insufficiency. Our results support the hypothesis that common mechanism(s) may be involved in CIMF-associated PAH pathogenesis, thus warranting studies regarding the role of bone marrow derived stem cells in angiogenesis.

Figure 1.

0243

JAK2V617F AND MPLW515L: PRESENCE OF MYELOPROLIFERATIVE DISEASE ASSOCIATED MUTATIONS DOES NOT NECESSARILY LEAD TO ACTIVATION OF DOWNSTREAM SIGNALLING PATHWAYS

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The JAK2V617F mutation occurs frequently in polycythemia vera (PV) (95% of reported cases), essential thrombocythemia (ET) (65%) and idiopathic myelofibrosis (IM) (50%). As reported extensively, JAK2V617F encodes for a constitutively active tyrosine kinase which stimulates downstream signalling pathways and confers cytokine-independent growth to hematopoietic cells. In accordance, we found that the JAK2 mutant cell lines SET-2 (DSM ACC 608) and UKE-1 were 100-fold more sensitive to JAK-inhibition than JAK2 wild-type cell lines. However, contradictory to the view of JAK2V617F acting as oncogene was that two of the four MPD-derived JAK2V617F cell lines described hitherto are cytokine-dependent. Removal of growth factors led to dephosphorylation of JAK2, of downstream STAT molecules and finally to cell death of cell lines MB-02 and MUTZ-8, both homozygous for the JAK2V617F mutation. Therefore we conclude that expression of JAK2V617F alone will not automatically lead to cytokine-independent cell growth. Recently, MPLW515L and MPLW515K mutations have been presented as further gain of function mutations in IM (5%) or ET (1%). We tested 6 acute myeloid leukemia derived cell lines established from patients with history of IM or ET for presence of MPL mutations: cell lines MARIMO and MONO-MAC-6 (DSM ACC 124) carried the MPLW515L mutation. Immunoprecipitation studies showed that, as for JAK2V617F, the MPLW515L mutated cell lines did not show constitutive phosphorylation of the affected proteins and of downstream signalling pathways. Both cell lines, MARIMO and MONO-MAC-6, express MPL mutated and MPL wild-type messenger RNA. Assuming that both variants of the MPL protein are expressed, the mutated version is not dominant and constitutively active. Our results do not contradict the view that the JAK2 or MPL mutations contribute to the etiology of the disease or to the advent of the described cell lines. On the contrary, the importance of these mutations is underscored by the observation that 5/6 cell lines with history of IM or ET either carry the JAK2V617F mutation (MB-02, SET-2, UKE-1) or the MPLW515L mutation (MARIMO, MONO-MAC-6). However, our data show clearly that the presence of JAK2V617F or MPLW515L mutations alone does not mean that the downstream signalling pathways are constitutively active. This is the first description of MPD-derived cell lines carrying the MPLW515L mutation. Our results indicate that they will be very useful tools to elucidate the role of this mutation for basic cellular processes, possibly contributing to onset of MPD.

0244

JAK2 MUTATIONS (V617F AND EXON 12) IN CHILDREN WITH ERYTHROCYTOSIS

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Background. Polycythemia vera (PV) is a myeloproliferative disorder of the median-advanced age, characterised by an high hematocrit often associated with increased WBC and platelets count. Recently, V617FJak2 mutation has been found in almost all PV patients. In children PV is extremely rare; most children with primary erythrocytosis are difficult to distinguish from real PV cases and at present no information are available regarding the Jak2 mutation in these patients. *Aims.* We report 6 children (2 females and 4 males, age at diagnosis 4 moths-15 years) with primary erythrocytosis (hematocrit range 53-71%). Two were siblings. On the basis of PVSG criteria all patients could be affected by PV. *Mathods.* Mutations of Jak2 was searched in peripheral blood granulocytes DNA with sequencing analysis and allele- specific PCR to confirm and determine homozygosis of V617FJak2 mutation. In the females the state of activation of the X-chromosome was determined the polymorphism the HUMARA, PGK and MPP1 gene (p55). Spontaneous erythroid colony formation (EEC) from peripheral blood mononuclear cells isolated on Ficoll-Hypaque density gradient was searched by placing a cell suspension into a semi-solid medium, such as methylcellulase, supplemented with nutrients and cytokines, followed by incubation at 37°C in 5% CO2 for 14 to 16 days. Colony evaluation can be done *in situ* by light microscopy. *Results.* Our results are summarized in the Table 1. Two of our cases are familial erythrocytosis. No molecular alteration have been observed at present in these patients. In 2 cases, oxygen sensing pathway genes alteration (heterozygous VHL mutation) has been found. Only one patient (VE) who had also increased WBC and platelets, exhibits V617FJak2 mutation, EEC formation, low serum erythropoietin (EPO) levels and monoclonal pattern associated with the clinical positive diagnostic criteria for PV. This is a pediatric case of PV and does not seem to differ from adult cases. In one male with sporadic erythrocytosis we failed to recognize the cause of the disease. *Conclusions.* Since the diagnosis of pediatric erythrocytosis requests a large panel of tests, we propose a new algorithm for the diagnosis of pediatric erythrocytosis. In this scheme, dosage of serum EPO, discriminates which mutational analysis (Jak2, oxygen sensing pathways and EPO-R genes).

Table 1.

0245

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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Background. A somatic point mutation in the JAK2 gene (JAK2V617F) has been recently recognized as the key pathogenetic lesion in Polycythemia Vera (PV) and Essential Thrombocythemia (ET). Histone-Deacetylase inhibitors (HDACi) are known inducers of cell differentiation, apoptosis and cell cycle arrest of neoplastic cells. ITF2357 is a new HDACi (Italfarmaco, Milano, Italy) which, at low micro molar concentration *in vitro*, inhibits the secretion of several cytokines such as IL-1, IL-6, VEGF and IFN-γ and exerts a potent anti tumor activity against multiple myeloma (MM) and acute myeloid leukemia cells (AML). For these latter properties, this drug is currently tested in Phase I/II trials in AML and MM patients. *Aims.* We investigated the ability of ITF2357 and the prototypic HDAC inhibitor 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.* The *in vitro* EEC assays was performed using mononuclear cells (MNC) from peripheral blood samples obtained from PV, ET and IE patients at the time of regular follow-up visits in our clinic. The inhibitory activity played by ITF2357 on the EEC colony growth was performed with or without the addition of exogenous cytokines including GM-CSF, IL-6, G-CSF, IL-3, EPO and Stem Cell Factor, in the presence of a log scale concentration of ITF2357 (from 0.001 to 0.75 mM). 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 (PCR) 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). On the contrary, MNC from JAK2V617F PV and ET patients invariably sustained the spontaneous EEC outgrowth with a marked hypersensitivity to exogenous cytokines. ITF2357 induced a 90% EEC inhibition in all JAK2V617F PV and ET patients at 0.01mM concentration while SAHA displayed a similar inhibitory activity only when used at 0.25 mM. When the JAK2V617F mutation analysis was performed on single colonies obtained from PV and ET cells plated with exogenous cytokines, the addition of ITF2357 allowed the reproducible outgrowth of normal hematopoietic colonies ranging from 30 to 60% in different experiments. *Conclusions.* ITF2357, at concentration easily attained after oral administration, shows a potent inhibitory activity on the autonomous proliferation of hematopoietic stem cells of PV and TE carrying JAK2V617F mutation. The inhibitory effect of ITF2357 is remarkably selective and more evident on JAK2V617F mutated hematopoietic progenitors. This warrants evaluating the clinical activity of this molecule in Phase II trials designed for patients with chronic myeloproliferative disorders carrying the JAK2V617F mutation.

0246

A SMALL DELETION AT 20Q13.13 INDICATES NFATC2 AS A CANDIDATE GENE IN ESSENTIAL THROMBOCYTHEMIA

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Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (MPD) characterized by megakaryocytic proliferation and an excessive production of platelets. Approximately half of ET and most other MPD patients show a somatic activating point mutation in JAK2 (JAK2V617F) which is believed to represent a secondary pathogenetic event. Recent data from the literature showed that JAK2V617F is significantly associated with deletion of the long arm of chromosome 20, suggesting that a yet undefined molecular lesion on 20q precedes JAK2V617F and is responsible for initiation of clonal hematopoiesis. We describe a cytogenetic and molecular study of an acquired three-way translocation t(X;20;16)(p11;q13;q23~24) identified in a JAK2V617F-positive ET patient. Using fluorescence *in situ* hybridization with 27 different probes mapping along the 20q12-20q13.2 region, we identified a 500-kilobase deletion associated with a breakpoint at 20q13.13. Subsequent deletion mapping using polymerase chain reaction (PCR) analysis confirmed deletion of 3 genes: nuclear factor of activated T-cells cytoplasmic 2 (NFATC2), SAL-like 4 (SALL4) and ATPase class II type 9A (ATP9A). The SALL4 gene has recently been implicated in the pathogenesis of acute myeloid leukemia, however, we have sequenced the SALL4 coding sequence and flanking intronic regions and demonstrate that it is not mutated in 28 patients with ET. We further found that neither SALL4 nor ATP9A are expressed in 2 JAK2V617F-positive cell lines (SET-2 and HEL) although their expression was detected in 16 different normal tissue samples. In contrast, NFATC2 was strongly expressed in SET-2, HEL and in 7 other leukemic cell lines and normal leukocytes, making it the most likely candidate gene from the deleted region. To further address the role of NFATC2 in megakaryocyte proliferation, we simulated the effects of a deletion by suppressing NFATC2 gene expression in SET-2 megakaryocytic cells using small interfering RNAs. After 24 hours of transfection, NFATC2 mRNA and protein levels were 48% reduced compared to SET-2 cells transfected with a control siRNA. The specific suppression of NFATC2 resulted in a 34% increase in cell number which was accompanied by an increase in megakaryocyte cell size. Furthermore, the suppression of NFATC2 correlated with an up-regulation of cyclin B1 and cyclin E protein levels, indicating that the higher cell number was a consequence of cell cycle progression. Since NFATC2 regulates the expression of several hematopoietic cytokines and is expressed at high levels in megakaryocytes, mutations that affect NFATC2 expression may have important consequences in the control of megakaryopoiesis and consequently, contribute to ET pathogenesis.

0247

EVIDENCE THAT OVERPRODUCTION OF CYTOKINES AND OTHER MOLECULES LINKED TO JAK2 ACTIVATION CONTRIBUTES TO ABNORMAL HEMATOPOIESIS IN POLYCYTHEMIA VERA

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Background. Polycythemia vera (PV) is a clonal myeloproliferative disorder arising from a multipotent progenitor and resulting in excessive erythropoiesis. Activating mutations of JAK2, a key signalling molecule for hematopoietic cytokines, are found in almost all PV patients. The V617F mutation (JAK2-V617F) is found in >90% of PV; other JAK2 mutations have been described in JAK2-V617F negative PV. However, there is evidence that onset of clonality and/or disease phenotype may be due to other molecular abnormalities. We previously described the partial dependence of PV erythroid progenitors on interleukin-11 (IL-11), a cytokine that activates JAK2, stimulates erythropoiesis and is overproduced in PV, notably by bone marrow (BM) stromal cells (BMSC). *Aims.* To identify other JAK2-activating molecules with altered production in PV. *Methods.* We used cytokine antibody arrays (Raybiotech Inc.) to establish the cytokine profile of serum from 20 PV patients and 27 patients with second erythrocytosis (SE) and of BM plasma (21 PV, 21 SE). Molecules of interest were then measured by ELISA in serum (32 PV, 33 SE) and in BM plasma (27 PV, 26 SE). Correlations between cytokine levels, blood cell counts and%JAK2-V617F, measured by quantitative allele-specific PCR in blood granulocytes, were analysed using Pearson's or Spearman's rank correlation tests. *Results.* In addition to IL-11, cytokine arrays revealed two new molecules linked to JAK2 activation and present at high levels in PV: tissue inhibitor of metalloproteases-1 (TIMP-1) and hepatocyte growth factor (HGF). Overexpression of TIMP-1 in PV was confirmed by ELISA in BM plasma (102 ng/ml vs 58 ng/ml in SE, p=0.013). Overexpression of HGF was confirmed by ELISA in BM plasma (PV: 7929 pg/ml vs 4438 pg/mL in SE, p=0.01) and in serum (PV: 5176 pg/mL vs 1765 pg/ml in SE, p<0.0001). As previously described in PV, the%JAK2-V617F correlated with counts of white blood cells (WBC) (n=26, r=0.41, p<0.05) and neutrophils (n=19, r=0.46, p<0.05) but not with hematocrit. Serum HGF levels correlated with the%JAK2-V617F (n=44, r=0.37, p<0.01) and, logically, with WBC (n=27, r=0.47, p<0.01) and neutrophils (n=21, r=0.71, p<0.001) but not with hematocrit. In contrast, serum IL-11 level correlated with hematocrit (n=45, r=0.43, p<0.01) but not with WBC or neutrophils. For TIMP-1, the only correlation found was between BM plasma level and the%JAK2-V617F (n=15, r=0.49, p<0.05). Finally, exposure of PV BMSC to HGF induced a dose- and time-dependent increase in IL-11 production, and IL-11 is known to induce TIMP-1 secretion. *Conclusions.* HGF, IL-11 and TIMP-1, three molecules acting sequentially and known to promote erythroid cell proliferation via JAK2, were found elevated in PV and correlated with the%JAK2-V617F (HGF, TIMP-1), WBC and neutrophils (HGF) and hematocrit (IL-11). These results, combined with reports from other groups that HGF acts in synergy with IL-11, suggest that the three molecules contribute to hematopoiesis deregulation in PV.

0248

COMPARISON OF WHOLE BLOOD VS PURIFIED BLOOD GRANULOCYTES FOR THE DETECTION AND QUANTITATION OF JAK2-V617F

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Background. Detection of the V617F mutation of JAK2 (JAK2-V617F) is now one of the first tests performed in the diagnostic work-up of polycythemia and thrombocytoses. When studied in purified blood granulocytes, the JAK2-V617F allelic ratio is typically >20% in Polycythemia vera (PV) but <15% in half of mutated essential thrombocythemia (ET). Such low levels of mutant, often missed by sequencing, are detected in purified granulocytes by allele-specific PCR (AS-PCR) techniques. However, many diagnostic laboratories opt to work on whole blood. Since lymphocytes do not express JAK2-V617F, the JAK2-V617F burden is likely underestimated in whole blood assays and patients with low levels of mutant might be found negative. *Aims.* To determine whether whole blood allows reliable detection of JAK2-V617F in patients with <15% JAK2-V617F in purified granulocytes. *Methods.* We used a quantitative AS-PCR that detects 0.1% JAK2-V617F in granulocytes (Lippert et al. Blood 2006;108:1865) to compare results obtained with genomic DNA extracted from whole blood or purified blood granulocytes of 48 patients: 12 PV, 26 ET, 3 idiopathic myelofibrosis (IMF), 7 with secondary erythrocytosis. Twelve patients had been receiving cytoreductive treatment; all others were examined at the time of diagnosis. *Results.* The JAK2 status (JAK2-V617F positive or negative) of patients, including 2 with 1% mutant, was identical in whole blood and in purified granulocytes. For the 24 patients studied at diagnosis, the%JAK2-V617F in whole blood correlated with the%JAK2-V617F in purified granulocytes (n=24, r=0.99, p<0.001) and with the white blood cell (WBC) counts (n=24, r=0.83, p<0.001) and% neutrophils in blood (n=24, r=0.51, p=0.01). The JAK2-V617F allelic ratio in whole blood (on average 15% lower than in granulocytes) could be corrected using the% neutrophils. The 12 patients with cytoreductive treatment were characterized by lower WBC counts, a lower% neutrophils and a higher% lymphocytes. In contrast to patients at diagnosis, the%JAK2-V617F in whole blood and the% neutrophils were no longer correlated and the variations between the%JAK2-V617F measured in whole blood and in purified granulocytes were not significantly reduced when adjusted for the% neutrophils in blood. *Conclusions.* Provided that the AS-PCR used detects <1% JAK2-V617F, whole blood is suitable for the diagnosis of MPD; the JAK2-V617F burden thus measured may be adjusted using the% neutrophils. For the follow-up of the mutated clone in treated patients, it is advisable to assess JAK2-V617F before and during treatment in the same population of purified cells as blood cell ratios are altered. For the purpose of assessing the JAK2-V617F burden during treatment, blood granulocytes remain the easiest cells to obtain and purify.

0249

TWO JAK2 V617F MUTATED ALLELES ARE NECESSARY FOR EPO INDEPENDENCE IN POLYCYTHEMIA VERA

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Background. JAK2 V617F is frequently found in Polycythemia vera (PV) and Essential Thrombocythemia (ET). Most PV patients have a mix of wild-type, V617F heterozygous, and V617F homozygous erythroid progenitors. In contrast, most ET patients have only wild-type and heterozygous progenitors. The duplication of the mutant allele that occurs in homozygous erythroid progenitors may modify their response to EPO, and therefore influence the disease phenotype. *Aims.* To test this hypothesis, we studied the effects of different JAK2 V617F levels on EPO hypersensitivity or independence in two models: ET and PV primary erythroid progenitors, and the Ba/F3-R-EPO cell line expressing various amounts of mutant JAK2. *Methods.* We analysed bone marrow $CD34^+$ progenitors from 5 PV, 5 ET and 3 healthy controls. We picked $CD34^+$ derived erythroid colonies grown in methylcellulose in the presence of 0, 0.001, 0.01, 0.05 and 0.5 IU/ml EPO, and genotyped them using real time PCR. We infected Ba/F3-R-EPO cells with a retrovirus encoding JAK2 V617F and the GFP. After single cell fluorescence activated cell sorting, seven clones, which expressed 0, <10%, 25%, 40%, 60%, 75%, 90%, and 100% of JAK2 V617F, were selected and expanded. EPO sensitivity, proliferation, cell cycle, apoptosis, and cell signalling were further studied. *Results.* Wild-type erythroid progenitors from healthy controls and patients were not EPO hypersensitive. In contrast, mutated cells were found EPO hypersensitive or independent. The effect was greater in V617F homozygous progenitors from PV patients: 69±3% of homozygous progenitors were EPO independent compared to only 22±3% of heterozygous ones. We then analyzed the response to EPO of Ba/F3-R-EPO clones. Two clones with 90% and 100% of JAK2 V617F expression were EPO independent. Clones expressing 0 to 75% of mutant were not EPO hypersensitive. In the presence of low EPO concentration we found a correlation between the JAK2 V617F level and the proportion of cells in the S phase of the cell cycle. In cells expressing less than 50% and 75% of V617F, 0.1 and 0.05 IU/mL of EPO were respectively necessary to promote G1/S phase transition. In contrast, G1/S phase transition was EPO independent for clones expressing more than 90% of mutant JAK2. Moreover, at low concentrations or in the absence of EPO, an increase in apoptosis was observed in clones that expressed less than 90% of JAK2 V617F, but not in clones with 90% and 100% of JAK2 V617F. In the later clone, EPO depletion resulted in a four-fold increase in Bcl-xl, that was not seen in clones with low JAK2 V617F levels. *Conclusions.* Only Ba/F3-R-EPO clones engineered to mimic homozygous cells (clones with 90 to 100% of mutant JAK2) were EPO independent, able to promote G1/S phase transition, and to increase Bcl-xl level in low EPO concentration conditions. In addition, most homozygous erythroid progenitors from PV patients seemed to be EPO independent. These observations are consistent with the hypothesis that EPO independence is induced by the biological consequences of the presence of the V617F mutation on two JAK2 alleles.

0250

INACTIVATION OF SUPPRESSOR OF CYTOKINE SIGNALING-1 AND -3 (SOCS-1 AND -3) AND SH2-CONTAINING PHOSPHATASE-1 (SHP-1) IN PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS (CMPD)

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Background. Ph-negative chronic myeloproliferative disorders (CMPD) are a clinically overlapping group of disorders 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 activation in response to cytokines. SHP-1, SOCS-1 and SOCS-3 may be silenced by aberrant DNA methylation and/or mutation in human malignancies. *Aims.* To test epigenetic and genetic inactivation of SOCS-1, SOCS-3 and SHP-1 in CMPD and acute myeloid leukaemia (AML). *Methods.* The study was based on: i) 112 CMPD, including 43 essential thrombocythemia (ET), 28 polycythemia vera (PV), 24 idiopathic myelofibrosis in pre-fibrotic phase (MF), 11 atypical chronic myeloid leukemia (ACML), and 6 chronic myelomonocytic leukemia (CMML); ii) 20 AML post-CMPD, including 10 AML from ET, 5 AML from PV and 5 AML from MF. For comparison, 20 normal bone marrow samples were also investigated. All cases were analysed for SOCS-1, SOCS-3 and SHP-1 aberrant methylation by methylation-specific PCR and for JAK2V617F mutation status by allele specific PCR. SOCS-1 and SOCS-3 mRNA levels were detected by real-time RT-PCR. Mutation of SOCS-1 and SOCS-3 were tested by DNA direct sequencing. *Results.* SOCS-3 aberrant methylation occurred with high frequency both in CMPD (46/112, 41%) and in AML post-CMPD (10/17; 59%), and was distributed throughout the different WHO categories: 20/43 (46%) ET, 13/28 (46%) PV, 5/24 (21%) MF, 5/11 (45%) ACML and 3/6 (50%) CMML. Methylation of SOCS-1 and SHP-1 occurred with lower frequency both in CMPD (14/112, 12.5% for SOCS-1; 8/112, 7% for SHP-1) and in AML post-CMPD (3/20, 15% for SOCS-1; 1/20, 5% for SHP-1). In particular, SOCS-1 methylation was detected in 5/43 (12%) ET, 5/28 (18%) PV, 3/24 (12.5%) MF, 1/11 (9%) ACML, 0/6 CMML. SHP-1 methylation was observed in 4/43 (9%) ET and 4/28 (14%) PV, while it was absent in MF, ACML and CMML. All normal bone marrow samples (n=20) scored negative for SOCS-1, SOCS-3 and SHP-1 methylation. JAK2V617F mutation was detected in 66/112 (59%) Ph-CMPD, including 24/43 (56%) ET, 23/28 (82%) PV, 19/24 (79%) MF, and in 5/20 (25%) AML post-CMPD. SOCS-3, SOCS-1 and SHP-1 methylation occurred in both JAK2V617F-positive (26/66, 39% for SOCS-3; 11/66, 17% for SOCS-1, 6/66, 9% for SHP-1) and JAK2V617F-negative CMPD (12/46, 26% for SOCS-3; 2/46, 4% for SOCS-1, 2/46, 4% for SHP-1). This pattern of SOCS-3, SOCS-1 and SHP-1 methylation was conserved also when the analysis was restricted to PV, ET and MF each as a single group and after stratification for JAK2V617F mutation. By combining the results of SHP-1, SOCS-1 and SOCS-3 methylation status, 29/66 (44%) JAK2V617F mutated cases carried SHP-1 and/or SOCS-1 and/or SOCS-3 methylation as opposed to 29/46 (43%) germline cases. Similar results were obtained in JAK2V617F-positive and JAK2V617F-negative AML post-CMPD. To verify the correlation between aberrant methylation and gene expression, we analyzed SOCS-3 and SOCS-1 mRNA levels by real-time RT-PCR. SOCS-3 mRNA levels were significantly higher in unmethylated samples (n=10) compared to methylated samples (n=6; p=0.005) and to normal bone marrow (n=10; p=0.03). Similar results were obtained for SOCS-1. SOCS-1 and SOCS-3 missence mutations were detected in 2/104 (2%) and 1/93 (1%) CMPD, respectively. *Conclusions.* 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.

0251

CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED MICRORNAS IN GRANULOCYTES FROM PRIMARY MYELOFIBROSIS

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Background. Despite advances in defining diagnostic and prognostic criteria in patients with Primary Myelofibrosis (PMF), and the recent description of Val617Phe mutation in JAK2 exon12 and of MPL W515K/L mutation, the molecular defect(s) associated with the development of PMF remain still largely to be defined. Comparative transcriptome microarray analysis has allowed us to evidentiate a complex pattern of aberrantly regulated genes in PMF. The underlying mechanism is still poorly understood, but microRNAs might be supposed to play a role in abnormal gene regulation. AIMS. As an approach to identify possibly aberrantly regulated microRNAs in PMF, we used a Human Panel including 150 individual miRNA assays. We performed a comprehensive transcriptome comparative microRNA analysis of normal and PMF granulocytes. METHODS. To this purpose, we prepared four pools, each comprising three subjects, of granulocytes from PMF subjects, two from JAK2V617F wild-type (WT) and two from homozygote patients; two pools from blood donors were used as controls. The method uses stem-looped primers for reverse transcription (RT) of the miRNA, followed by quantitative real-time PCR. The cDNA was analysed with the aid of a TaqMan MicroRNA Assay Human Panel Kit (Applied Biosystem). *Results.* Ninety six differentially expressed microRNAs were identified; 87 were decreased and 9 were increased. In order to validate these data, we have selected 7 miRNAs which were extremely aberrantly regulated in PMF and we performed a Real-time PCR (RT-PCR) using single TaqMan MicroRNA Assay (Applied Biosystem); these were carried out in an indipendent cohort of normal controls and patients with PMF, polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic erytrocytosis (IE). Three of these gene (miR-150, miR-95 and miR-183) allowed to discriminate patients with PMF from both healthy subjects, IE and others chronic myeloproliferative disorders. We found no difference in expression profile between WT and JAK2V617F homozygote patients. With the aim to confirm the biological effects of these microRNAs, we have combined miRNA expression data with previously described transcriptosome of CD34+ PMF using microarray analysis (Guglielmelli et al, Stem Cells, 2007:165-73). After identified a number of predicted miRNA 'Targets, we have measured gene expression levels in granulocytes by RT-PCR. We found that MYB, MYCN, LEPR and PRAME were significantly increased in PMF compared with healthy controls as expected for the low levels of miR-150 and miR-183; on the other hand, both DTR and FOSB, and their putative regulatory miR-95 and miR-31, were found concurrently decreased. *Conclusions.* Recent studies have implicated miRNAs in a number of fundamental cell processes and in hematopoiesis. These data show an unique expression profiling of PMF patients as compared to normal controls, with an observed overall miRNA downregulation, as reported in other cancer cells. Moreover, 3 miRNAs were identified as a predictive signature of PMF as opposed to other myeloproliferative disorders. Finally, we showed correlation between some microRNAs and abnormally regulated putative target genes, suggesting their possible role in disease pathogenesis.

Myeloma and other monoclonal gammopathies - Clinical I

0252

THALIDOMIDE INDUCED IMPOTENCE IS A COMMON BUT FREQUENTLY UNRECOGNIZED COMPLICATION IN MALE HEMATOLOGY PATIENTS

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Background. Thalidomide has recently become an important therapy in myeloma. Well recognized complications of this drug include somnolence, venous thromboembolic disease, constipation and peripheral neuropathy. Male sexual dysfunction is described as a 'rare complication' of thalidomide, despite absence of any scientific data on its true incidence. *Aims.* We wished to ascertain the frequency and severity of erectile dysfunction induced by thalidomide therapy in male patients attending our hematology department who had been exposed to this drug. *Methods.* All male patients attending the hematology department at Beaumont Hospital who were taking or had taken thalidomide were asked specifically about erectile dysfunction associated with the use of this agent. Severity of erectile dysfunction was graded according to the National Cancer Institute Common Toxicity Criteria (version 2). *Results.* 16 male patients were receiving or had received thalidomide: 14 had myeloma, one had angioimmunoblastic lymphadenopathy and one had massive inoperable abdominal haemangioma. 5 cases (median age 74, range 59-79) were impotent (grade 3 erectile dysfunction) prior to diagnosis of myeloma and commencement of any therapy. 5 further cases of myeloma denied problems of erectile dysfunction during thalidomide therapy: 3 relapsed myeloma cases received up to thalidomide 100mgs daily in combination with intermittent high dose corticosteroids for periods of between 2 months and 21 months, and the other 2 patients received single agent thalidomide 50 mgs daily as maintenance therapy post autologous peripheral stem cell transplant (PSCT) for 1 month and 18 months respectively. 4 of these 5 cases developed grade 1 peripheral neuropathy during thalidomide therapy. All 6 remaining patients developed erectile dysfunction (grade 3 in 5 cases) within 4 weeks of starting thalidomide. Only 2 of these cases had mentioned this side effect prior to direct questioning about this complication. All 6 cases also developed thalidomide induced grade 1 peripheral neuropathy. Of the 2 patients developing erectile dysfunction whilst taking single agent thalidomide 50 mgs daily as maintenance post PSCT, one patient (aged 44) developed grade 1 erectile dysfunction which has resolved following drug discontinuation after 14 months of therapy, whilst the second individual (aged 51), who discontinued thalidomide after 15 months, remains impotent. 2 further patients (aged 58 and 64), who had received a combination of thalidomide (maximum dose of 200 mgs in both cases) and intermittent high dose corticosteroids for 3 months and 24 months respectively, remain impotent despite discontinuation of thalidomide for 12 months and 23 months respectively. The final 2 patients with thalidomide induced impotency remain on this medication: one patient (aged 55) with massive intraabdominal hemangioma has had a marginal clinical improvement since starting thalidomide 100mgs daily 6 months ago, whilst a patient (aged 63) with relapsed myeloma is responding to a combination of thalidomide (maximum dose 150 mgs daily) and intermittent high dose corticosteroids, started 2 months ago. *Conclusions.* The results from this study of a small number of patients suggest that erectile dysfunction in male hematology patients may be a much more common problem than previously suspected. The probable reason for this *blind spot* is that embarrassing questions about erectile dysfunction are not asked at hematology review of these patients, who are equally unlikely to volunteer such information. However, as this complication could be regarded as important by some such patients and their sexual partners, the issue needs to be taken seriously by the hematology community. As many male myeloma patients are likely to receive thalidomide in the foreseeable future, studies on larger numbers of patients are required to establish the true incidence of thalidomide induced sexual dysfunction, its relationship to thalidomide dose, patient age, other therapies and its liklihood of recovery on drug discontinuation.

0253

BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE COMBINATION THERAPY FOLLOWED BY THALIDOMIDE AND DEXAMETHASONE AS A SALVAGE TREATMENT FOR RELAPSED MULTIPLE MYELOMA: PRELIMINARY ANALYSIS OF EFFICACY AND SAFETY

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Introduction. PAD was reported to be highly effective regimen as an induction therapy before high dose therapy. TD is an another effective regimen with no cross resistance. We conducted a phase II study with PAD followed by TD in relapsed MM to test effectiveness of this combination. *Method.* Patients were planned to receive 6 cycles of PAD, (bortezomib 1.3 mg/m^2 days 1, 4, 8 and 11, doxorubicin 4.5 mg/m^2 days 1-4, dexamethasone 40 mg days 1-4, every 21 days). Responders following 6 cylces of PAD received 12 cycles of TD (thalidomide 100 mg days 1-28 and dexamethasone 40 mg days 1-4, every 28 days). In patients with progression during PAD therapy, regimen was changed to 12 cycles of thalidomide 200 mg days 1-28 and dexamethasone 40 mg days 1-4, every 28 days. Response was assessed by EBMT criteria, with additional categories of nCR and VGPR. Adverse events were graded by the NCI-CTC, Version 3.0. *Results*. This study aimed to enroll 35 patients till Oct 2007 and we are reporting preliminary result with 25 patients. Efficacy could be assessed in 20 patients. After two cycles of PAD, 14 patients showed response with 4 CR. Overall response rate to 6 cylces of PAD was 84% with 32% CR. Five of total 11 patients with TD showed further improvement of response status with 2 additional CR. Overall response to PAD followed by TD was 90%: CR 42%, nCR 11%, VGPR 5%, PR 32%, MR 5%, PD 5%. There was no prognostic factor for CR+nCR achieving in the univariate analysis. The median follow-up was 7.7 months with 1 year PFS 64% and 1 year OS 85%. Ninty-five PAD cycles in 24 patients were assessable for safety. The most common hematologic toxicity was thrombocytopenia, with grade 3/4 in 21%. Grade 3/4 neutropenia was observed in 20%. Sensory neuropathy occurred with grade 2 in 38% and grade 3 in 4%. The median dose intensity was 1.52 mg/m^2/week for bortezomib and 5.37 mg/m^2/week for doxorubicin, which correspond 88% and 90% of the planned dose intensities, respectively. A total of 42 TD treatment cycles (median 3, range 1-6 cycles) was administered. One patient developed grade 3 neutropenia and thrombocytopenia. Non-hematology toxicities occurred infrequently and mild. *Conclusions.* PAD followed by TD in patients with relapsed multiple myeloma is very active and tolerable.

0254

COMBINATION OF BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE FOR PATIENTS WITH ADVANCED MULTIPLE MYELOMA

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Background. Bortezomib has significant activity in patient with multiple myeloma (MM); its efficacy is increased by the addiction of Dexamethasone and it demonstrates synergy with Doxorubicin. Two different studies evaluated the efficacy of the combination of Anthracyclines, Dexamethasone and Bortezomib showing encouraging response rate of 72% in relapsed-refractory patients and 95% in newly diagnosed patients. *Aims.* We assessed the safety and efficacy of the association of Bortezomib, Doxorubicin and Dexamethasone (PAD) in patients with advanced MM. *Methods.* Sixty-four patients with relapsed/refractory MM (median age 65 years; range 41-85 years) were treated with PAD:

Bortezomib 1.3 mg/m^2 days 1,4,8,11; Doxorubicin 20 mg/m^2 i.v. days 1,4 or Pegilated Lyposomal Doxorubicin 30 mg/m^2 i.v. day 1; and Dexamethasone 40 mg p.o, days 1,2,3,4. Each cycle was repeated every month. *Results.* Patients received a median number of 4 cycles (range 1-8). Median number of prior therapies was 2 (range 1-7) and median time from diagnosis was 31 months (range 2-181 months): 27% patients received prior Bortezomib based-regimens, 75% prior Thalidomide based-regimens and 58% prior transplantation. Forty-three patients (67%) achieved at least partial response (PR) including 16 patients (25%) who showed at least very good partial response (VGPR) and 6 patients (9%) who achieved a complete response (CR). In patients who received PAD as 2nd line treatment, the PR and VGPR rates were 80% and 27%, respectively; CR was reported in 13%. Responses were equal or superior to that induced by previous treatment schedules in 69% of patients. The PR (59% vs 66%) and VGPR (18% vs 28%) rates were similar between patients who received or not prior Bortezomib regimens. Similarly, the PR (67% vs 69%) and VGPR (27% vs 19%) rates were analogous between patients who received prior Thalidomide regimens or those who did not. From the start of PAD therapy, the 1-year progression free survival (PFS) and the 1-year overall survival (OS) were 34% and 67%, respectively. Patients treated with PAD as 2nd line, showed a 1-year PFS of 57%. The 1-year PFS was not different between patients who received prior Bortezomib based-regimens and those who did not (16% vs 39%, p<0.1). Grade 3 or more hematological toxicities included trombocitopenia (48%), neutropenia (36%), anemia (13%); non-hematological toxicities ≥ grade 3 included infections (17%), neuropathy (13%), gastrointestinal (11%), fatigue (6%) and cardiac (3%). Sixteen patients died: 2 for infections, 1 for heart failure and 13 for progressive disease. *Conclusions.* The PAD regimen showed a high proportion of responses. Both responses and PFS were not influenced by previous Bortezomib treatments. Toxicities were predictable and manageable.

0255

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 30 MM patients. Median age was 71 years (range 47-83 years). *Results.* Before starting Thal and Dex schedule, 2 patients (7%) were in complete response (CR), 15 (50%) in partial response (PR) while the remaining 13 patients (43%) in stable disease (SD). After a median Thal/dex administration of 12 months (range 1-42 months), 2 patients (7%) 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 (11/30 pts), peripheral neuropathy (9/30), fatigue (8/30), dizziness and/or somnolence (8/30). Although no patient received anti-thrombotic prophylaxis, no case of DVT occurred during Thal/Dex therapy. Six out of 15 patients (40%) 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, 12 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 12 months and 23 months, respectively. *Conclusions.* The results of this study indicate that Thal/Dex treatment, given as consolidation/maintenance treatment in elderly MM patients, is feasible and tolerable, with a cumulative incidence of grades III and IV toxic events of only 7%. Moreover, this treatment produced a noticeable clinical benefit. Additional randomized studies need to be designed to explore if low dose Thal associated with monthly Dex, given as consolidation/maintenance treatment, may affect life expectancy of MM patients not eligible for aggressive treatment modalities.

0256

COMBINATION OF TCD (THALIDOMIDE, CYCLOPHOSPHAMIDE AND DEXAMETHASONE); AN EFFECTIVE AND SAFE ALTERNATIVE TO CONVENTIONAL VAD (VINCRISTINE, ADRIAMYCIN AND DEXAMETHASONE) AS A FIRST-LINE THERAPY IN MULTIPLE MYELOMA

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Background. Conventional VAD (vincristine/adriamycin/dexamethasone) has been shown to induce rapid remissions in patients with newly diagnosed multiple myeloma. However, the high toxicity and short duration of the response after VAD warrants the search for alternatives. *Aims.* To examine the efficacy and safety of TCD (thalidomidecyclophosphamide/dexamethasone), compared with conventional VAD, in patients with newly diagnosed multiple myeloma. *Methods.* A total of 71 patients with multiple myeloma were initially treated with TCD or VAD between July 1997 and October 2006. TCD regimen: thalidomide (50 mg/day, daily), cyclophosphamide (150 mg/m^2 P.O. on D1-4), and dexamethasone (20 mg/m^2 I.V. on D1-5, D15-19) and VAD regimen: vincistine (0.4 mg C.I.V. on D1-4), adriamycin (9 mg/m^2 C.I.V. on D1-4), and Dexamethasone (40 mg P.O. on D1-4, D9-12, D17-20). Autologous peripheral blood stem cells (PBSC) were collected after mobilizing with G-CSF with or without cyclophosphamide. *Results.* 71 patients (TCD regimen: 35 patients, VAD regimen: 36 patients) who received at least 4 cycles or more were evaluated for response and toxicity. No significant difference showed in age and sex between two treatment groups. There were 11 (31.4%) complete responses and 18 (51.4%) partial responses for TCD regimen and 10 (27.8%) complete responses and 18 (50%) partial responses for VAD regimen, respectively. There was no significant difference in overall response rate between two treatment groups (TCD: 82.8% vs. VAD: 77.8%, p=0.77). A trend for longer progression-free survival (PFS) was observed in patients treated with TCD compared with VAD, but there was no statistical significance (19.4±4.8 ms vs. 10.4±4.1 ms, p=0.12). In addition, there was no significant difference in overall survival (OS) between two groups (NA. vs. 45.5±10.3 ms, p=0.24). However, there was a significantly lower incidence of NCI-CTC (grade 3/4) neutropenia (14.3% vs. 36.1%, p<0.05) and treatment-related mortality (5.7% vs. 22.2%, p<0.05) in patients treated with TCD compared with those treated with VAD. 2 patients (5.7%) with thrombosis in TCD group and 1 patient (2.8%) with dilated cardiomyopathy (DCMP) in VAD group were observed. 11 (TCD group) and 10 (VAD group) patients who achieved more than partial response to induction proceeded to PBSC collection and the median number of CD34+ cells collected failed to show significant difference between two groups (p=0.78). *Conclusions.* Low dose thalidomide containing regimen (TCD) was as effective as conventional VAD regimen for patients with newly diagnosed multiple myeloma. Further, TCD may emerge as the superior regimen in terms of tolerability and safety.

0257

FREE LIGHT CHAIN ASSAYS FOR EARLY DETECTION OF RESISTANCE TO BORTEZOMIB-BASED REGIMENS

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Background. Several studies have shown that immunoassays measuring free light chains (FLC) in the serum are useful for diagnosis and monitoring of multiple myeloma (MM). Because of short half-life of light chains, FLC assay is also a promising tool for the early detection of responses to treatment. The early detection of responses would be helpful in making the clinical decision whether to continue the treatment, especially with expensive agents. The role of FLC assay in the detection of responders and non-responders to bortezomib-based regimens was evaluated by us in 2006 and now an update of this analysis is presented. *Aims.* To evaluate the FLC assay as an early indicator of sensitivity or resistance to bortezomib-based regimens. *Methods.* Patients with at least one relapse of MM were prospectively evaluated on days 1 and 11 of every cycle of bortezomib-based regimens. The results of serum FLC

assays (for lambda or κ chains) and M-Ig analysis using immunofixation electrophoresis were analysed for three categories (time to reduction of parameters to 25% (minimal response - MR), 50% (partial response - PR) and 75% (very good partial response - VGPR) of the pre-treatment values) to establish their value for the detection of early response. The cohort of 24 patients from our pilot analysis (2006) was enlarged to a total of 39 patients who had had at least 5 cycles of therapy. *Results.* A total of 21 patients (54%) responded to the therapy (3% complete response, 15% VGPR, and 36% PR). Further 20% patients achieved minimal response (MR). Overall treatment responses (i.e. at least PR) were detected on days 22/33/44/55/66 of the treatment in 41%/49% /56%/59%/59% of patients using FLC assay versus 13%/18%/41%/ 46%/51% of patients using electrophoresis. The differences were statistically significant for all reported days up to day 55 (p=0.004-0.019). The results of the two methods became similar on day 66 (59% vs. 51%; p=0.060). Response after day 66 was only seen in one patient, who achieved PR after 88 days (FLC), or 99 days (M-Ig) of the treatment. Moreover, the patients who did not respond with at least 25% reduction of serum single chain as detected by FLC during the first 33 days never achieved PR. *Conclusions.* We confirmed our pilot data in that we did not observe PR in patients who did not have at least 25% reduction in serum free chain as measured by FLC by day 33. Thus, FLC assay can be used to detect resistance early during bortezomib treatment. By day 66 (day 1 of cycle 4 in the routine schedule of bortezomib treatment) 20 out of 21 responding patients achieved response (at least PR). With some limitation, day 66 can be suggested as a cut-off time for the chance of achieving long-term response to bortezomib-based therapy.

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0258

EFFICACY AND SAFETY OF MELPHALAN, PREDNISONE, THALIDOMIDE AND DEFIBROTIDE IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS: RESULTS OF A MULTICENTER PHASE I/II TRIAL

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Background. Defibrotide (DF) is a novel orally bioavailable oligonucleotide with protective effects on endothelial cells but without significant systemic anticoagulant effects and bleeding risk. *in vitro*, DF showed minimal inhibitory effect on multiple myeloma (MM) cells growth but *in vivo* (human MM xenografts in SCID/NOD mice) markedly increased the responsiveness of MM cells to cytotoxic agents (melphalan or cyclophosphamide) and dexamethasone. DF may therefore abrogate tumor cell interaction with marrow stromal cells and enhance sensitivity to chemotherapy, thus improving the activity of Melphalan, Prednisone and Thalidomide, while protecting against thrombosis. *Aims.* We designed a study to determine the efficacy and safety of Melphalan, Prednisone, Thalidomide and Defibrotide (MPTD) as salvage treatment in patients with relapsed/refractory MM. 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 of febrile neutropenia, or Grade 4 neutropenia ≥ a week, or any other Grade 4 hematologic toxicity, or any ≥ Grade 3 non-hematologic toxicity, in >30% of patients at first cycle. MTD was the dose level prior to that resulting in DLT. *Methods.* Between March and November 2006, 24 patients in first or second relapse (excluding primary refractory patients and/or patients receiving therapeutic anticoagulation) were enrolled. MPTD consisted of 6 X 35 days cycles of oral melphalan (0,25 mg/Kg day 1-4), prednisone (1,5 mg/kg day 1-4), thalidomide (50-100 mg/day continuously) and DF at 3 different dose levels (17 mg/Kg i.v. or 2.4 g p.o. D 1-4, 1.6 g p.o. D 5-35; 34 mg/Kg i.v. or 4.8 g p.o. D 1-4, 3.2 g p.o. D 5-35; 51 mg/Kg i.v. or 7.2 g p.o. D 1-4, 4.8 g p.o. D 5-35), without prophylaxis against deep vein thrombosis (DVT). *Results.* Nineteen pts (median age 69 years ;range: 47 - 88) completed at least one cycle and were evaluable for response. According to EBMT/IBMTR criteria, after a median of 3 cycles, 42% of patients achieved at least partial response (including 16% very good partial response). To date no significant difference in response rate was noted among the 3 DF dose levels, but follow up remains short. DLT consisted of grade 3 ileus in the 1st dose level, 1 acute myocardial infarction (AMI) in the 2nd (both considered unrelated to DF) and none in the 3rd. MTD was therefore not reached in any cohort. Greater than or equal to grade 3 hematological toxicities included neutropenia (47%), thrombocytopenia (10%), and anemia (21%). Non-hematological toxicities ≥grade 3 were observed in <5% and no DVTs were reported. Three patients stopped treatment because of adverse events : AMI (requiring additional anticoagulation as treatment), ileus (because of the finding of amyloidosis AL and disease progression), and persistent G4 neutropenia in one heavily pre-treated patient. In none of these cases was DF thought contributory and no significant bleeding was reported. Pharmacokinetic studies and analysis of surrogates are ongoing. *Conclusions.* MPTD provided promising evidence of anti-tumor activity in relapsed/refractory MM, with manageable toxicities. A protective role of DF in reducing thrombosis is suggested, although the thrombotic risk from thalidomide-based therapy is more variable in advanced MM than that seen in the up-front setting. The absence of other significant non-hematologic toxicity, including neuropathy, is encouraging. An update of these data will be presented at the meeting.

0259

LIPOSOMAL DOXORUBICIN INCREASES THE ANTI-TUMOR EFFICACY OF LOW DOSE BORTEZOMIB, THALIDOMIDE AND DEXAMETHASONE THERAPY IN ADVANCED MULTIPLE MYELOMA

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Background. standard chemotherapeutics plus bortezomib may have higher anti-tumor activity than bortezomib alone. The combination with anthracyclines is attractive but it may have a significant toxicity in advanced myeloma. Liposomal doxorubicin has a longer half-life than standard doxorubicin, lower cardiac toxicity and comparable efficacy. To improve outcome while minimizing therapy-related toxicity, we added non-pegylate liposomal doxorubicin (Myocet®) to the low dose Bortezomib, Dexametasone and Thalidomide regimen (LD-VTD) that we previously applied. *Aims*. Safety, ORR, TTP and OS in respect to LD-VTD. *Methods.* since February 2004, all refractory myeloma patients were enrolled in the study once they had a measurable disease, irrespective of PS. Planned therapy was: Bortezomib (Velcade®) 1.0 mg/m^2 i.v. bolus days 1, 4, 8 and 11 of a 28-d cycle, oral Dexamethasone 24 mg on the day of and the day following each bortezomib dose and Thalidomide 100 mg/d if non contraindicated (LD-VTD). In patients enrolled from January 2005, liposomal doxorubicin (Myocet®) 50 mg/m^2 i.v. was added on day 4 (LD-VTD/My).

Table 1. Patients characteristics (#65).

	LD-VTD Group 1 (# 28)	LD-VTD+Myocet Group 2 (#36)	*p*
Age, median	67(53-78)	66(42-81)	n.s.
Peripheral neuropathy>2	4(14%)	8(22%)	n.s.
Hb<10 gr/dL (#pts)	7(25%)	15(41%)	n.s.
Plt<100x10^9/L (#pts)	7(25%)	8(22%)	n.s.
β2 microglobulin 4 mg/L	11(39%)	11(39%)	n.s.
Stage III	19(68%)	29(80%)	n.s.
Yrs from diagnosis, median	5	6	n.s.
Number of prior treatments	4(2-8)	3(2-6)	n.s.
MP	22	9	
VAD	17	11	
VED	4	3	
Thal/Dex	28	36	
Autologous transplant	5	6	
Allogeneic transplant	2	2	
Primary refractory	10(36%)	17(47%)	n.s.
Relapsed/refractory	18(64%)	19(53%)	n.s.
#pts valuable for response	28	33	
CR+nCR	6(21%)	14(42%)	n.s.
PR	4	10	
MR	4	0	
ORR	50%	73%	0.04
PD/SD	3/11	0/9	
Median time to response, months	2(1-4)	1,2(1-3)	n.s.
Time to progression (TTP), months	7	16	0.02
Overall survival, months	23	Not reached	n.s.

Response was defined according to EBMT criteria. Patients with PD were removed from the study, the others continued until best response for a maximum of 6 cycles. LVEF was evaluated before and at the end of treatment. Time to response was from the date of study entry to the first evidence of response, time to progression (TTP) from the date of first response to progression. *Results.* as of February 28th 2007, 65 patients were enrolled, 28 received LD-VTD (group1) and 36 LD-VTD+My (group2). 13(20%) pts did not receive thalidomide due to previous neurotoxicity. Patients characteristics and results are reported in Table 1. As expected, haematologic toxicity was more frequently recorded in group2 and resulted in a major incidence of infections. Extra-haematologic toxicity was negligible in both groups and no patient experienced progression of PN. No case of DVT or cardiac failure was recorded and most patients were treated on an outpatient basis. Median time to response was 2 and 1,2 months, ORR 50% and 73% (p=0,04), median TTP 7 and 16 months (p=0,02) in group1 and 2, respectively. OS of the two groups was comparable. At the day of last follow-up, 11 group1 and 27 group2 patients were alive. *Conclusions.* liposomal doxorubicin increases the antimyeloma efficacy of bortezomib, thalidomide and dexametasone combination regimen with acceptable toxicity in elderly and heavily pre-treated patients. This might translate in a prolonged OS but an extended follow-up is needed.

0260

FEASIBILITY AND EFFICACY OF BORTEZOMIB RE-TREATMENT IN MULTIPLE MYELOMA

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Background. despite improved response and survival, multiple myeloma (MM) still relapse and remain an incurable disease. Safety and efficacy of bortezomib in previously treated MM, either as single agent or with thalidomide or standard chemotherapeutics, have been well established. However, no therapy is curative and upon relapse myeloma cells develop resistance to therapies that had been previously effective. Until recently, few data are available on the feasibility and benefits of re-treatment with bortezomib which is a weak substrate for multi-drug resistance efflux pumps and has the potential to avoid resistance. To assess the safety and efficacy of bortezomib re-treatment in advanced MM, we reviewed the outcome of patients who were re-challenged with bortezomib after a previous response in our institution. *Methods.* since January 2005, all myeloma patients previously treated with a bortezomib based therapy and achieving at least a stable disease were considered suitable for re-treatment. They were required a measurable disease and a life expectancy >1 months. Planned therapy was: bortezomib (Velcade®) 1.0 mg/m^2 i.v. bolus days 1, 4, 8 and 11 of a 28-d cycle, oral dexamethasone 24 mg on the day of and the day following each Velcade dose. Patients receiving thalidomide (50/100 mg) as maintenance continued the drug, if not contraindicated. Adverse events were assessed at each visit and graded according to NCI criteria. Efficacy was assessed after each cycle and response defined according to EBMT criteria. Patients with PD were removed from the study, the others continued until best response for a maximum of 8 cycles. Overall survival (OS) was from the date of bortezomib re-treatment to the date of death or last follow-up. Time to progression (TTP) from the date of first response to progression. *Results.* as of February 28th 2007, 11 were the patients re-treated. They all had a progressive disease, were heavily pre-treated and in most of them neuropathy of any grade was present. Patients characteristics: median age 64 yrs (42-80), 8 stage III (2 stage IIIB), 5 β2microglobulin >4 mg/L, WBC 3.7×10^9/L ((2.0-9.7), haemoglobin 10 gr/dL(7,8-14,5), platelets 135×10^9/L(10-258). Median time from diagnosis to first bortezomib was 4 yrs (1-15,8) and a median of 4 (1-8) were the previously delivered therapy lines. 4 were the patients relapsed after a bone marrow transplant prior to first bortezomib. Toxicity was negligible, not increased in comparison with first bortezomib therapy. Grade 1 neutropenia was recorded in two patients, grade 2 thrombocytopenia in 2. A negative impact on haemoglobin level was never registered. Overall, the most commonly reported adverse events of grade >1 were fatigue (50%) and nausea (22%). There was not a clinical progression of pre-existing neuropathy. At the date of last follow-up, 10 patients were valuable for response. 6 patients, who had achieved a response with first bortezomib, again responded (4 nCR , 2 PR) while 4 SD with the previous bortezomib, again achieved a SD. After a medium follow-up of 13 months, 5 patients were alive. Median TTP was 9 (2-12) months. *Conclusions.* bortezomib re-treatment is safe, effective and not associated with new or cumulative toxicity. Responsive patients may benefit from re-challenging the drug.

0261

SALVAGE THERAPY WITH INTRAVENOUS BORTEZOMIB, MELPHALAN AND DEXAMETHASONE IN PREVIOUSLY TREATED MYELOMA PATIENTS

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Background. Bortezomib, Melphalan, and Steroids are among the most effective drugs for treatment of multiple myeloma and their combination has already tested in elderly myeloma patients. However, so far the contemporary administration of these drugs has not been explored. *Aims.* We therefore designed a protocol with monthly courses of contemporary intravenous administration of these drugs in patients with advanced multiple myeloma. *Methods.* Bortezomib was given at dosage of 1.3 mg/m^2 i.v. days 1,4,8,11, Melphalan 5 mg/m^2 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) including autologous bone marrow transplant in 7 patients and and Bortezomib alone or in combination in 5 patients. 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. *Results.* Hematological toxicity grade 3 and 4 occurred in 38%, 36%, 33%, and 50% after I, II, III, and IV cycle respectively of patients after the first cycle. Three patients developed also grade 3 non-hematological toxicity (Herpes zoster, vomit). So far, 4 patients have stopped treatment for toxicity after 1, 3, 3, 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. *Conclusions.* The contemporary intravenous administration of Bortezomib, Melphalan, and Dexametasone, appears to be an highly effective treatment even for heavily pretreated patients. However, in these patients, haematological toxicity was the limiting factor. Therefore, the dosage of the drugs or their schedule has to be modified.

0262

SALVAGE TREATMENT WITH MELPHALAN 100 MG/M² IN FULMINANT PROGRESSION OF MULTIPLE MYELOMA

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Background. Patients with a rapid fulminant progression of multiple myeloma (PGMM) have poor prognosis. The treatment options are very limited due to a pancytopenia and/or a general poor status of patients. The treatment with melphalan (MEL) 100 mg/m^2 as a salvage therapy up front followed by infusion of stored autologous peripheral blood stem cells (PBSCs) can stop PGMM almost immediately. This strategy provides a window of opportunity for the application of new immunomodulatory or targeted drugs, such as thalidomide and bortezomib as consolidation treatment. *Aims.* The aim of our analysis was to evaluate the efficacy and toxicity of MEL 100 mg/m^2 (MEL 100) with PBSC support in the PGMM after the first autologous transplantation. *Methods.* We have retrospectively evaluated 18 patients with fulminant PGMM treated with MEL 100 in our centre from 2004 to 2006. Median follow-up from MEL 100 was 5 months (range: 3-14 months). The baseline characteristics of the patients are as follows: median age 58 years (range: 40-68); median number of relapse 1 (range: 1-4); clinical stages according to Durie and Salmon 17%-II/83%-III; clinical stages according to ISS 1-28%/2-44%/3-28%; extramedullary disease in 40% (7/18), renal impairment in 22% (4/18), high degree of bone marrow infiltration with peripheral pancytopenia in 61% (11/18). Following the MEL 100 salvage regimen, the patients were treated mostly with bortezomib (5/18) or with thalidomid based regimen (10/18). *Results.* There was no treatment-related mortality. The median time to the neutrophils engraftment (above 0.5×10^9/L) was 11 days (range: 7-12 days); the median duration of hospitalization after MEL 100 was 21 days (range: 13-90). Mucositis grade 3-4 developed in 27% (5/18) of patients, and febrile neutropenia occurred in 44% (8/18) of patients. Overall response rate (ORR) was 61% (11/18) and only 1 patient had continuous progression. Very rapid

progression within 3 months after MEL 100 occurred in 39% (7/18) of patients. Time to progression (TTP) and overall survival (OS) were evaluated in 78% (14/18) of patients who had median follow-up above 4 months or who died due to event. Median of TTP after MEL 100 was +3.3 months (range 1-11 months) but TTP was +11, 5, 5, +4,+4 months in patients at least in PR after MEL 100. Median OS was +5 months (range 1-12 months) but will be further improved as 44% (8/18) of patients still alive. *Conclusions.* Fulminant PGMM is usually fatal event when conventional chemotherapy as well as new agens are used due to very aggressive features of this event. MEL 100 is safe salvage regimen for PGMM with good response rate (61% ORR) and acceptable toxicity. However outcome is poor for substantial part of patients it is opportunity for 1/3 of patients to achieve at least PR and stop rapid continuation of progression. Such a strategy followed by optimal combination with new drugs (Velcade, IMIDs) can further prolong survival of patients with this fatal form of PGMM.

0263

THALIDOMIDE-DEXAMETHASONE AS EFFECTIVE TREATMENT OF THE FIRST RELAPSE AFTER TANDEM AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS TRANSPLATATION OF MULTIPLE MYELOMA

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Background. Thalidomide-Dexamethasone (TD) is an efficient drug combination, which is used in primary treatment of patients with multiple myeloma (MM). We have attempted to evaluate the use of this protocol in pts with MM relapsing after tandem autologous peripheral blood stem cells transplantation (auto-PBSCT). *Aims.* The end points of the study were: response, EFS, OS and toxicity. *Metods.* An analysis was conducted after the relapse MM in 30 patients (15F/15M) with a median age of 50 years (range 44-60), who entered the study between February 2003 and October 2006. Nineteen patients had IgG producing MM, 4-IgA, 1-nonsecretory, 6-light chain. Clinical stage according ISS: 21 pts in stage 1, 3-stage 2, 6-stage 3. Prior treatment included primary VAD protocol (vincristin, adriamycin, dexamethasone) followed by cyclophosphamide mobilization and tandem autoPBSCT after conditioning with high dose melphalan. Patients were then observed until first signs of disease progression and then treated with TD. Thalidomide was used in doses 200 mg/day po. continuously until any sign of progressive disease or relapse, and dexamethasone 40 mg po. for 4 days every 3 weeks. Fifteen (50%) patients received enoxaparin prophilaxis. The response rate was assessed using EBMT/IBMTR criteria. *Results.* Four out of 30 patients (13.3%) achieved complete remission (CR), 10 patients (33.3%) partial remission (PR) (M-protein reduction 50-99%), 16 patients (53,4%) stable disease (SD) (M-protein reduction 0-49%). Time to response was 6 weeks. Six pts, who had initially stable disease a progression was observed after 6 months. These pts underwent another mobilization treatment with cyclophosfamide 4 g/m^2 and the 3rd autoPBSCT. For remaining patients on TD the median EFS was 14 months (4-31.3). Duration of treatment TD was 14.6 months (median), (3-48.6). The median OS has not been reached. The major adverse events of TD were: constipation-2 patients, somnolence-3 patients, deep-vein thrombosis-1 patient. We did not observed neuropathy after TD in this particular group of patients. *Conclusions.* The chemotherapy according TD protocol is effective in pts with relapsing MM after tandem auto-PBSCT. Time to response was short-median 6 weeks. TD was well tolerated.

0264

PRACTICAL RECOMMENDATIONS ON THE MANAGEMENT OF VTE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA WHO ARE TREATED WITH LENALIDOMIDE AND DEXAMETHASONE

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Background. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who have received at least one prior therapy. *Aims.* To present practical commendations for prevention and management of venous thromboembolism (VTE) in these patients. *Methods.* A moderated round table discussion. *Results.* Treatment with lenalidomide/dexamethasone (Len/Dex) in patients with relapsed/refractory MM has been reported to be associated with a rate of VTE of 12%. Therefore, it is important to assess the VTE risk and consider the use of prophylaxis. There was consensus that the following factors increase the risk for VTE in this setting: high-tumour mass, concomitant chemotherapy, doxorubicin, high-dose dexamethasone, erythropoietin use, ongoing infection/inflammation, older age, thrombophilia, previous VTE, or pre-existing coagulation disorders. Neither baseline coagulation studies nor screening for VTE in asymptomatic patients are recommended. Sonography for diagnosis of VTE is recommended in symptomatic patients. We recommend prophylactic anticoagulation if any of the above risk factors is present at treatment with Len/Dex and no prophylaxis in patients without risk factors. The risk of VTE is particularly high in the first 4'6 months of therapy. At present there is no evidence of the best prophylaxis: daily aspirin, low-molecular-weight heparin (LMWH), or therapeutic doses of warfarin, are the options. The panel suggests the use of low-dose aspirin (ASA) (81-100 mg) or prophylactic dose of LMWH. Low-dose warfarin is not recommended, therapeutic-dose warfarin seems to be associated with an increased risk of severe haemorrhage. All patients need to receive clear instructions on how to proceed in case clinical symptoms of VTE occur. When VTE has occurred, the patient can be continued on treatment with Len/Dex or retreated after stabilization depending on the severity of the VTE. For therapeutic anticoagulation, patients previously on ASA should be switched to LMWH; and patients already on prophylactic doses of LMWH should receive therapeutic doses. *Conclusions.* In relapsed/refractory MM patients who receive lenalidomide, VTE prophylaxis with ASA or LMWH is suggested if at least one of the above listed risk factors is present.

0265

PRACTICAL RECOMMENDATIONS ON THE MANAGEMENT OF CYTOPENIAS IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA WHO ARE TREATED WITH LENALIDOMIDE AND DEXAMETHASONE

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Background. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who received ?1 prior therapy. *Aims.* This communication focuses on recommendations regarding the management of cytopenias in these patients. *Methods.* Moderated round table discussion. *Results.* In published studies, 11.6% of patients treated with lenalidomide/dexamethasone (Len/Dex) had NCI grade 3 neutropenia and 3.3% grade 4 neutropenia (Chen et al., Blood 2006; 108:3556). This was the most frequent reason for discontinuation of therapy and dose reduction. The rate of grade 4 febrile neutropenia was <1%. Thrombocytopenia occurred in 11.1%. Risk factors for cytopenia during Len/Dex include low counts at baseline, previous chemotherapy and response to treatment. Patients with renal insufficiency were reported to suffer from more severe thrombocytopenia, but age has not been reported to be a risk factor. Recommendations regarding monitoring and management of cytopenia during treatment with Len/Dex for relapsed/refractory MM have been developed. In case of a normal baseline full blood count (FBC), biweekly monitoring is recommended. If baseline FBC is abnormal because of MM infiltration, treatment should still be pursued with full dose and at least weekly monitoring. Standard dose-reduction strategies should be followed for all other causes of abnormal baseline and follow-up FBC. As a general rule,

G-CSF can be used in neutropenic patients. In case of neutrophils <1000/∝L, G-CSF is recommended to prevent dose reduction and febrile neutropenia aiming at >500/µL neutrophils. If neutrophils fall <500/∝L, Len should be interrupted and restarted (same dose for first fall and no other toxicity; lower dose for subsequent falls) once neutrophils >500/∝L. Similarly, if platelets fall <50,000/∝L, anticoagulation should be stopped and in case of thrombocytopenia <30,000/∝L, Len should be interrupted and restarted at a lower dose once platelets >30,000/∝L. Also, antibiotic prophylaxis with cotrimoxazole should be applied if patients receive Len with high dose Dex. Patients should receive clear instructions to seek medical care within 3 hours if febrile while neutropenic. *Conclusions.* A stringent strategy for the management of cytopenia due to treatment with Len/Dex is recommended.

0266

PRACTICAL RECOMMENDATIONS ON THE MANAGEMENT OF NON-HAEMATOLOGICAL ADVERSE EVENTS IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH LENALIDOMIDE/DEXAMETHASONE

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Background. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who have received at least one prior therapy. *Aims.* This communication focuses on recommendations for management of non-hematological adverse events (NHAE) in these patients. VTE management recommendations are presented separately. *Methods.* A moderated round table discussion. *Results.* Treatment with lenalidomide/dexamethasone (Len/Dex) is well tolerated in patients with relapsed/refractory MM. Nevertheless, some NHAE may occur. The most common NHAE of all grades in two randomised studies were fatigue (38%; versus 37% with dexamethasone alone), constipation (39% vs. 19%), diarrhoea (29% vs. 25%), nausea (22% vs. 19%), muscle cramps (30% vs. 21%), rash (16% vs. 8%); and paraesthesia (12% vs. 13%). The most common NHAE leading to dose reduction/interruption were fatigue (4%) and pneumonia (2%). A randomised study comparing lenalidomide combined with high-dose or low-dose dexamethasone demonstrated considerably less toxicity with low-dose dexamethasone; however, efficacy data from this study are not yet available. Atrial fibrillation (AF) appears to be more common in patients treated with Len/Dex (grade 3/4 NCI: 3% vs. 1%), especially if high-dose dexamethasone is used or if patients had previous AF, and regular monitoring is recommended. In contrast to monotherapy with lenalidomide alone, rash is less frequent with Len/Dex. In case of grade ¿2 rash, we recommend treating the patient with antihistamines. If persistent, continuous low-dose prednisone (10-20 mg/d) should be added. Rash is mostly self-limiting with a duration of several weeks but in some cases dose reduction or discontinuation of lenalidomide is necessary. In case of fatigue, other causes such as anaemia, infection, depression or hypothyroidism should be ruled out. Also, patients benefit from counselling. Dose reduction may be considered for severe fatigue. Dexamethasone therapy may predispose patients to infection; therefore we recommend the use of routine antibiotic prophylaxis for all patients upon initiation of Len/Dex treatment. In addition, vaccinations (influenza, pneumococci, meningococci and haemophilus) should be considered. *Conclusions.* A concise strategy for NHAE management of Len/Dex is presented, which will aid safe and efficient administration.

0267

PATIENT ELIGIBILITY FOR LENALIDOMIDE/DEXAMETHASONE TREATMENT IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who have received at least one prior therapy. *Aims.* This communication deals with patient-eligibility for treatment with lenalidomide and dexamethasone. *Methods.* A moderated round table discussion. *Results.* Response rates of combination therapy with lenalidomide/dexamethasone (Len/Dex) in the setting of relapsed/refractory MM are approximately 60% and lead to a survival advantage over treatment with dexamethasone alone. This effect was seen in all groups of patients, including patients of all age groups, independent of disease stage, duration of disease prior to Len/Dex treatment, ECOG performance status, cytogenetics including t(4;14) and del(13q), level of β2-microglobulin and renal or hepatic impairment. Of note, patients with a creatinine >2.5 ng/dL had not been included in clinical trials. Response to Len/Dex was superior to Dex alone, independently of the type of prior therapy (bortezomib, thalidomide, or prior transplant). It is important to note that patients refractory to thalidomide still respond to Len/Dex, although the overall response might be less than in patients not refractory to thalidomide (not significant). Patients with only 1 previous line of therapy had a greater survival advantage than patients with more than 1 previous line of therapy. There is some evidence that a lower dose of Dex may result in less toxicity (Rajkumar *et al.,* Blood 2006; 108:799), but efficacy data are not yet available. However, the dexamethasone dose may be adjusted in elderly, fragile patients >75 years. Also, as lenalidomide is mainly renally excreted, dose reduction of lenalidomide depending on the severity of renal impairment will be provided. Adjustments for mild to moderate hepatic dysfunction or potential drug-interactions are not required. *Conclusions.* Len/Dex is recommended for patients with relapsed/refractory MM regardless of baseline factors.

0268

EARLY BORTEZOMIB TREATMENT IMPROVES RESULTS IN RELAPSED MYELOMA PATIENTS. BETTER SECOND THAN FURTHER LINES

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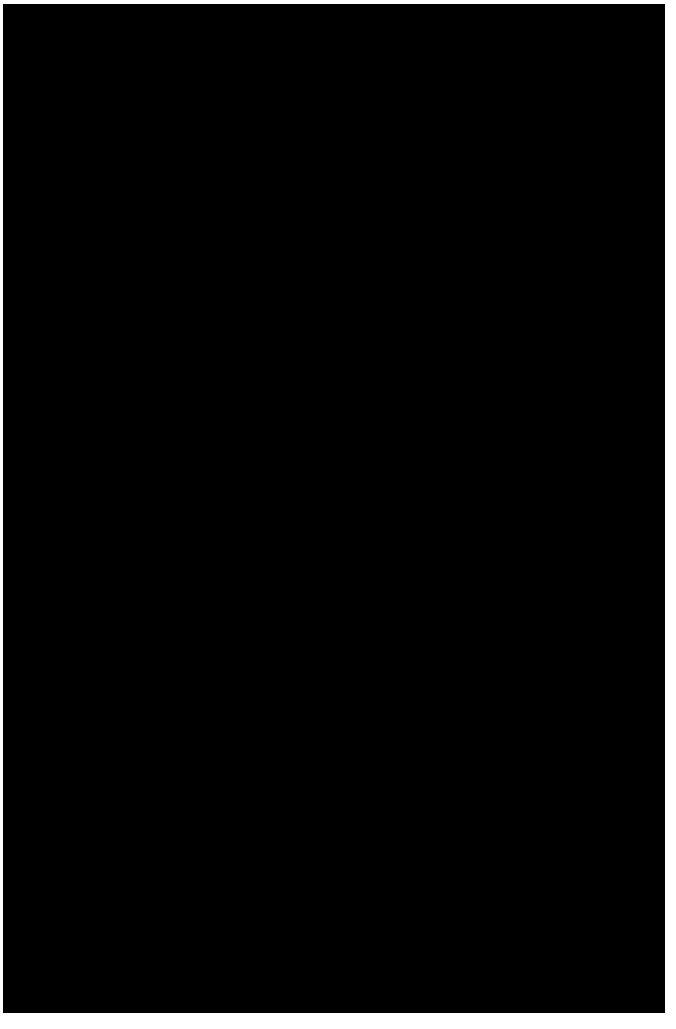
Background. Proteasome inhibition with bortezomib (VELCADE) is the standard of care in patients with relapsed/refractory multiple myeloma (MM) who have received at least one prior therapy. The phase II SUMMIT trial of patients with relapsed and refractory MM showed that bortezomib is active, with a response rate of 27% (CR/PR) as a single agent, median TTP of 7 months, and median OS of 17 months. The phase 3 APEX trial in pts with relapsed MM following 1 3 prior therapies showed btz to be superior to high-dose dexamethasone (dex) in terms of response rate, TTP, and OS. Updated APEX data show a CR/PR rate of 43%, median TTP of 6.2 months, and median OS of 29.8 months. Preliminary data from the APEX trial showed that earlier treatment (second vs further lines of therapy) resulted in better RR,TtPD and OS. *Aims.* To compare patients receiving bortezomib as second line therapy vs those receiving it as third or subsequent lines of treatment. *Methods.* 63 patients with relapsed or refractory MM were treated with bortezomib at a dose of 1.3 mg/m^2 on days 1, 4, 8, and 11 in a 21-day cycle. 80% of patients received dexamethasone at a dose of 20 mg on days 1, 2, 4, 5, 8, 9, 11 and 12. Median age was 68.9 years old (range 43-87). Twenty-four patients received only one line of therapy with melphalan-prednisone, and 39 of them received previously 2 or more lines of therapy;

median of 3 lines, range 2-6 (37% BMT and 40% Thalidomide).Treatment was administered till disease progression, unacceptable toxicity or complete response (CR), up to a maximum of 8 cycles. *Results.* 321 cycles were administered; median 4 cycles (range 2-10). Dose reductions were done in 18% of cycles, mainly due to neuropathic pain or peripheral neuropathy. The most frequent grade 3-4 toxicity was thrombocytopenia in 28% of patients. The most frequent grade 1-2 non-hematological toxicity was neuropathic pain and peripheral neuropathy (in 25% of patients) and asthenia in 17% of them. Response rate by treatment branch are shown in Table 1. Median TtPD was 200 days in the group of patients treated in third or subsequent lines of therapy, and was not reached in the second line group. *Conclusions.* Treatment with bortezomib and dexamethasone is hignly efficacious in patients with refractory MM. Earlier treatment produced better results.

Table 1.



Myeloma and other monoclonal gammopathies - Plasma cell biology

0269

IN VITRO AND *IN VIVO* ACTIVITY OF THE VEGF INHIBITOR PAZOPANIB IN MULTIPLE MYELOMA

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Background. Vascular endothelial growth factor (VEGF) and its receptors play an important role in the pathogenesis of multiple myeloma (MM). VEGF present in the MM bone marrow microenvironment induces neovascularization; triggers tumor cell growth, survival and migration; inhibits dendritic cell maturation; and promotes osteoclastogenesis. VEGF therefore provides a potential therapeutic target in MM. *Aims.* To evaluate the therapeutic potential of the novel, potent VEGF receptor inhibitor pazopanib in MM. *Methods.* Effects of pazopanib on MM and endothelial cell growth, survival, and migration were evaluated using western blot analysis, 3H-thymidine uptake, Boyden- modified chamber, tubule formation assays, FACS analysis as well as microarray analysis, RT- PCR, and specific cMyc knockdown by siRNA. *in vivo* effects of pazopanib were evaluated using a MM xenograft mouse model. *Results. In vitro*, pazopanib inhibits VEGF- triggered VEGF receptor phosphorylation and activation of downstream signaling molecules including Src kinase in MM cells and blocks MM cell migration, growth, and survival. Moreover, gene expression and signaling network analysis in pazopanib- treated cells demonstrate transcriptional changes of several signaling pathways, including marked downregulation of cMyc. siRNA targeting cMyc blocked VEGF production and secretion in MM cell lines. In addition, pazopanib reduced VEGF in the microenvironment and directly inhibited endothelial cell growth, migration, and vessel formation. Furthermore, pazopanib inhibited VEGF- induced upregulation of adhesion proteins on both endothelial and MM cells, thereby abrogating endothelial cell -MM cell adhesion and associated tumor cell proliferation. Pazopanib also strongly sensitized tumor cells bound to endothelial cells to DNA- damaging chemotherapeutic agents (i.e. melphalan), immunomodulatory drugs, and bortezomib. Similar activity of pazopanib was demonstrated *in vivo* using a MM xenograft mouse model. Ongoing studies evaluate effects of pazopanib on MM bone disease and immune deficiency. *Summary and Conclusions.* In summary, this is the first report showing anti- MM activity of an anti-VEGF compound in both *in vitro* and *in vivo*, strongly supporting its clinical evaluation either as a single agent or in combination with other therapies.

0270

IDENTIFICATION AND CHARACTERIZATION OF HLA CLASS I RESTRICTED T-CELL EPITOPES IN THE PUTATIVE TUMOR ASSOCIATED ANTIGENS P21 ACTIVATED SERIN KINASE 2 (PAK2) AND CYCLIN DEPENDENT KINASE INHIBITOR 1A (CDKN1A)

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Multiple myeloma (MM) is one of the most common hematological malignancies. Despite a variety of therapeutical approaches including high dose cytostatic treatment with subsequent autologous or allogeneic stem cell transplantation, as well as vaccination, cures remain rare exceptions. An important issue for future immunological treatments is the identification and characterization of appropriate tumor associated antigens. However, the number of tumor associated antigens in MM is limited. PBK/TOPK and activated serin kinase 2 (PAK2) are novel serin kinases that have recently been identified. PBK/TOPK is overexpressed in Burkitt Lymphoma, acute lymphoblastic leukemia and MM, PAK2 is expressed on malignant lymphatic cells. The cyclin kinase inhibitor 1A (CDKN1A) is overexpressed in MM compared to normal plasma cells. We here identified and characterized for the first time HLA class I restricted immunogenic peptides in the amino acid sequences of PAK2 and CDKN1A. Using two independent prediction algorithms we identified 2 peptides in PAK2 and 3 peptides in CDK1NA with high binding to HLA-A2. Using an IFN-γ Elispot assay we could demonstrate the pres-

ence and functional activity of CD8 peptide specific T cells with all tested peptides. To show HLA-A2 restricted antigen recognition, specific inhibition of T cell recognition was demonstrated with an anti HLA-A2 blocking antibody. By analysis of peripheral blood of 34 healthy donors for presence and functional activity of CD8 T cells specific for these peptides, we could demonstrate that 50-60% of the tested donors contain peptide T-cell precursors specifically recognizing at least one of the tested peptides and that these T-cell precursors can be expanded *in vitro*. We conclude that PAK2 and CDKN1A derived peptides can elicit a strong and consistent CD8 T cell response in an *in vitro* model. Further investigations will examine the presence and functionality of such T-cells in the tumor bearing host.

0271

CELLULAR IMMUNE RESPONSES AGAINST THE CANCER TESTIS ANTIGEN SPAN-XB IN HEALTHY DONORS AND PATIENTS WITH MULTIPLE MYELOMA

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The cancer-testis antigen SPAN-XB has been recently identified in multiple myeloma. In the present study we identified and characterized for the first time a cytotoxic cellular immune response against SPAN-XB in healthy donors and patients with multiple myeloma. Using two independent computer algorithms two SPAN-XB derived peptides (peptides 624 and 626) with predicted binding to HLA-A2 were identified. To further improve the immunogenicity of peptide 626 we designed a heteroclitic peptide (peptide 627) by modifying one amino acid on the HLA binding position 2 of peptide 626. Using an IFN-γ Elispot assay we could demonstrate the presence and functional activity of CD8 peptide specific T cells with all tested peptides. By analysis of peripheral blood of 13 healthy donors and 5 patients with multiple myeloma peptide specific T-cell precursors specifically recognizing at least one of the tested peptides could be detected and expanded in 9 of 13 of tested donors and 3 of 5 tested patients. Importantly, in two donors specific peptides could be generated against the heteroclitic peptide 627 but not against the native peptide 626. We conclude that SPAN-XB derived peptides can elicit a consistent CD8 T cell response in healthy donors and patients with multiple myeloma.

0272

CLINICAL RELEVANCE OF SOLUBLE HLA CLASS I IN WALDENSTROMS MACROGLOBULINEMIA AND IGM MGUS

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Background. Waldenstrom's Macroglobulinemia (WM) is a B-cell neoplasm characterised by excess secretion of IgM by lymphoplasmacytic bone marrow cells and characterized by a broad heterogeneous clinicobiological presentation throughout the disease history. Serum β-2-microglobulin level is one of the best survival prognostic factors in WM, but other HLA class I molecules might be of interest. Soluble HLA class I molecules (HLA-Is) as well as the main soluble HLA-G isoform (HLA-Gs) are known to have immunosuppressive properties and have been implicated in the pathogeny of several malignancies, including recently in Multiple Myeloma. We hypothesized therefore that expression of soluble HLA class I molecules may help to better characterise IgM-related disorders. *Methods.* We assessed the serum soluble HLA molecules levels in 111 patients with IgM-related disorders [WM (n=42) and IgM-MGUS (n=63), and compared the results to 30 healthy subjects. *Results.* We found higher levels of HLA-Is in WM compared to IgM-MGUS and healthy donors. HLA-Gs levels were similar in WM and in IgM-MGUS, but higher than in healthy donors. HLA-Is, but not HLA-Gs, correlated with markers of tumour burden and with markers of adverse prognosis in WM. HLA-Is also separated patients with symptomatic to asymptomatic diseases within the WM subgroup. High levels of HLA-Is were observed in patients with presence of autoimmune complications, especially cryoglobulinemia, although independently of IgM-MGUS or WM diagnostic. In contrast, low levels of HLA-Is related to IgM-related neuropathy, another complication linked to the presence of the IgM monoclonal protein. Finally, HLA-Is (high) and HLA-Gs (low) levels were inversely associated to neutropenia and lymphopenia. *Conclusions.* HLA-Gs is a marker linked to the presence of a clonal B lymphocyte, with no survival prognostic impact in IgM-disorders and especially in WM, in our study. In contrast, HLA-Is level isolated subsets of patients within the very heterogeneous group of patients with IgM disorders. HLA-Is was related to the occurrence of autoimmune complications and disorders linked to the presence of the monoclonal IgM protein, such as cryoglobulinemia syndrome and neuropathies, in addition to its previously published survival prognostic impact in plasma cell disorders. Together our results suggest a role for soluble MHC class I molecules in IgM-related disorders. Further studies are necessary to confirm those results in a larger population of patients.

0273

PLASMA CELLS FROM MULTIPLE MYELOMA PATIENTS EXPRESS B7-H1 (PD-L1) AND INCREASE EXPRESSION FOLLOWING STIMULATION WITH IFN- AND TLR LIGANDS VIA A MYD88-, TRAF6-, AND MEK-DEPENDENT PATHWAY

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Background. Multiple Myeloma (MM) cells inhibit certain T-cell functions. A possible candidate responsible for such T-cell inhibitory mechanisms in MM plasma cells is B7-H1. B7-H1 (also known as PD-L1 or CD274) is a B7 family member and is the ligand for PD-1 (programmed death-1), a member of the CD28 family. *Aims.* We wanted to determine the role of B7-H1 in CTL inhibition and the mechanisms that control its expression in malignant plasma cells. *Methods.* We examined the expression of B7-H1, in CD138-purified plasma cells isolated from 82 MM patients, 42 monoclonal gammopathy of undetermined significance (MGUS) patients, and 20 healthy donors. *Results.* We observed that B7-H1 was expressed in most MM plasma cells, but not cells isolated from MGUS or healthy donors (Figure 1).

Figure 1. B7-H1 in MM, MGUS, and Healthy controls (HD).

This expression was increased or induced by IFN-γ and Toll-Like Receptor (TLR) ligands in isolated MM plasma cells. Blocking of the MEK/ERK pathway inhibited IFN-γ- and TLR-mediated expression of B7-H1. Inhibition of the MyD88 and TRAF6 adaptor proteins of the TLR pathway not only blocked B7-H1 expression induced by TLR ligands, but also that mediated by IFN-γ. IFN-γ induced STAT1 activation, via MEK/ERK and MyD88/TRAF6, and inhibition of STAT1 reduced B7-H1 expression. MM plasma cells stimulated with IFN-γ or TLR ligands inhibited CTLs generation and this immunosuppressive effect was inhibited by pre-incubation with an anti-B7-H1 antibody, the UO126 MEK inhibitor, or by transfection of a dominant-negative mutant of MyD88. *Conclusions.* We have shown that B7-H1 is expressed on malignant plasma cells, that it is involved in inhibition of T-cell responses by these malignant cells, and is upregulated by IFN-γ and TLR ligands through a common pathway involving MEK/ERK and MyD88. B7-H1 represents a new immune escape mechanism in MM.

0274

NEOPLASTIC PLASMA CELLS ARE DEMONSTRABLE AT BONE MARROW SITES DISTANT TO SOLITARY PLASMACYTOMA OF BONE AND PREDICT FOR PROGRESSION TO MULTIPLE MYELOMA

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Background. Solitary plasmacytoma of bone (SPB) typically present as single destructive lesions within the spinal column or long bones. Local radiotherapy is the treatment of choice but approximately 50% of patient's progress to myeloma. Many patients have a monoclonal protein detectable in their serum and/or urine at diagnosis and persistence of this for >1 year after radiotherapy predicts for progression to myeloma. Identification of high risk patients at the time of diagnosis is clearly desirable as it would enable risk stratification and more careful monitoring of patients. Although prophylactic adjunctive chemotherapy has not definitively been shown to benefit unselected patients with SPB, future therapeutic advances might be targeted on patients with a high risk of progression. Interestingly it has recently been shown that patients positive for serum free light chains are at greater risk of progression to myeloma. We and others have previously demonstrated that neoplastic bone marrow plasma cells are distinguishable from their normal counterparts by virtue of their lack of CD19 expression and/or their aberrant expression of CD56. We have developed a multiparameter flow cytometry assay which predicts outcome following autologous transplantation in myeloma patients and risk of progression in patients with MGUS. *Aims.* In this study we have applied this assay to assess the staging bone marrow specimens from patients with biopsy proven SPB for the presence of occult disease at sites distant to the primary lesion. *Methods.* 52 patients were included in this analysis (31 male, 21 female, median age 65) and in each case the staging bone marrow aspirate and trephine biopsy (obtained from a site distant from the SPB, typically the right iliac crest) was not indicative of myeloma (<10% plasma cells). *Results.* Plasma cells comprised a median of 0.6% (0.05-6.2%) of bone marrow leucocytes while distinct populations with a neoplastic immunophenotype (>30% CD19- and/or $CD56^+$ as per convention) were demonstrable 35/52 (67%). Neoplastic plasma cells when present comprised a median of 70% (35-100%) of bone marrow plasma cells. 21 patients (40%) developed myeloma with a median time to progression of 476 days (range 18-1632). Progression occurred in 18 of the 35 (51%) patients with neoplastic plasma cells in their staging marrows and in 3/17 (18%) patients with a normal phenotypic profile. The difference was significant using Chi-square analysis with Yates' correction for continuity (p=0.04). The overall risk of progression was similar in patients with a 'myeloma pattern- in which >90% of bone marrow plasma cells had a neoplastic phenotype (5/12, 42%) and those with an MGUS or mixed pattern in which distinct populations of both normal and neoplastic cells are demonstrable (13/23, 57%). *Conclusions.* Neoplastic plasma cells are frequently found at bone marrow sites distant to SPB and their presence predicts for progression to multiple myeloma. Trials of adjuvant systemic therapy are warranted in this group.

0275

INHIBITION OF CELL PROLIFERATION BY LENALIDOMIDE IS ASSOCIATED WITH STIMULATION OF EGR1 TRANSCRIPTIONAL ACTIVITY IN A CHROMOSOME 5 DELETED BURKITTS LYMPHOMA AND MULTIPLE MYELOMA CELL LINE

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Background. The mechanism by which lenalidomide exerts its antiproliferative effects in deletion 5q MDS clones or MM cells is not yet fully elucidated. Early growth response (Egr1) gene is a tumor suppressor gene located on chromosome 5q31.1 that encodes a transcription factor involved in the regulation of cell proliferation and apoptosis. *Aims.* The present study examines the hypothesis that lenalidomide may act by enhancing the expression or activity of Egr1 in sensitive hematopoietic tumor cells, especially those with only a single copy of the Egr1 gene. *Methods.* Luciferase assay. Cells were transfected with Egr1-luciferase (Stratagene) using Lipofectamine 2000 (Invitrogen) reagent according to manufacturer's instructions. Six hours post-transfection, cells were treated with 1 ÌM lenalidomide or DMSO for 16 hours. Luciferase activity was assayed using luciferase substrate (Promega) and measured using a luminometer (Turner Designs). qRT-PCR and siRNA silencing. Cells were transfected with Egr1 or non-targeting siRNA (Dharmacon) using Dharmafect2 (Dharmacon) reagent according to manufacturer's instructions. Gene expression analysis used gene expression assays (Applied Biosystems) and expression was measured on a real-time PCR System 7500 (Applied Biosystems). Relative quantifications were calculated with SDS v.1.3.1 software. Cell proliferation assay. Cells were incubated in 96-well cell culture plates with compounds for 72 hours and assayed by 3H-thymidine incorporation. IC_{50}s were calculated by nonlinear regression analyses with GraphPad Prism. *Results.* Lenalidomide stimulated the transcriptional activity of Egr1 in the lenalidomide-sensitive chromosome 5 deleted Burkitt's lymphoma Namalwa CSN.70 and in the MM cell line LP-1. Egr1 siRNA Namalwa cells proliferated more than mock controls, indicating that Egr1 functions as a tumor suppressor in Namalwa cells. Lenalidomide had no effect on expression of Egr1, but augmented Egr1 nuclear transport in a dose-dependent manner. Lenalidomide did not affect expression of the Egr1 downstream effector genes ATF3, fibronectin, p53, PTEN, and TGF-β1, while p21 levels increased. However, lenalidomide-induced p21 expression was not affected in Egr1 siRNA Namalwa cells. Interestingly, lenalidomide's anti-proliferative potency was greater in Egr1 siRNA Namalwa but not in Egr1 siRNA LP-1 cells. *Conclusions.* Lenalidomide induces nuclear transport and transcriptional activation of the tumor suppressor Egr1, which may contribute to lenalidomide's anti-proliferative activity. This activity may be related to the levels of Egr1 expression, explaining why del 5q31 myelodysplastic clones are especially sensitive to the cytotoxic effects of lenalidomide.

0276

DEPLETION OF BLOOD DENDRITIC CELLS (BDC) AND ITS PROGNOSTIC SIGNIFICANCE IN PATIENTS WITH MULTIPLE MYELOMA

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Background. The number and function of dendritic cells (DC), which are an important part of anti-tumor response, may be crucial for development and natural course of malignancy. There are several types of DC, including those circulating in the peripheral blood (blood DC; BDC), as was recently distinguished by the panel of monoclonal antibodies against BDC antigens (named BDCA-1, -2 and -3). *Aims.* Since these BDC subtypes have not been assessed in multiple myeloma (MM) we aimed to investigate their frequency and absolute numbers in this disease. *Material and Methods.* The study was performed in 41 untreated MM patients. Circulating BDC were detected in blood (MM, control) and bone marrow (MM) samples by the flow cytometry. Three BDC subsets were determined: plasmacytoid (PDC), BDCA-2+/CD123+/HLA-DR+, myeloid 1 (MDC1), BDCA-1+/CD11c+/CD14-/HLA-DR+, and myeloid 2 (MDC2), characterized by BDCA-3+/CD32-/CD19-/HLA-DR+ immunophenotype. The rates and absolute numbers of all DC (total DC; tDC) as well as their particular subsets were calculated. Results obtained in MM patients were compared with age- and sex-matched healthy controls. Moreover, they were correlated with the outcome. The study endpoints were response to first line treatment, progression free survival (PFS) and overall survival (OS) time. *Results.* Objective response to first line treatment was achieved in 27 (66%) patients, including 9 (22%) complete and 18 (44%) partial responses. The remaining 14 (34%) patients were either resistant or even progressive upon chemotherapy. The median follow up was 18 months (5-28). The median PFS of responders was 13.5 months (8-21). In MM patients total frequency and absolute numbers of BDC were significantly lower than in healthy subjects (0.69±0.37% vs. 1.24±0.40%; p=0.002, and 16.2±13.5 cells/μL vs. 31.7±13.3 cells/μL; p=0.005, respectively). Significantly lower rates and counts in MM patients concerned all BDC subtypes, including MDC1 (p=0.035 and 0.029, respectively), MDC2 (p=0.033 and p=0.009, respectively) and PDC (p=0.007 and p=0.0005, respectively). Moreover, all those BDC subtypes were also found in the bone marrow of MM patients. Importantly, a frequency of blood DC in the bone marrow was even higher than in the peripheral blood (for tDC - p=0.017, for MDC2 - p=0.015, and for PDC - p=0.021, respectively). Total DC rates and counts were significantly higher in responders than in resistant patients (0.90±0.40% vs. 0.41±0.29; p=0.009, and 25.3±14.7 cells/μL vs. 9.3±6.0 cells/μL; p=0.032, respectively). The difference was due to prevalence of blood MDC1 in responders (responders vs. non-responders - p=0.011 for rates and p=0.008 for numbers, respectively). Lower (less than median) MDC1 number at the diagnosis correlated with shorter PFS of patients (log rank test: p=0.027) and showed a trend toward longer OS (log rank test: p=0.073). *Conclusions.* This is the first study demonstrating a profound deficiency of all subtypes of BDC in patients with MM. It may reflect either an impairment of immune system, facil-

itating tumor development, or engagement of DC in anti-tumor response. Low pretreatment numbers of BDC may be considered as an biological, negative prognostic marker for further studies.

0277

HIGH HEPATOCYTE GROWTH FACTOR LEVELS AND LOW THROMBOSPONDIN LEVELS IN MYELOMA PATIENTS CORRELATE CLOSELY WITH POOR POSTRANSPLANT RESPONSE

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Backround. Angiogenesis is involved in the development and progression of multiple myeloma (MM). It has already been shown that angiogenesis as assessed by micro-vascular density in the bone marrow of MM patients plays a significant role in the prognosis of MM. Vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF) plays a key roles as angiogenesis activators in MM. Role of angiogenesis inhibitors in MM remains very unclear. The most important inhibitors seem to be thrombospondin, endostatin and angiostatin. *Aims.* Aim of this study was to evaluate the role of VEGF, HGF, bFGF, thrombospondin, endostatin, and angiostatin peripheral plasma (PP) and bone marrow plasma (BM) levels in a group of patients with MM who underwent autologous stem cell transplant (ASCT) and their influence on treatment response. *Methods.* We studied PP and BM levels of factors in 92 MM patients (43M/39F, median age: 51 years) who underwent an ASCT after high dose melphalan conditioning. PP and BM levels of VEGF, HGF, bFGF, thrombospondin, endostatin, and angiostatin were measured in time of diagnosis MM. Patients were divided into three groups according to treatment response: group A) 43 patients who achieved at least very good partial response (VGPR); group B) 35 patients who achieved partial response (PR); group C) 14 patients who did not achieved even PR. The levels of factors in the time of diagnosis in different groups were compared using nonparametric Kruskal-Wallis ANOVA *Results.* HGF concentration at the time of diagnosis in both PP and BM is significantly lower at group A (PP-median=472 pg/L; 95%IS 417-823pg/L. BM-median= 886 pg/L; 95% IS 928-2111pg/L) than at group B (PP-median=623 pg/L; 95%IS 493-990pg/L;/p=0,025/. BM-median= 1165 pg/L; 95% IS 1140-2656 pg/L/; p=0,001/) and group C (PP-median=1870 pg/L; 95% IS 522-4971pg/L;/p=0,001/. BM-median= 2605 pg/L; 95%IS 1328-5355pg/L/;p=0,001/). Thrombospondin concentration at the time of diagnosis only in BM not in PP is significantly lower at group C (BM-median= 188 pg/L; 95%IS 38-678pg/L) than at group B (BM-median= 303 pg/L; 95%IS 249-705p/L/;p=0,036) and group A (BM-median= 351pg/L; 95%IS 437-916pg/L/; p=0,001/. VEGF, bFGF, endostatin and angiostatin concentrations at diagnosis time did not differ significantly in PP and BM in patiensts with VGPR, PR and no response. *Conlusions.* Our results confirmed that key angiogenesis activators in MM is HGF. If the treatment is successful low levels of HGF occurs only. There is important finding that thrombospondin in BM is higher in patients with successful treatment. It means, that angiogenesis is more inhibited in patients with VGPR than in other. Hovewer thrombospondin concentrations in PP are influenced with activated endothelium and platelets, so only BM level of thrombospondin is good candidate for angiogenesis monitoring. Postransplant response is strong prognostic factor and PP HGF levels and BM thrombospondin levels at diagnosis time corelates closely with it. Such as they could became a new prognostic and predictive factor in MM .

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0278

MULTIPLE MYELOMA PLASMA CELLS SHOW DIFFERENT CHEMOKINE RECEPTOR PROFILES AT SITES OF DISEASE ACTIVITY

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Background. Chemokines and their receptors play a pivotal role in the regulation of B-lymphocyte trafficking. *Aims.* This study was aimed at investigating the pattern of chemokine receptor expression, and the migration ability of malignant plasma cells (PC) obtained from Multiple Myeloma (MM) patients. *Methods.* PC were recovered from the bone marrow of 29 MM patients, from extramedullary sites of 10 MM patients and from the bone marrow of 5 controls. 3 myeloma cell lines were also analyzed. By flow cytometry analysis the chemokine receptor expression, including CCR1 to CCR3, CCR5 to CCR7, CXCR1 to CXCR5, was investigated. Moreover, the functionality of the most expressed receptor (CXCR4) was tested by a cell migration assay. *Results.* Flow cytometry analysis showed that the receptors mainly expressed on malignant bone marrow PC were represented by CXCR4 (70% of patients), CCR1 (25%), CCR2 (25%), CCR5 (17%) and CXCR3 (20%), while other receptors were commonly lacking. The analysis performed on extramedullary (peripheral blood and pleural effusion) malignant PC demonstrated that the most represented receptors were CXCR4 (100%), CCR2 (66%) and CXCR1 (60%). The migratory capability evaluation of malignant PC at resting conditions identified 3 groups of patients with different migration (low, intermediate and high). Since CXCR4 was the relevant chemokine receptor expressed by MM PC, its ligand CXCL12 induced their migration. *Conclusions.* These data suggest that malignant PC from MM display different chemokine receptor profiles and that CXCR4 is fully functional and might play a role in the spreading of the disease.

0279

EVALUATION OF CYTOKINE NETWORK PROBABLY INVOLVED IN EVOLUTION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE INTO MULTIPLE MYELOMA

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*Backround.*Monoclonal gammopathies are lymphoproliferative diseases characterised by the production of immunoglobulins or only light or heavy chains, known as *M component*, by a neoplastic B-cell clone. Gammopathies are classified on the basis of their degree of malignancy and their M component. Monoclonal gammopathy of undetermined significance (MGUS) is characterised by being asymptomatic and having a modest M component (<3 g/dL), with bone marrow infiltration <10% and a low proliferative activity, without other alterations typical of multiple myeloma (MM). The risk of progression of MGUS to multiple myeloma or related disorders is about 1 percent per year. MM is typically characterised by osteolytic lesions, the presence of Bence-Jones protein and kidney failure, while plasma cell infiltration of bone marrow is variable. The mechanisms of neoplastic transformation and evolution of monoclonal gammopathies have been studied by many authors, who have predominantly focused their attention on an involvement of programmed cell death, on anomalies of cell adhesion mechanisms and on the role of cytokines. *Aims.* The aim of this study was to evaluate the cytokine network in patients with MGUS and MM and to identify any changes in cytokine concentrations in patients developing a MM from MGUS in order to establish whether the cytokines are involved in this evolution. *Methods.* Fifty-one patients with multiple myeloma (MM) divided into 3 groups according to disease stage (MM1, MM2, MM3), 60 with monoclonal gammopathy of undetermined significance (MGUS) and 50 healthy controls (C) were studied in Caserta's Hospital and in University. The levels of sCD138 (Syndecan-1), TGF-β1, sVCAM-1, IL-13, Fas/APO-1, IL-6, β2-microglobulin (β2-M) and C-reactive protein (CRP) were assayed. Seven of the 60 cases of MGUS (11.6%) evolved into MM3 during the 5-year follow-up; these cases were studied in order to identify any changes in the cytokine network. *Results.* β2-M and CRP concentrations increased significantly through C, MGUS and the three stages of MM. TGF-β1, sVCAM-1, Fas/APO-1 and IL-6 levels were significantly higher, while IL-13 concentration was significantly lower, in MGUS and in MM than in C (p<0.001). The level of sCD138 was significantly lower in MGUS than in C and significantly higher in MM than in C (p<0.001). On the other hand, TGF-β1 concentration was significantly higher, while IL-6 and sCD138 concentrations were significantly lower, in MGUS than in MM (p<0.001). Only sCD138 was significantly higher in MM3 than in MM1 and in MM2 (p<0.001), while the concentrations of all the other cytokines did not differ significantly between MM1, MM2, and MM3. There were no significant differences in cytokine values between MGUS which evolved into MM3 and MGUS which did not evolve. Moreover, in the 7 cases of MGUS which did evolve into MM3, the cytokine levels were not significantly different between the start of the study and at the end of the follow-up. *Conclu-*

sions. We conclude that syndecan-1, like the better recognised β2-M, CRP and IL-6 markers, has a prognostic value for the evolution of monoclonal gammopathies.

0280

HIGHLY PRESERVED POLYCLONAL REGULATORY T CELLS (TREGS) CAN DOMINATE EFFECTOR FUNCTIONS IN MULTIPLE MYELOMA PATIENTS

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Naturally occurring $CD4^+CD25^+$ T regulatory cells (Tregs) are a subpopulation of $CD4^+$ T cells vital to homeostasis and maintenance of tolerance. Tregs play a key role in hampering anti-tumor immune surveillance by regulating T-cell immune responses against tumor cells. Depletion of Tregs brings to improved anti-tumor immune responses in both preclinical and clinical settings. Contradictory data have been reported in the frequency and function of Tregs cells in multiple myeloma (MM). The aim of this study was to characterize MM Tregs from the phenotypic, molecular and functional standpoints. We studied both peripheral blood (PB) and bone marrow (BM) samples from patients with symptomatic MM and from healthy donors. $CD4^+CD25^{high}$ Tregs were normally represented in both PB and BM of MM patients and expressed a memory and activated phenotype. No differences were observed between PB and BM Tregs of healthy donors and MM patients, based on the expression of CD45R0, HLADR, CD40L and CD69 cell surface antigens. Flow cytometry was also used to assess the intracellular expression of Foxp3, a transcription factor which has been shown to be crucial for the inhibitory function of Tregs cells. More than 90% of $CD4^+$CD25high T cells were Foxp3-positive in PB and BM of MM patients and healthy donors. Numbers and distribution of BM Treg were further confirmed by immunohistochemical analyses of Foxp3-positive cells in whole tissue paraffine embedded sections. $CD4^+CD25^+$ T cells were purified from PB of newly diagnosed MM by MACS sorting. These cells were anergic to the stimulation via TCR and they were as effective as normal donor-derived $CD4^+CD25^+$ cells in inhibiting the TCR-mediated proliferation of autologous $CD4^+CD25^-$ counterparts. Moreover MM Tregs equally inhibited autologous and allogeneic CD4+CD25- from normal donors. To investigate whether clonal restriction had occurred in MM Tregs, we studied TCR diversity of $CD4^+CD25^+$ and $CD4^+CD25^-$ cells by determining the reciprocal usage of BV gene segments (TCRBV repertoire) with a novel multiplex polymerase chain reaction assay, Our results demonstrate an highly preserved polyclonal TCRBV repertoire, providing the first evidence in cancer patients that TCR diversity of Tregs is not skewed by the long lasting exposure to tumor cells. Based on these data, we propose that inhibitory signals delivered by the highly preserved Tregs subset in MM can easily overwhelm the effector mechanisms of antitumor immunosurveillance which are deeply compromised in MM. Thus, depletion or neutralization of Tregs should be considered in future trials aimed at controlling the disease in MM patients by immune intervention.

Non-Hodgkin lymphoma - Biology

0281

PERSISTENCE OF LYMPH NODE T(14;18) BEARING CELLS IN FOLLICULAR LYMPHOMA PATIENTS IN CLINICAL COMPLETE REMISSION: PROOF OF PRINCIPLE FOR MAINTENANCE THERAPY?

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Background. Despite many new therapeutic options, follicular lymphoma (FL) is a chronic relapsing malignant disease rarely cured by conventional treatments. Some institutions use PCR t(14;18) blood and/or bone marrow monitoring, however, its real importance remains controversial. *Aims.* We hypothesized that lymph nodes are much more better compartment for FL monitoring than peripheral blood/bone marrow. We asked whether it would be possible to monitor regularly the disease in patients by ultrasound-guided fine needle aspirations (UG-FNA) like we usually monitor acute leukemias by bone marrow aspirations. *Methods.* First we asked about the efficacy of FNA to detect FL by FISH and/or PCR. For this purpose we investigated either autopsy material or surgically removed lymph nodes both by FNA and classical histology.Then, upon informed consent, we performed UG-FNA in patients either with advanced disease or in clinical remission in order to search for lymph node t(14;18) bearing cells in involved nodes, or in previously involved node areas. Real time (RQ) PCR (Taqman) gives results as copies/10^6 cellular equivalents. *Results.* In 22 tested paired samples (histology vs. FNA) by PCR, 20/22 samples gave identical results. In one case each, histology vs. FNA or vice versa, gave discordant negative results. We performed 31 UG-FNA on out-patients basis without any complications. In 15 cases, both FISH and PCR were available, with FISH being positive in 13/15 samples and PCR in 8/15 samples. In only 4/31 cases, the FNA was not eventually carried-out for very small nodes. In 3/31 samples, the material did not contain vital nodal cells. All unsuccessful UG-FNA were performed in patients in remission (see below). Therefore, 24/31 samples could be examined by FISH and/or PCR: 10/24 in newly diagnosed patients, 9/24 in relapsed patients, and 5/24 in patients in remission. RQ PCR gave much higher copy numbers in lymph nodes (median: 2.3×10^6)> bone marrow (16×10^3)>peripheral blood (4.2×10^3). There were 12 UG-FNA in 11 remission patients (CT and usually also PET, and PCR from blood/or marrow, when available). 4/12 were technically unsuccessful and 3/12 samples did not contain material suitable for further examination. Four of these 7 patients had had PCR positivity at the time of diagnosis, and 6/7 are still in sustained remission. In 5 aspirations in 4 patients, there was detection of t(14;18). One patient is in long lasting remission despite two UG-FNA positive *Results.* Remarkably all other 3 patients relapsed 3, 6, and 12 months after an UG-FNA. *Summary*. This study is the proof of principle of the detection of residual (lymphoma progenitor, stem?) t(14;18) bearing cells in the previously involved lymph nodes despite patients being in well defined remission. Further study is needed to define the biological and clinical importance of this finding. This study is also the theoretical basis for maintenance therapy with rituximab, however, with the current strategy, some patients might be overtreated and the best schedule is not known.

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0282

NK-CELL ASSOCIATED RECEPTORS EXPRESSION IN EPSTEIN-BARR VIRUS-POSITIVE GAMMA-DELTA T-CELL LINES DERIVED FROM PATIENTS WITH NASAL T-CELL LYMPHOMA AND CHRONIC ACTIVE EBV INFECTION

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Background and Aim. The contribution of chronic antigen stimulation to the occurrence of lymphoproliferative disorder (LPD) with the γ-δ T-cell lineage is unclear, despite the fact that Epstein-Barr virus (EBV) positive T-cell LPD is derived from antigen-stimulated cytotoxic T-cells. Given the possible association of antigen stimulation with the development of cytotoxic T-cell LPD, we compared gene expression patterns in Epstein-Barr virus (EBV)-positive γ-δ T-cell lines derived from patients with nasal T-cell lymphoma and chronic active EBV infection and those in γ-δ T-cells from healthy volunteers. [METHODS] Three EBV-positive γ-δ T-cells lines, SNT cells (SNT-8, SNT-13 and SNT-15), were used in this study. SNT-8 was established from patients with nasal T-cell lym-

phoma and SNT-13, -15 were established from patients with chronic active EBV infection (Zhang Y, *et al.,* Br J Cancer 94:599-608, 2006). All the SNT cells exhibits common rearrangement of Vγ9-JγP and Jδ3 genes. The γ-δ T-cells obtained from healthy volunteers were expanded *ex vivo* by 1 μM of zoledronate (ZOL) plus IL-2 for 14 days incubation. Global gene expression was analyzed using the Affymetrix Human Genome U133 2.0 Plus GeneChip Set. Analysis of variance (ANOVA) was done using GeneSifter® (VizXLabs). Values of $p<0.05$ were considered to be a statistically significant difference. *Results.* SNT cells share a common molecular signature, including transcriptional regulation of genes related to cytokine-cytokine receptor interaction and natural killer cell mediated cytotoxity: up-regulated genes in SNT cells include chemokine (C-C motif) receptor 7, CSF1 receptor, and major histocompatibility complex class II. Expression of killer cell lectin-like receptor subfamily B, member 1(KLRB1:CD161), NK-cell IRC1c (CD300A) were significantly down-regulated in SNT cells. Expression of killer cell immunoglobulin-like receptors (KIRs) , i.e. KIR3DL1, KIR3DL2, KIR2DL4, were evident in SNT-13 cells as well as γ-δ T-cell obtained from healthy volunteers. *Conslusions*. Our results suggest that γ-δ T-cell expansion in EBV-positive T-LPD is in principle due to inappropriately expressed NK-cell associated receptors (NKRs), thereby, being resistant to activation-induced cell death. Further study of NKRs may provide insights into the pathogenesis of EBV-positive LPD with γ-δ T-lineage, which may lead to the development of novel therapeutic strategies.

0283

AMPLIFICATION AT 7Q22 IN THE ANAPLASTIC LARGE CELL LYMPHOMA CELL LINE SU-DHL-1 TARGETS CYCLIN-DEPENDENT KINASE 6

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Background. Genomic amplification is microscopically visible extrachromosomally as double-minute chromosomes (DMIN) or intrachromosomally as homogeneously stained regions (HSR). Both DMIN and HSR comprise iterated copies of short genomic regions effecting copy number increases associated with upregulation of target genes which in cancer cells are usually well known oncogenes such as cMYC. *Results.* Cytogenetic analysis of the anaplastic large cell lymphoma (ALCL) cell line SU-DHL-1 revealed an HSR on the long arm region of chromosome 7 (7q). Chromosome analysis of this region by fluorescence *in situ* hybridization using tilepath BAC clones identified an amplicon corresponding to 86-95 Mb (NCBI Build 36.1) at 7q22. Expression analysis using reverse transcription (RT)-PCR of candidate genes mapped within the amplified genomic region comprising, DBF4, SRI, AKAP9, GATAD1, CDK6 and PPP1R9A, highlighted cyclin-dependent kinase 6 (CDK6) as a plausible amplification target. With reference to another ALCL cell line SR-786 which lacks this amplicon, SU-DHL-1 displays upregulation of CDK6 at both the RNA and protein levels as indicated by semiquantitative RT-PCR and immuno-cytochemistry. In contrast to SR-786, SU-DHL-1 cells are resistant to the effects of rapamycin on proliferation and G1 cell cycle arrest, implying that CDK6 overexpression may confer proliferative advantage. *Summary.* Taken together, we identified an amplicon at 7q22 in ALCL cells targeting CDK6 expression. CDK6 is an important cell cycle regulator probably connected to the increased proliferative capacity of ALCL cells and susceptible to small-molecule inhibition which may represent a potential therapeutic target in this disease entity.

0284

LOCALIZED FOLLICULAR LYMPHOMA: SPREAD OF BCL-2/IGH+ CELLS IN THE BLOOD AND BONE MARROW FROM THE PRIMARY SITE OF DISEASE AND POSSIBILITY OF CLEARANCE AFTER INVOLVED FIELD RADIOTHERAPY

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Background. Localized forms of follicular lymphoma (FL) have a good prognosis being curable in 40-50% of cases with local radiotherapy alone. Polymerase chain reaction (PCR) of Bcl-2/IgH rearranged cells provides a sensitive measure of peripheral blood (PB) and bone marrow (BM) minimal non-Hodgkin's lymphoma (NHL) cell contamination in FL. *Aims.* To evaluate whether a more sensitive technique could document the presence of circulating NHL cells in limited stage FL and the possibility of inducing the disappearance of PCR$^+$ cells from the PB and BM after involved field radiotherapy. *Patients and Methods.* Between April 2000 and April 2006, 24 consecutive patients with FL, Ann-Arbor stage I or IIA, entered the study. Median age was 57 years (range: 27-74), with 9 males and 15 females. Involved field radiotherapy was performed in all patients. PCR was evaluated in the BM and PB of all patients at diagnosis and was re-evaluated 3 months after treatment and, thereafter, every 6 months. *Results.* PCR analysis performed at presentation demonstrated the presence of Bcl-2/IgH$^+$ cells in the PB and/or BM of 16 of the 24 patients studied (66.6%). The rearrangement fell in the major breakpoint region (MBR) in 15 cases and in the minor cluster region (mcr) in 1. Cases Bcl-2/IgH negative at baseline were all negative for JH rearrangement. Of the 16 Bcl-2 positive patients at baseline, at least one post-treatment molecular evaluation was performed in 15 (1 patient refused). Among these evaluable patients, 9 (60%) achieved a PCR negativity in the PB and BM, while 6 (40%) remained positive. RQ-PCR could be performed on the baseline material of 7 Bcl-2/IgH positive cases. The molecular clearance of BCL2/IgH$^+$ cells occurred, following therapy, only in the 3 patients who at diagnosis had a PB or BM tumor infiltration lower than 1 positive cell/100,000. Conversely, the 4 patients with a higher tumor contamination (1 positive cell in 10,000 or more) remained PCR positive after irradiation and 2 patients ultimately relapsed. After treatment, all patients achieved a clinical complete remission (CR) that still holds, except for 2 persistently Bcl-2$^+$ patients who had a clinical relapse 39 and 9 months after treatment, and 1 patient who obtained a PCR negativity but relapsed 36 months after diagnosis. The follow-up ranged from 11 to 70 months (median 43.5). Among the 6 patients persistently positive for Bcl-2/IgH rearranged cells in the BM and/or PB after treatment, 2 (33.3%) have relapsed, while among the 17 patients negative either at baseline or after treatment only 1 (5.8%) has so far relapsed. *Conclusions.* In the majority of stage I/IIA FL patients at presentation, PCR analysis demonstrates the presence of viable Bcl-2$^+$ cells in the BM and PB. A durable disappearance of Bcl-2$^+$ cells from BM and/or PB samples can be obtained in a high proportion of patients after local radiotherapy alone. The possibility of a persistent lymphoma cell clearance is proportional to the amount of cells detected at presentation by quantitative PCR.

0285

HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN ADULTS: A SERIES OF 19 PATIENTS

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Background. Haemophagocytic Lymphohystiocytosis (HLH) is a rare, fatal disease, usually of early infancy, characterized by uncontrolled hyperinflammation on the basis of inherited or acquired immune deficiencies. Both acquired and genetic forms are associated with impaired natural killer (NK) and cytotoxic T- cell (CTL) function. Perforin (PRF) mutations have been demonstrated in a proportion of patients diagnosed with the familial form of HLH. Impaired PRF activity results in ineffective killing, sustaining NK and CTL activation with a hypersecretion of pro- inflammatory cytokines, leading to macrophage activation and organ infiltration. Cardinal symptoms of HLH are fever, cytopenias and hepatosplenomegaly. Characteristic laboratory findings include elevated triglyceride, ferritine, transaminases, bilirubin and low fibrinogen. *Aims.* Data on the occurrence of HLH in adult patients are limited to single cases or small series. The aim of this study is to evaluate the occurrence of HLH in adult patients, focusing on the biological and clinical features and the underlying diseases. *Patients and Methods.* From February 2003 through August 2006 19 patients (11 male and 8 female) were diagnosed with HLH, using the revised criteria of the Histyocyte Society. The median age at diagnosis was 55 years (31-71). Thirteen (68%) patients presented a hematological underlying disease (1 Castleman's disease, 2 AIHA, 5 DLBCL, 2 B-CLL, 2 PTCL and 1 ALCL). Perforin expression was performed on NK cells by flow cytometry. NK activity of PBMCs was assessed by standard 4-hour51Cr-release assay with K562 cells. Perforin mutations were detected by polymerase chain reaction and sequencing. *Results.* All patients presented fever, cytopenia and elevated ferritin. Eleven (58%) patients showed hypertriglyceridemia and hypofibrinogenemia. Fifteen (79%) patients had splenomegaly. Bone marrow biopsy showed obvious hemophagocytosis detected both morphologically and immunohistochemically (anti-CD68R and anti-CD163) in 12 (63%) patients. EBV DNA, CMV DNA and HHV8 DNA were detected in 4, 3 and 8 cases respectively. CTL activity resulted defective in 7/9 patients evaluated: 3 patients showed no PRF mutation; 1 patient

without PRF mutation showed normal PRF expression by flow cytometry; 2 patients showed A91V heterozygous polymorphism with reduced and absent PRF expression, respectively; 1 patient showed heterozygous G1070A mutation; genetic analysis could not be performed in 3 patients. *Summary and Conclusions.* In this series of patients the occurrence of HLH in adults was higher than expected and it was associated with a high incidence of lymphoma, also of B cell lineage, and of Herpes virus DNA detection. Impaired CTL appears to be, at least in some patients, independent from PRF mutations, raising the question of whether and how these genetic events can be related, in acquired HLH of adults, to the deficiency of the lytic activity of PRF.

0286

THE PROGNOSTIC SIGNIFICANCE OF CELL CYCLE REGULATORY GENES EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Background. Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy of mature B cells with heterogeneous prognosis. Biological markers have been used in an attempt to improve the discriminative capacity of the International Prognostic Index (IPI). Abnormalities of cell cycle constitute a marker of clonal expansion and may contribute to disease progression; thus the investigation of cell cycle regulatory genes may be of prognostic relevance. *Aims.* The aim of the present study was the investigation of gene expression levels of cell cycle regulatory genes in DLBCL and their relation to prognosis. *Methods.* The analysis included 45 newly diagnosed cases of diffuse large B-cell lymphomas and 15 cases of non-neoplastic lymphadenopathies used as controls. The IPI was available for all lymphoma cases. RNA was extracted from fresh frozen tissues and analyzed by RNase Protection Assay (RPA) with Riboquant kit (BD Biosciences). Two sets of probes were used that included cyclins, cyclin dependent kinases and their inhibitors. In more detail h-CC1 set of probes detected mRNA for the genes cdk-1,cdk-2,cdk-3,cdk-4,p16,p27,p21,PISSLRE and h-CYC1 set of probes detected mRNA for the genes of cyclins A,B,C,D1,D2,D3 and A1.The intensity of each band was compared to that of the housekeeping genes GAPDH and L32 using the Image Master Software(Pharmacia). Moreover, immunohistochemistry was performed on paraffin-embedded tissue sections in order to verify the expression of germinal center proteins, bcl6 and CD10. *Results.* The expression levels of the cell cycle regulatory genes did not correlate with any of the clinical parameters nor the prognostic markers. 85% of patients that didn't have complete remission to first line therapy, expressed the cyclinA1 (p=0,03). 92% of the patients with low IPI (0,1) expressed PISSLRE, while only 56% of the patients with high IPI (2,3,4,5),expressed this gene. Moreover, cdk2 and cdk4 expression levels were significantly higher in cases expressing bcl-6 protein (p=0,013, p=0,004 respectively). The mean expression level of cyclin B was higher in cases with positive expression of protein CD10 (p=0,007). All patients with negative expression of CD10 had a lower expression level of cyclinA1 compared to normal RNA pool (p=0,037). Moreover, patients expressing cyclinA1 had significantly shorter disease free survival (p=0,03), while the expression of PISSLRE was associated with significantly better overall survival (p=0,0329). *Summary and Conclusions.* Taking into consideration the heterogeneity of clinical behaviour of DLBCL, the cell cycle regulatory genes may serve as biologic markers with prognostic significance. Our study has shown that the expression level of cyclinA1 is associated with significantly lower response to first line therapy and lower disease free survival. Moreover the expression of PISSLRE seems to be associated with low risk disease and higher overall survival, which emphasizes the potential role of this gene as a tumour suppressor.

0287

Z-GUGGULSTERONE DOWNREGULATES SURVIVIN AND INDUCES CELL DEATH IN LARGE B CELL LYMPHOMA CELLS *IN VITRO*

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Background. Survivin is a member of the Inhibitor of Apoptosis Proteins and has recently gained attention as a possible therapeutic target in neoplasia, due to its dual role both as an antiapoptotic protein and as a cell cycle regulator. It is overexpressed in malignant cells and confers resistance to chemotherapy and other stimuli triggering apoptosis. Survivin as well as other antiapoptotic proteins are under NF-κB control. Z-Guggulsterone (Z-GGS) is a plant sterol, which has been used in inflammatory conditions, is a potent NF-κB suppressor and might affect Survivin. *Aims.* To evaluate Survivin expression by two large B cell lymphoma cell lines and study the effect of Z-GGS on Srvivin expression and cell viability. *Methods.* DB and HT cell lines were treated with increasing concentrations (10 μM, 20 μM and 30 μM) of Z-GGS, for 24, 48 and 72 hours. Survivin expression was tested by flow Cytometry and quantitative real time PCR using the Universal Probe Library hydrolysis probes and expressed as Survivin/abl ratio. Cell viability was assessed by the MTT assay. *Results.* Both cell lines were positive for Survivin at baseline by flow cytometry (66% of total cells for DB and 95% for HT). Treatment of DB cells with 10, 20 and 30Ìª Z-GGS resulted in a 44%, 49% and 68% reduction of Survivin expression at 24 hours, respectively, whereas the effect on HT was less prominent with a 10% reduction at 24 hours with 30 μM Z-GGS. Survivin transcripts decreased as well, with the maximum effect observed at 72 hours with 3 μM Z-GGS for both cell lines: Survivin/abl was 0.009 for untreated cells vs 0.0008 with 30 μM Z-GGS for DB cells and 0.0135 vs 0.0005 for HT cells. Linearity was observed for increasing concentrations of Z-GGS at 72 hours. Cell viability was practically unaffected at any time point with 10 and 20 μM Z-GGS for both cell lines, whereas 30 μM Z-GGS resulted in a 63% and 78% cell death at 48 and 72 hours respectively for DB cells and 67% and 83% for HT cells. *Conclusions.* The steroid Z-GGS downregulates Survivin expression in B-lymphoma cells *in vitro* and induces cell death at 30 μM concentration. Further experiments will clarify its possible role in the treatment of B-cell malignanciies

0288

CD5 POSITIVE SPLENIC MARGINAL ZONE LYMPHOMA : CLINICAL, CYTOLOGICAL, IMMUNOLOGICAL, MOLECULAR AND CYTOGENETICAL FEATURES OF A SERIES OF 35 CASES

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Splenic marginal zone B-cell lymphoma (SMZL) has been recently recognized owing to clinical, cyto-histological, immunological, cytogenetical characteristics. The immunological profile, usually CD5, CD10, CD23, CD43, cyclinD1 negative B-cells, is however in lack of specific markers which could be useful in doubtful cases. For instance, the report of CD5 positive cases without histological control is still a matter of debate, and in fact the differential diagnosis with B-CLL or MCL is sometimes difficult. Nevertheless CD5 postivity has been reported in some histologically typical MZL cases and proposed as a marker for a more aggressive and disseminated disease. Here we described a series of 35 CD5 positive SMZL histologically proven in 26 cases and without t(11;14) detected by FISH, that were compared to a series of 44 *typical* CD5 negative SMZL. From a clinical, cytological and immunological point of view, the CD5 positive SMZL cases presented many similarities with the group of CD5 negative SMZL. The main differences consisted in a higher lymphocytosis at diagnosis (4,95 G/L in CD5 positive group and 1,60G/L in CD5 negative group, respectively -p=0.003-) and a more frequent IgM isotype of the monoclonal component (75% vs 53%). Interestingly, all CD5 positive cases in peripheral blood were CD5 positive on splenic specimen studied by flow cytometry and no CD5 negative B-cells were detected in spleen compartment. The tumoral mass assessed by spleen weight seemed similar between both groups.These results suggest that CD5 expression gives B-cells a higher propensity to recirculate from spleen. A good correlation was observed with the CD5 expression detected by immunohistochemistry. The few CD5 negative cases on sections corresponded in fact with a faint CD5 expression by FCM. Karyotypic changes were similar in both groups and included the chromosomal abnormalities previously described in SMZL. However, some differences were observed : trisomy 18 was more frequent (29% vs 11%) and the 7q deletion was less frequent (23% vs 36%) in the CD5 positive group than in the CD5 negative group, respectively. The frequency of 17p deletion, leading to an alteration of P53 and a more aggressive clinical course was similar in both groups. Most cases were mutated in the CD5 positive group (82%) in contrast to the CD5 negative group (55%). The IgVH analysis showed a VH4 usage gene in 4 cases (23%) of the 17 available CD5 positive cases, whereas in the CD5 negative group, an underrepresentation of VH4

usage (2/20 ie 10%) were observed. No difference in outcome and overall survival was found between both groups. In conclusion, this study allowed to confirm the existence of CD5 positive SMZL, closely related to classical CD5 negative SMZL and distinct from CLL and MCL. The differences observed with CD5 negative cases, in particular cytogenetical abnormalities and usage of IgVH gene, suggest that this entity may arise from a distinct cell in the marginal compartment, Obviously further studies of more cases are required to confirm and precise these data.

0289

ABERRANT METHYLATION OF SHP-1 (SH2-CONTAINING PHOSPHATASE 1) AND SOCS-1 (SUPPRESSOR OF CYTOKINE SIGNALLING 1) GENES IN IMMUNODEFICIENCY-RELATED LYMPHOMAS

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Background. SHP-1 and SOCS-1 are members of a family of negative regulators of signalling that act downstream of cytokine receptors, receptor tyrosine kinases and receptor complexes of the immune and hematopoietic system. SHP-1 and SOCS-1 act through the inhibition of the JAK2/STAT pathway and their inactivation is considered to be a relevant mechanism in JAK2/STAT pathway induction. Several observations point to induction of the JAK/STAT pathway as an important mechanism of lymphomagenesis. *Aims.* Since DNA methylation and DNA somatic mutation are mechanisms of gene silencing and gene inactivation involved in the pathogenesis of human cancers, including lymphoma, here we tested the involvement of SHP-1 and SOCS-1 methylation and of SOCS-1 mutation in immunodeficiency-related lymphoma. *Methods.* Tumor samples from 48 HIV-related non-Hodgkin lymphoma (HIV-NHL) and 25 post-transplant lymphoproliferative disorders (PTLD) were analyzed for SHP-1 and SOCS-1 methylation by methylation-specific polymerase chain reaction and for SOCS-1 mutation status by DNA direct sequencing. The tumor panel included 21 HIV-diffuse large B cell lymphoma (HIV-DLBCL), 17 HIV-Burkitt lymphoma (HIV-BL), 10 HIV-primary effusion lymphoma (HIV-PEL), 10 PTLD-immunoblastic (PTLD-IB), 7 PTLD-centroblastic (PTLD-CB), 4 polymorphic PTLD (P-PTLD), 3 PTLD-BL, and 1 PTLD multiple myeloma (MM). *Results.* Among HIV-NHL, SHP-1 methylation occurred in 23/48 (48%) cases, including 12/21 (57%) HIV-DLBCL, 3/17 (17.6%) HIV-BL, and 8/10 (80%) HIV-PEL. SOCS-1 methylation occurred in 7/48 (14.6%) HIV-NHL, including 5/10 (50%) HIV-PEL and 2/21 (9.5%) HIV-DLBCL. When considering EBV status, SHP-1 was methylated in 10/11 (91%) EBV-negative HIV-NHL and in 12/22 (54%) EBV-positive HIV-NHL. Among PTLD, SHP-1 methylation was detected in 19/25 (76%) cases, including 7/10 (70%) PTLD-IB, 6/7 (86%) PTLD-CB, 3/4 (75%) P-PTLD, 2/3 (66%) PTLD-BL and 1 PTLD MM. SOCS-1 methylation was detected in 3/25 (12%) PTLD, including 2/10 (20%) PTLD-IB and 1/7 (14.3%) PTLD-CB. When considering EBV status, SHP-1 was methylated in 12/13 (92%) EBV-negative PTLD and in 6/11 (54%) EBV-positive PTLD. Missense mutations of SOCS-1 were detected in 1/15 HIV-DLBCL tested and in 1/25 PTLD. *Conclusions.* The implications of our data are threefold. First, SHP-1 methylation is involved in the majority of HIV-NHL and PTLD. Second, it is remarkable that virtually all EBV-negative HIV-NHL and EBV-negative PTLD carry SHP-1 methylation. In EBV-positive B-cells, EBV infection activates the STAT pathway. It is conceivable that, in EBV-negative HIV-NHL and in EBV-negative PTLD, SHP-1 inactivation through aberrant methylation may surrogate EBV infection for STAT activation. Third, similar to observations in NHL of the immunocompetent host, SOCS-1 methylation and mutation is rarely implicated in the pathogenesis of immunodeficiency-related NHL. This notion is consistent with the phenotype of SOCS1-deficient mice, that die of a myeloproliferative disease but do not develop a lymphoproliferative disease

0290

P53 STABILIZATION BY THE MDM2 INHIBITOR NUTLIN-3A INDUCES P53-DEPENDENT CELL CYCLE ARREST AND APOPTOSIS IN ANAPLASTIC LARGE CELL LYMPHOMA

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Background. Mutations of the p53 tumor suppressor gene is the most frequent genetic alteration in human cancer. However, p53 mutation rate is relatively low in most haematologic malignancies including non-Hodgkin lymphomas. Previously we have shown that p53 mutations are uncommon in anaplastic large cell lymphoma (ALCL), an aggressive T/null cell lymphoma type, despite the variable level of expression of wild-type p53 protein [Leukemia 2005;19(9):1663]. MDM2 (HDM2 in humans) is a physiologic negative regulator of p53 levels within the cell. We hypothesized that p53 stabilization using a novel Mdm2 inhibitor, nutlin-3A, would activate p53-dependent apoptotic and cell cycle regulatory pathways in ALCL cells carrying wild-type and potentially functional p53 gene. *Methods.* Two ALCL cell lines, SupM2 and DEL, carrying wild-type p53 (wt-p53) and 2 ALCL cell lines harboring a mutated p53 (mt-p53) gene, Karpas 299, and SR-786, were used. All cell lines were treated with different concentrations of nutlin-3A (0, 2.5 , 5, 10 mM). The effects of p53 stabilization by nutlin-3A on cell viability and proliferation of ALCL cells were assessed using trypan blue and MTS assays, respectively, at two time points (24 and 48 hours). Cell cycle and apoptosis (annexin V binding) analysis was also performed using flow cytometry. To further investigate the underlying mechanisms of cell cycle arrest and apoptosis following nutlin-3A treatment, Western blot analysis was performed. *Results.* Treatment with increasing concentrations of nutlin-3A resulted in a dose-dependent increase of p53 levels, which was associated with increased levels of MDM2 a known transcriptional target of p53 gene. P53 stabilization resulted in a concentration-dependent decrease in cell viability and total cell number in ALCL cells carrying wt-p53 (SupM2 and DEL) but not in cells harboring p53 mutations (Karpas 299 and SR-786). At a concentration of 10 mM nutlin-3A, cell viability was dramatically decreased at 48 after treatment in SupM2 and DEL. Changes in cell viability were largely attributed to apoptosis, since the percentage of Annexin V+ cells was increased from 8% to 87% in SUPM2 and from16% to 50% in DEL.. By contrast, no changes in cell viability or apoptosis were observed in Karpas 299 and SR-786 cell lines(mt-p53). Apoptosis was associated with cleavage of caspase 3 and 9 in immunoblots. Cell cycle analysis revealed that nutlin-3A treatment in SupM2 and DEL resulted in cell cycle arrest. The S-phase fraction of cell cycle was decreased by approximately 50% at a concentration of 5 mM, 24 hours following treatment. Cell cycle arrest was associated with a concentration-dependent increase of the cyclin-dependent kinase inhibitor p21, which is trancriptionally regulated by p53 gene. *Conclusions.* Selective inhibition of Mdm2 by nutlin-3A leads to non-genotoxic activation of p53 pathway and may represent a novel therapeutic modality in patients with ALCL.

0291

PREVALENCE OF CHLAMYDIA PSITTACI INFECTION IN NODAL AND EXTRANODAL NON-HODGKIN LYMPHOMAS

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Background. Ocular adnexal lymphomas (OAL) are malignancies with rapidly increasing incidence rates. Recently, we demonstrated an association between Chlamydia psittaci (Cp) persistent infection and OAL. The condition of the systemic subclinical Cp infection encountered in many OAL patients provided the rationale for searching alternative sites of lymphoma development, an uncovered issue particularly for extranodal localizations. *Methods.* 172 non-consecutive cases of non-Hodgkin lymphomas were analyzed. 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, which simultaneously detects the DNA of the three major Chlamydiae species (i.e. C. trachomatis, pneumoniae, and psittaci). DNA of C. trachomatis L2, C. pneumoniae TW-183 or C. psittaci ORNI were included as positive controls. Amplicons encompass part of the 16S rRNA gene and the 16S-23S spacer region in the ribosomal genes. PCR products were analyzed by electrophoresis and DNA fragment size was quantified by image analysis. Samples were considered positive when Chlamydia DNA was amplified in at least two of three independent experiments. Specificity of the amplified fragments was confirmed by direct sequencing. Sequence specificity was assessed by BLAST search. In each sample negative to PCR, amplification of α-globin control gene was carried out. Prevalence of Cp DNA for each lymphoma category was compared with the prevalence in the 74 controls. *Results.* Overall, Chlamydiae DNA was detected in 20 (12%) cases of NHL. Cp turned out to be the most

prevalent in NHL tissues (17 cases; 10%); *Chlamydia pneumoniae* and trachomatis DNA was detected in 3 cases (2%) (p=0.001). Cp DNA was present in 5 (7%) out of 74 controls; it was detected in 15 (11%) out of 141 extranodal lymphomas and in 2 (6%) of 31 nodal lymphomas (p=0.48) (Table 1). Among extranodal lymphomas, the prevalence of Cp DNA was 10% and 11% respectively for B- and T-cell lymphomas. According to the histotype of extranodal B-cell lymphomas, Cp DNA was present in 4 (7%) of the 56 marginal zone B-cell lymphomas (MZL) and in 9 (15%) of 73 diffuse large B-cell lymphomas (DLBCL) (p=0.33). Cp infection was not randomly distributed among extranodal B-cell lymphomas: it was detected in 6 (25)% of cutaneous lymphomas and in 18% of DLBCL arising in the Waldeyer's ring, while it was rarely detected in gastrointestinal sites. *Conclusions.* This is the largest study revealing that Cp-related lymphomas may occur outside the ocular adnexae; these lymphomas may arise in the skin and Waldeyer's ring, two extranodal sites considered as *first-barrier* to antigen exposure. This finding may have obvious clinical implications, in view of the encouraging results offered by Cp-eradicating therapy with doxycycline 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.

Table 1.

0292

SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES (VL>20%) CONSTITUTES A VARIANT OS SPLENIC MARGINAL ZONE LYMPHOMA WITH DISTINCT CLINICO-PATHOLOGICAL AND MOLECULAR FEATURES

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Splenic marginal zone lymphoma (SMZL) presents some degree of molecular heterogeneity, which suggests the coexistence of different diseases. Especially splenic lymphoma with villous lymphocytes (SLVL) defined at the beginning by hematologists on blood films and incorporated in WHO classification as the leukaemic form of almost all SMZL, could play a role in this heterogeneity. In our experience, the aspect and proportion of villous lymphoid cells (VL) in blood may be quite variable, and typical VL are often lacking or rare indeed in SMZL. Thus, with the aim to define more homogeneous entities, we selected among our files of SMZL, 37 cases displaying more than 20% of typical VL and for which cytogenetic and molecular data were available, and were compared them to a control group of classical non-villous SMZL. Patients presented a male predominance (sex ratio: 1.76) with a median age of 76 years old, a moderate lymphocytosis (from 4.3 G/L to 25.5 G/L) with splenomegaly (97%) without lymphadenopathy (5%) or pancytopenia (32% anemia, 15% thrombocytopenia, 3% neutropenia). Immunophenotypically, the monoclonal B cells expressed IgM (20%), IgG (20%), IgM+G (22%) or IgM+D (30%), CD76 (86%), CD11c (97%), and usually lack CD25 (one positive case) or CD5 (5 faintly positive cases). Interstingly CD103 and CD123, known as markers of hairy-cell leukemia (HCL), were expressed in 13 of 34 and in 3 of 19 available cases, respectively. Besides, the fluorescence intensity (RFI) of the CD11c and CD22 appeared different in SLVL, SMZL and HCL. On VL, the CD11c and CD22 expression was moderate (RFI CD11c=40 and CD22 RFI=138), whereas in SMZL the CD11c and CD22 staining was lower (RFI CD11c=15 and CD22 RFI=62) and in HCL the CD11c and CD22 staining was very strong (RFI CD11c=211 and CD22 RFI=268) (p<0.05). Thus typical SLVL as defined here display a characteristic immunological profile. Interstingly spleen sections showed in all available cases (14 cases) a pattern distinct from classical SMZL and HCL with predominant red pulp infiltration by small cells with round hyperchromatic nuclei. Bone marrow infiltration was essentially interstitial and intrasinusoidal without fibrosis. Cytogenetic analysis showed a frequent absence of clonal aberrations (9/26 -34%-). Most cases were mutated (84%) with a surrepresentation of VH3 and VH4. Clinically true SLVL differed from classical SMZL by a longer progression free survival (p<0.05) without significant change in overall survival. Altogether theses results support the view that these cases need to be individualised as a variant of SMZL sharing some features with HCL, which would not be surprising considering the marginal origin of these 3 entities.

0293

PATIENTS WITH AIDS-RELATED LYMPHOMA HAVE HIGHER EPSTEIN-BARR VIRUS VIRAL LOAD THAN THOSE HIV-INFECTED WITHOUT LYMPHOMA

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Background. The incidence of lymphomas is increased among patients with AIDS. Epstein-Barr virus (EBV) has been associated with non-Hodgkin's lymphomas (NHL) as well as Hodgkin's lymphoma (HL), especially in those patients with HIV infection. Owing to EBV seems to play a role in the lymphomagenesis of AIDS-related lymphoma (ARL), the viral load of this virus might be elevated in HIV-infected patients when they develop lymphoma. *Aims.* To compare the viral load of EBV in HIV-infected patients with lymphoma with that of HIV-infected individuals without lymphoma. To study the correlation between EBV viral load and the presence of EBV in the tumor. To consider the usefulness of EBV viral load for lymphoma diagnosis and follow-up in the HIV-infected population. *Methods.* 113 HIV-infected patients were studied: 39 with lymphoma (32 NHL and 7 HL) and 74 without lymphoma. Samples from patients with lymphoma were collected at diagnosis and before any treatment. EBV viral load was measured in plasma by real-time PCR using Real Art EBV kit (QIAGEN Diagnostics, Hamburg, Germany) for LightCycler Instrument. HIV RNA load was measured using the NASBA method NucliSens Easy Q HIV-1 (bio-Mérieux, Boxtel, The Netherlands). The presence of EBV-EBER mRNA in the lymphoma was investigated by FISH. The main clinical and biological parameters were recorded, including CD4 lymphocyte count. *Results.* HIV-infected patients with lymphoma (n=39) had higher EBV viral load at lymphoma diagnosis than those HIV-positive without lymphoma (n=74) [mean (SD) 24,180 (73,387) copies/mL versus 3 (27.7) copies/mL]. The difference was statistically significant evaluating either NHL and LH together (p=0.047) or separately (p=0.013). Among patients with lymphoma, no correlations were found between EBV viral load and CD4 lymphocyte count, HIV RNA load or any clinical or biological parameter. The presence of EBV-EBER mRNA in the tumor was investigated in 17 cases: 11 were negative and 6 positive. EBV viral load were 37,935 (124,333) copies/mL and 50,652 (74,671) copies/mL respectively (p=0.022), and all 7 cases with negative EBV viral load were EBER negative. *Conclusions.* HIV-infected patients with lymphoma have higher EBV viral load at lymphoma diagnosis than those HIV-infected without lymphoma. Patients with EBER positive lymphomas have higher EBV viral load than those who are EBER negative. Serial measurement of EBV viral load might be useful for lymphoma detection in HIV-infected patients.

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0294

MYCOSIS FUNGOIDES. IMMUNOHYSTOCHEMISTRY ANALYSIS OF LYMPHOID AND MICROENVIREMENT CELLS BY MACROTISSUE ARRAY

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Background. Cutaneous lymphomas (CL) represent a unique group of lymphomas and are the second most frequent extranodal lymphomas. CL probably is the result of a multifactor and multistep process. Firstly an inflammatory reactive process is developing secondary to various chronic stimuli that may be genetic, environmental, infectious and immunologic. The consequences are the negative effects in cell regulation and deregulation of oncogenes and/or suppressor genes later promotes transition from pre-neoplastic conditions to neoplasia. Detailed molecular expression analysis of cutaneous T-cell lymphoma is not available. Some oncogenic alterations have been demonstrated, such as functional inactivation of the Fas receptor, constitutive activity of STAT 3, or the inactivation of the p16 gene via deletion or promoter hypermethylation. Objective: To study the expression of p16, c-myc, Ki67, bcl-2, CD1a, CD123, TCL1, CD68, STAT 3, STAT 4 and MAL1 in an homogeneous group of. CL diagnosed in one tertiary Hospital in order to know more about the characteristics of this neoplastic process. *Methods.* We have study 30 CL diagnosed consecutively as Mycosis Fungoides (14 early and 16 advanced stages) between January 2005-December 2006. By macrotissue array techniques and immunohistochemistry protocol with p16, c-myc, Ki67, bcl-2, CD1a, CD123, TCL1, CD68, STAT 3, STAT 4 y MAL1 was applied in all paraffin histological samples. *Results.* In 28 samples (92%) we have observed p16 positive over expression, the two negative samples corresponding to early affectation. In 14 samples from early stages (48%) c-myc was negative. In 29 samples (96%) CD1a was positive in dermal and epidermal layer. CD123 (interleukine 3 receptor) was negative in 16 samples (52%) and TCL1 was positive in 12 cases (39%) in small cells with oval and cleaved nucleus and scarce cytoplasm. Over expression of MAL1 was observed in advanced patients with aggressive CL. *Conclusions.* In our study an over expression of p16 is observed in the majority of cases and high c-myc expression in advanced stages. Probably dendritic plasmacytic cells are involved in the pathogenesis of skin lymphoproliferative disorders with cutaneous T cell infiltration. MAL1 could be a predictor of agressive CL but it is necessary more studies and more cases in order to confirm it.

0295

EXPRESSION OF NPM-ALK LEADS TO ONCOGENIC EFFECTS OF JUNB

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Background. The oncogene NPM-ALK arises due to a chromosomal rearrangement in human T-cells, resulting in activation of MAPK, PI3K and PLCγ signaling pathways. Enhanced activity of multiple signalling cascades and subsequently ALC(L)- lymphoma formation are the result. High expression of CD30 and JunB are a hallmark of NPM-ALK positive ALCL. In contrast to the prototypic AP-1 transcription factor c-Jun, JunB exerts an antioncogenic function in most cell types. Its functional role in the context of NPM-ALK remains uncertain. *Results.* Here we show that aberrant NPM-ALK expression leads to IL-3 independent outgrowth of Ba/F3 cells. Furthermore NPM-ALK expression induces JunB and CD30 expression, which is undetectable in the corresponding wild type cells and can be reversed by MEK-inhibition. Interruption of the NPM-ALK kinase domain impedes JunB and CD30 expression. Specific down-modulation of JUNB mRNA using small hairpin (sh) RNA avoids CD30 expression and arrests the cell cycle in G1 phase of NPM-ALK expressing cells. Conversely, ectopic JunB expression in NPM-ALK transgenic Ba/F3 cells leads to enhanced proliferation in the absence of IL3, whereas ectopic JunB expression in WT Ba/F3 is not sufficient to provoke IL3 independence and even leads to reduced proliferation in the presence of IL3. *Conclusions.* Thus, both, NPM-ALK and JunB are essential to induce CD30 expression and malignant transformation. The presence of NPM-ALK determines the oncogenic role of JunB.

0296

RITUXIMAB ENHANCES CELL-MEDIATED CYTOTOXICITY IN PATIENTS WITH B-CELL LYMPHOMA: *IN VIVO* STUDY OF NK/T-LYMPHOCYTES ACTIVITY

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Background. Rituximab dramatically improves the outcome of patients with B-cell lymphomas. The major mechanism of its action is antibody-dependent cellular cytotoxicity (ADCC) mediated by degranulation of cytotoxic lymphocytes (CTL) after binding to the Fc receptor. Although rituximab has been routinely used for 10 years, the published studies of natural killer (NK) and T-lymphocyte cytotoxic activity are limited to *in vitro* and *ex vivo* studies. The novel flow cytometry method is capable of visualizing the degranulation of T/NK cells via surface expression of lysosomal-associated membrane protein 1 (CD107a). *Aims.* To investigate changes in NK/T lymphocytes subsets and *in vivo* anti-lymphoma activity of normal NK/T lymphocytes in patients with B-cell lymphoma during the administration of chemoimmunotherapy. *Methods.* We investigated peripheral blood samples from 44 patients with newly diagnosed lymphomas (diffuse large B-cell lymphoma, DLBCL=21, follicular lymphoma, FL=16, mantle cell lymphoma=3, marginal zone lymphoma=2, B-cell lymphoma, unspecified=2). The patients were treated with chemoimmunotherapy (CHOP-like regimen=42, fludara-based regimen=2) repeated every 21 days. Rituximab was given in standard dose of 375 mg/m^2. Blood samples were obtained during the 4th therapy cycle - before and within 1 hour after the administration of rituximab. Patients with active infection or inflammation were excluded from the study. Flow cytometric analysis was performed on a FACSCalibur cytometer (BD Biosciences). Anti-human fluorescein-conjugated monoclonal antibodies: CD 3 FITC/ CD16+56+ PE, CD 4 FITC/CD 8 PE, CD 16 FITC/ CD 56 PE/ CD 3 EDC, (all Beckman Coulter) and CD 107a PE-Cy5 (BD Biosciences) were used for multi-color sample analysis. Antibody concentrations were adjusted according to the manufacturer's protocol. The results were expressed as the percentage of cells in a gated lymphocyte region. The collected data were analyzed using the Cell Quest Pro software by BD Biosciences. *Results.* We did not find any difference in the numbers of T/NK cell subsets when comparing patients with FL and DLBCL. The administration of rituximab is connected with a significant increase of all NK subsets ($CD16^+$ 12.8±9.1 vs 20.8±14.4, p=0.007; $CD56^+CD16^+$ 14.9±7.4 vs 23.1±13, p=0.03), the proportion of CD8+ lymphocytes did not change, $CD4^+$ cells decreased (14.5±2.2 vs 12.1±1.8, p=0.01). The proportion of degranulated CTL increased more than twice (CD107a+ 3.07±1.9 vs 6.37±3.8, p<0.001). No differences in absolute lymphocyte count before/after rituximab administration were observed. *Summary.* Degranulation of cytotoxic T/NK lymphocytes is the last step in innate antitumor immunity. The published data demonstrate close correlation between CD107a expression and tumor cell lysis *in vitro*. Our data show that rituximab administration *in vivo* has an direct impact on ADCC - mostly by increasing the proportion of degranulated cells (CD107a) and the numbers of $CD16^+$, $CD16^+CD56^+$ cells. The ADCC activity and numbers of NK/Tc cells vary individually. Therefore, the subgroup of low rituximab responders may be threatened by lower efficacy of induction or maintenance immunotherapy.

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Therapy of non-Hodgkin's lymphoma

0297

QUALITY OF LIFE IN PATIENTS WITH NON-HODGKINS-LYMPHOMA DURING MAINTENANCE THERAPY WITH THE ANTI-CD20 ANTIBODY RITUXIMAB

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The introduction of rituximab into the treatment of malignant lymphomas of the B-cell lineage has had a major impact on the management of these diseases. In diffuse large B-cell lymphomas (DLBCLs) and follicular lymphomas (FLs) several multicenter prospective randomized trials consistently demonstrated an improved outcome when rituximab was added to chemotherapy. In addition, prolonged exposure to rituximab as maintenance therapy has been benefical in patients with FL and mantle cell lymphoma (MCL). For patients, the effect of any prolonged antitumor therapy on the quality of life (QoL) is a very important question. However, so far the question whether rituximab maintenance therapy may impair QoL in patients with Non-Hodgkins-lymphoma remains unanswered. To investigate this subject , we have performed a prospective randomized trial of rituximab maintenance therapy (8 cycles rituximab 375 mg/m^2 every 3 months) versus observation in patients with CD20$^+$ B-cell Non-Hodgkins-Lymphoma in our institution. Between July 2002 and December 2005, 106 patients (pts) were included into the trial. QoL was assessed with the standardized questionnaires EORTC-QLQ-C30 and EuroQol-5D. After statistical analysis with the Wilcoxon signed-rank test, we found no significant differences of the QoL between the rituximab treatment group and the observation group. We conclude that rituximab maintenance therapy is safe and does not impair quality of life in this patient population.

0298

RITUXIMAB MAINTENENANCE THERAPY IN CD20+ B-CELL NON-HODGKIN-LYMPHOMA FIRST RESULTS OF A MULTICENTER PROSPECTIVE RANDOMISED PHASE II STUDY

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Clinical and pharmacokinetic data suggest that the effect of rituximab could be improved by prolonged exposure to the drug. To test for this hypothesis we performed a prospective randomized trial of rituximab maintenance therapy in patients with CD20$^+$ B-cell Non-Hodgkins-Lymphoma. After completion of standard treatment patients were randomized to either observation or maintenance therapy with rituximab (375 mg/m^2) every 3 months for 2 years. Patients after first line therapy as well as relapse patients were included in the study. Patients with aggressive lymphoma were enrolled if they had achieved a complete response (CR) after initial treatment. Patients with aggressive lymphoma with residual tumor mass were examined with positrone emission tomography (PET) and qualified for randomization if PET showed no signs of tumor activity. Patients with indolent lymphoma qualified for the study if at least a partial response (PR) was achieved. After recruitment of 172 patients a planed interim analysis was performed. Complete data sets of 162 patients (pts) with CD20$^+$ B-cell Non-Hodgkins-Lymphoma were evaluable for analysis. Histological subtypes included diffuse large cell lymphoma (69 pts), follicular lymphoma (41 pts), mantle cell lymphoma (18 pts), primary mediastinal lymphoma (15 pts), marginal zone lymphoma (9 pts), Burkitt's lymphoma (3 pts), immunocytoma (2 pts), primary intestinal lymphoma (1 pt), hairy cell leukemia (1 pt), chronic lymphocytic leukemia (1 pt) and unclassified B-cell lymphoma (2 pts). The interim analysis showed that event free survival was significantly prolonged in the rituximab maintenance group compared to the observation group (p<0.05). However, no difference in overall survival between the two groups was observed so far. Two patients in the treatment group developed WHO grade III adverse events (1 leucopenia, 1 infection). Both pts recovered shortly after appropriate treatment. We conclude that rituximab maintenance therapy is feasable, safe and well tolerated in patients with CD20+ B-cell Non-Hodgkins-Lymphoma and may prolong event free survival in this patient population.

0299

PROGRESS IN TREATMENT RESULTS OF CHILDHOOD B-CELL LYMPHOMAS IN THE 15 YEARS EXPERIENCE OF POLISH PEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP

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Objectives. The efficacy of the LMB-89 protocol for children with B-NHL has been investigated. The patients (pts) were treated in 10 oncohematological centers of PPLLSG between 1992 and 2007. We have registered a total number of 149 children with B-NHL: 105 (70%) of them with Burkitt and 44 (30%) with non - Burkitt histopathological types (17 - Burkitt-like, 6 - Large B-cell, 4 -immunoblastic, 6 - centroblastic, 11 - others). Primary sites were: abdomen (50%), peripheral lymph nodes (17%), Waldeyer ring (13%), head - neck (7%),bones (4%), thorax (3%), other sites (6%). *Methods.* The diagnosis was based on histomorphological investigation and supplemented with immunophenotyping. The S. Murphy staging system was used for prognostic stratification. Treatment intensity was adapted to 3 risk groups (A,B,C), according to LMB-89 protocol. *Results.* In 1992 62% of B-NHL pts presented on admission I-III and 38% IV stages. In 2007 80% of them presented I-III (more than in 1992) and 20% IV (less than in 1992) stages. In 1992 72% pts were classified to A+B and 28% to C risk groups, whereas in 2007 87% to A+B and only 13% to C risk groups. Pts number, achieved complete remission (CR), increased from 87% (1992) to 91% (2007). Number of non-responders, early deaths, relapses and deaths in RC decreased from 11% (1992) to 9% (2007), from 13% to 5%, from 21% to 8% and from 13% to 5%, respectively. Probability of EFS for all B-NHL was 0,69 (1992) and 0,86 (2007). EFS of III and IV stages were: 0,80 and 0,56 (1992); 0,87 and 0,71 (2007). EFS for Burkitt and non-Burkitt types increased from 0.82 and 0,62 (1992) to 0.83 and 0,87 (2007), respectively. EFS of B and C risk groups were: 0,79 and 0,50 (1992); 0,89 and 0,54 (2007). *Conclusions.* The treatment outcome of children with B-NHL, estimated in 2007, has improved in comparison to previously reported observations in 1992. Higher EFS and overall survival of B-NHL in 2007 compared to this in 1992, could be achieved thanks to quick diagnosis after first tumor clinical symptoms and an improvement of intensive supportive care (adequate blood product substitutions, regular infection specific prophylaxis, amelioration of MTX therapy monitoring) for therapy toxicity elimination. The worst results are still observed in children with bone marrow involvement and in those with large tumor burden at diagnosis.

0300

RADIOIMMUNOTHERAPY IN RELAPSED OR REFRACTORY B-CELL NON-HODGKIN'S LYMPHOMA WITH I-131-LABELED CHIMERIC ANTI CD20 C2B8 (I-131 RITUXIMAB)

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Background and Aims. Recently, the native chimeric human-mouse anti CD20 antibody IDEC-C2B8 (Rituximab) has been widely applied in NHL. This phase II study was performed to evaluate of efficacy and safety of radioimmunotherapy (RIT) with I-131 rituximab in relapsed or refractory B-cell non-Hodgkin's lymphoma (NHL). *Methods.* Inclusion criteria were as follows: B-cell NHL with relapsed or refractory to primary standard therapy, measurable disease, adequate hematologic, renal, and hepatic function, informed consent. The rituximab (Mabthera, Roche) was radiolabeled with iodine-131 (I-131) using a modified chloaramine T method with high radiochemical purity (95%) and preservation of immunoreactivity. All patients received loading doses of unlabeled rituximab (median, 70 mg; range, 20-70 mg) immediately prior to administration of therapeutic dose (median 7.3 GBq; range, 3.70-8.51 GBq), and then underwent gamma camera scan. *Results.* 20 patients were enrolled (9 low-grade B-cell NHL, 11 DLBCL, median age 63 years). Patients had received a median of three prior chemotherapy regimens

(range, 1-6) and 11 treatment cycles (range, 6-22). All patients were evaluated for response and toxicities. The total objective response rate was 35.0% (1 CR, 6 PRs), and 55.6% in low-grade lymphoma, 18.2% in DLBCL. There was a trend of higher response in low-grade lymphoma than DLBCL, but this difference was statistically insignificant (two sided Fisher's exact test, p=0.16). Adverse events were primarily hematologic toxicities; the incidence of grade 4 neutropenia and thrombocytopenia was each 25.0%. The treatment-related mortality was observed in one patient, who had been previously treated with high-dose chemotherapy plus TBI with autologous stem cell transplantation. *Summary and conclusions.* RIT with I-131 rituximab seems to be effective and tolerable in refractory low-grade B-cell NHL, although modest activity in refractory DLBCL. Further studies to define the efficacy of I-131 rituximab in DLBCL are warranted, such as consolidation RIT followed by standard chemotherapy or high-dose RIT with autologous stem cell transplantation.

0301

RITUXIMAB AND CHLORAMBUCIL AS FIRST LINE TREATMENT OF LOW-GRADE OCULAR ADNEXAL LYMPHOMAS

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Background. Ocular adnexal lymphomas (OALs) represents 5-15% of primary extranodal lymphomas, and are constituted largely by extranodal marginal zone lymphomas (EMZL). Radiotherapy is associated to high rates of local disease control, but also to the risk of relapse and immediate and delayed complications, such as xerophtalmy, corneal ulcerations, cataract and retinal damage. Surgery is a feasible option, but not always satisfactory in terms of disease control. Single-agent chemotherapy with alkylating agents is used for the treatment of low-grade lymphomas, including OALs. Rituximab, a chimeric anti-CD20 monoclonal antibody, is effective in EMZL and in OALs. *Aims.* We investigated the efficacy and the safety of a combination of chlorambucil and rituximab as first line treatment in patients with OALs. *Methods.* Patients with histologically proven low-grade OALs were enrolled in this study. Staging included CT-scan of the orbit, neck, chest and abdomen, MR of the orbit, and bone marrow biopsy, and was done according to Ann Arbor classification. Treatment consisted of chlorambucil (0,1 mg/Kg/die for 45 days, then on days 1 to 15 monthly for 4 months) and rituximab (375 mg/sqm weekly for 4 doses, then monthly for 4 infusions). Toxicities were reported according to WHO criteria. At the end of treatment patients were restaged clinically and with a MR of the orbit. *Results.* Since November 2003 to December 2005 nine consecutive histologically proven low-grade OALs (eight EMZL, a grade I follicular lymphoma) have been treated according to this protocol. The median interval between onset of the first symptoms and diagnosis was 13 months (range, 4 months - 3 years). Six patients were female (66%). Median age at diagnosis was 78 years (range, 56-86 years). Disease was localized in the conjunctiva in four patients, in the lacrimal glands in 3 patients and in other orbital sites in the last two patients. Eight patients had a stage I disease, one stage IV, and no patient presented B-symptoms. LDH was within normal range in all patients, ECOG-PS was 0 in all patients. All patients completed the treatment without delay; there was no grade III-IV toxicities nor hospitalizations. Five patients had grade 1-2 rituximab infusion-related reactions usually during the first infusion. We did not observe infectious complications. Haematological toxicity was mild. At the end of treatment local symptoms were not present: eight patients (89%) were in CR, and the remaining patient (11%) obtained a PR. After a median follow-up of 22 months (range, 10-38 months) all patients are alive and we did not observe disease progressions; the median EFS was 19 months (range, 8-36 months). All patients underwent periodic ophthalmologic follow-up visits: we did not report ocular toxicities, and all patients conserved a normal visual function, including acuity. *Conclusions.* The combination of rituximab and chlorambucil proved to be a safe, feasible and effective therapy for primary OALs. However, a longer follow-up would be necessary to determine the long-term efficacy of this treatment.

0302

PHASE I STUDY OF SMILE CHEMOTHERAPY FOR ADVANCED-STAGE OR RELAPSED/REFRACTORY EXTRANODAL NK/T-CELL LYMPHOMA/LEUKEMIA

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Background. Extranodal NK/T-cell lymphoma, nasal type, and aggressive NK-cell leukemia are rare, and their standard therapy has not been established. They are Epstein-Barr virus (EBV)-associated lymphoid malignancies, and lymphoma/leukemia cells express P-glycoprotein leading to multi-drug resistance (MDR) of the disease. Almost all patients with stage IV, relapsed, and refractory diseases die within 1 year. *Aims.* To establish a more effective induction therapy for these neoplasms with untreated stage IV or relapsed/refractory state, we conducted a dose escalating feasibility study of a newly designed chemotherapeutic regimen, SMILE [Steroid=dexamethasone (DMS) 40 mg/body d2-4 IV, Methotrexate (MTX) 2 g/m^2 d1 6hr IV, Ifosfamide (IFM) 1.5 g/m^2 d2-4 IV, L-asparaginase (L-asp) 6000U/m^2 d8,10,12,14,16,18,20 IV, and Etoposide (ETP) 100 mg/m^2 d2-4 IV; every 28 days]. SMILE comprised MDR-unrelated agents and ETP that shows both *in vitro* and *in vivo* efficacy for EBV-associated lymphoproliferative disorders. *Methods.* Primary endpoint is MTD (maximum tolerated dose), and secondary endpoints are the overall response rate (ORR) and the complete response (CR) rate. Pts with newly diagnosed stage IV diseases (including aggressive NK-cell leukemia), first relapsed/recurrent diseases after CR/PR, refractory (either NC or PD) diseases with first-line chemotherapy, 15-69 years of age, and PS 0-2 were eligible. A standard 3+3 design was used to evaluate dose-limiting toxicities (DLTs). The doses of DMS, IFM, and L-asp were fixed. Four dose levels of MTX/ETP were planned to be evaluated. *Results.* At Level 1, 6 pts with extranodal NK/T-cell lymphoma, nasal type were enrolled, and showed the following features; age 28-69 yrs (median 48), M:F=5:1, newly-diagnosed stage IV: 3, 1st relapse: 2, primary refractory: 1, BM involvement: 2, elevated serum LDH: 4, and PS0: 2 , PS1: 3, PS2: 1. Among the first 3 pts, 1 pt died from sepsis with grade 4 neutropenia due to disease and delayed initiation of G-CSF administration. 1 pt developed grade 3 infection and hypofibrinogenemia. The remaining 1 pt did not develop DLT other than transient grade 3 hyponatremia, and obtained a CR. We made a protocol amendment to initiate G-CSF administration from day 6, and proposed to register additional 3 pts in Level 1. It was approved by the Data and Safety Monitoring Committee. Additional 3 pts developed DLTs (grade 3 APTT 1, grade 3 gamma-GTP 1, grade 3 hyponatremia 2, and grade 3 hyperglycemia 1) that were all manageable, and pts recovered from the toxicities rapidly. The incidence of grade 4 neutropenia was comparable, but grade 3/4 leukopenia/ anemia/ thrombocytopenia were less frequent than the first 3 pts. Of all 6 enrolled pts, 3 achieved CR, 1 PR, 1 NR, and 1 NE.%ORR was 67%, and%CR was 50%. *Summary and Conclusions.* Our results suggest that dose level 1 of SMILE is feasible and promising for advanced, refractory or relapsed NK/T-cell lymphoma. To determine the efficacy and feasibility of SMILE in a large number of cases, we will start the subsequent phase II study of SMILE in spring 2007.

0303

RITUXIMAB MAINTENANCE THERAPY FOR PATIENTS WITH FOLLICULAR LYMPHOMA. A COST-EFFECTIVE STRATEGY?

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Background. Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown to significantly improve overall survival (OS) (p=0.011) and progression free survival (PFS) (p<0.001) when compared to observation alone (OA) in patients with relapsed or refractory follicular lymphoma (FL) who attain a response with either cyclophosphamide, doxorubicin, vincristine,

and prednisone (CHOP) alone, or Rituximab + CHOP (EORTC 20981 study). *Aims.* To determine, from a Spanish perspective, if the maintenance treatment with Rituximab is cost-effective alternative compared to the current clinical practice of OA for the described population. *Methods.* Incremental cost-effectiveness of Rituximab maintenance therapy (375 mg/m^2 every 3 months until progression or for 2 years) versus OA was assessed through a deterministic, three health states (progression-free, progression and death) transition model. Base case assumptions for the model included: clinical evidence based on EORTC 20981 trial (extrapolation of PFS and OS data using a Weibull distribution), Rituximab maintenance benefit to last 5 years, 10 years time horizon, 3,5% discount rate on costs and benefits, and Spanish National Health Service perspective (direct costs only). Resource use (study drug, adverse events, treatments at relapse, surveillance/monitoring) was estimated from a Spanish expert panel and EORTC 20981 trial. Unit costs were obtained from local databases (€ May 2,006). Health states utility values were derived from an ad hoc study. Sensitivity analyses were performed for all mentioned variables. *Results.* For the base case (Table 1), more quality-adjusted life years (QALY), life-years (LY) and progression-free survival years per patient on maintenance therapy were obtained versus OA (incremental values of 0.85, 0.94 and 1.46, respectively). Total cost per patient was higher with Rituximab than with OA (+8,026 €). Incremental cost per QALY gained was 9,358 €, with a cost per LY gained of 8,493 € and a cost per PFS year gained of 5,485 €. Sensitivity analyses demonstrate results remains cost-effective for all variable tested. *Conclusions.* Rituximab maintenance therapy, when compared to observation alone, improves overall survival and progression free survival, and is a cost effective strategy in patients with relapsed or refractory follicular lymphoma who attain a response with further therapy.

Table 1. Results for base case (€ May 2, 2006)

Input	Rituximab	Observation	Difference
Cost per patient(€)	22,458.20	14,432.40	8,026.06
Quality adjusted life years (QALY)	4,1133	3.2557	0.8576
Cost per QALY(€)	**9,358.49**	-	-
Lyfe Years (LY)	5.6891	4.7441	0.9450
Cost per LY gained (€)	8,493.18	-	-
Progression free survival (PFS) years	3.1952	1.7320	1.4632
Cost per PFS year gained (€)	5,485.39	-	-

0304

ACTIVITY OF SINGLE-AGENT FLUDARABINE IN GASTRIC MALT LYMPHOMA: IMPACT OF T(11;18)(Q21;Q21) IN MOLECULAR RESPONSE RATE

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Background. Fludarabine (F) is an active agent for the treatment of indolent lymphoma. However, its clinical activity in the treatment of gastric MALT lymphoma has not been studied in depth. Moreover, there is scarce information regarding molecular response (MR) in gastric MALT lymphoma treated by purine analogs and the prognostic value of t(11;18) in this setting. *Aims.* The current study was undertaken to determine the activity of single-agent fludarabine in gastric MALT lymphoma and to analyze if these patients achieve molecular remission after this treatment. *Methods.* Treatment consisted of fludarabine (25 mg/m^2 IV) given on days 1-5, every 4 weeks, for 6 cycles; after the first cycle, oral fludarabine was allowed to be given orally at 40 mg/m^2 with the same schedule. Molecular response (MR) was assessed by RT-PCR analysis of t(11;18) or by PCR assays for analysis of IgH gene rearrangements analyzing FR1, FR2 and FR3 in endoscopic biopsies. *Results.* Eight consecutive patients were entered on this trial. All but one patient were chemonaïve. Median follow-up was 26 months (range 12-46 mo); median age was 60 years (range: 45-77). Three pts were in stage I, 2 stage II-1 and 3 stage IV according to Lugano system. Four out of 5 (80%) pts achieved a CR after three cycles and all eight cases achieved a CR after six cycles, for an overall response rate of 100%. No patient has shown clinical or endoscopic relapse at last follow-up. Hematological toxicity occurred in 75% of pts, mainly mild neutropenia and generally after the third cycle. Three cases received G-CSF (after the 2nd, 3rd and 6th cycle) and three patients required dose modification or delay (3-7 days) in the delivery of the following cycle. No cases of autoimmune hemolytic anemia developed. No blood transfusions were required. Only one patient had to be admitted because of non-neutropenic fever that resolved with broad-spectrum antibiotics. Four out of 8 pts (50%) achieved MR during the study-period. Four out of 5 (80%) pts without t(11;18) achieved MR (Figure 1). However, none case carrying t(11;18) achieved MR. Sequencing of PCR products will be presented. *Conclusions.* Monotherapy with intravenous or oral fludarabine is safe in gastric MALT lymphoma and achieves a high response rate (100%). Only pts without t(11;18) achieved MR and all those pts carrying t(11;18) had evidence of clonal residual disease by PCR.

Figure 1.

0305

RITUXIMABS IMPACT ON LARGE B CELL LYMPHOMA TREATMENT (ANALYSIS OF 116 PATIENTS OF A LYMPHOMA UNIT)

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Background. A possible selection bias occurring in multicentric trials could cause their outcomes not to be appliable to the real, nonselected population of patients or to different environments. *Aims.* To validate the results of chemotherapy added Rituximab in a population of non selected large B cell lymphoma patients. *Methods.* We perform a comparative analysis of response, time to first treatment failure and survival among all large B cell lymphoma patients who received CHOP or alike chemotherapy in our Lymphoma Unit since January 1997 until March 2002 (Historical Cohort:HC) and the same outcomes in patients treated with Rituximab added to similar schedules of chemotherapy since April 2002 until January 2007 (Rituximab Cohort: RC). Suspension due to intolerance, progression, relapse or death was considered *primary treatment failure*. *Complete remission* was defined by disappearance of disease or long term observation and/or PET validated inactive residual mass. Different outcomes were defined as *non response*. Demographic and prognostic variables, primary chemotherapy response, time to primary treatment failure and overall survival were analysed. Statistical *Methods.* Student's T, chi*2, Kaplan-Meier's survival tables and log-rank test. *Results.* 116 patients were included: 57 in the first period and 59 in the second one. No significant differences in the distribution of sex (male/female ratio: 1,19 in HC and 0,69 in RC) or age (58 years mean,15-84 years range in HC; 63 years mean, 17-88 years range in RC) were observed between the two cohorts. The clinical stage (1-2 versus 3-4: 23/34 in HC and 27/32 in RC) and IPI score were also similar (0-2/3-5:26/31 in HC and 27/32 in RC). A non significant higher number of patients with AIDS (5 versus 2) and mediastinal lymphoma (9 versus 1) were detected in HC. A lower, non significant number of grade three follicular lymphoma (2 versus 7), was found in HC. The extranodal lymphoma's rate was balanced (ten patients each). High dose chemotherapy and stem-cell autotransplantation was administered as consolidaton therapy in ten patients of HC and one of RC. A significantly higher proportion of patients achieved complete remission in RC (93 versus 79%; p<0,05). The probability of survival without treatment failure was significantly higher in RC (75 versus 55% at three years; p<0,01). The median overall survival wasn't achieved by neither of the groups, but the probability of survival was higher in RC (89% versus 59% at three years; p<0,005). *Conclusions.* Our results confirm the superiority of standard chemotherapy added Rituximab versus classical chemotherapy schedules, regarding proportion of response, time to treatment failure and overall survival. Further observation is required to determine whether this greater or equal to 20% difference in time to treatment failure and overall survival is sustained.

0306

RISK-ADAPTED IMMUNOCHEMOTHERAPY OVERCOMES THE NEGATIVE PROGNOSTIC IMPACT FCGRIIIA OF THE RECEPTOR GENOTYPE IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Background. Antibody (rituximab) dependent cellular cytotoxicity (ADCC) is a key mechanism in killing CD20+ lymphoma cells. The Fc portion of the rituximab molecule is bound by Fc receptors on the surface of cytotoxic lymphocytes (NK cells). FCGR3A-158 V/F gene polymorphism leads to expression of 3 variants of the FcγIIIa receptor (FcγRIIIa) with different receptor affinity. The published studies with FL patients treated with rituximab monotherapy demonstrated better outcomes of patients with the V/V or F/V genotypes over the F/F genotype. *Aims.* To assess whether the FcγIIIa receptor genotype influences the treatment response quality (molecular remission) in newly diagnosed patients with FL treated with risk-adapted immunochemotherapy. *Methods.* We studied 34 patients with newly diagnosed FL (grades I-IIIa) who had detectable bcl-2/IgH (using standard or long-distance PCR) rearrangement in bone marrow and/or peripheral blood. The median age was 55 years (31-84), 31 out of 34 patients had advanced (III/IV) clinical stages. The FLIPI scores were as follows: low 4/34, intermediate 13/34 and high 17/34. The first-line treatment was stratified according to the generally used risk factors (FLIPI, GELF, β-2-m level, bulk disease). Patients under 60 (65) years of age with high-risk disease (FLIPI equal or more than 3, or additional risk factors) were indicated to autologous stem cell transplantation (ASCT). Whereas 16 patients underwent ASCT, 18 patients were treated conventionally (R-CHOP- or R-fludarabine-based regimens). The treatment response was classified according to the standard Cheson criteria (1999). Patients who achieved CR/CRu plus PCR bcl-2/IgH negativity were classified as CRm (molecular CR). Genotyping of the FCGR3A-158 V/F gene was performed using the PCR followed by allele-specific restriction enzyme digestion. *Results.* Complete or unconfirmed complete response with Bcl-2/IgH negativity (molecular remission) was achieved in 83.5% of patients, 1 patient achieved CR, 4 patients had partial remission and one patient stable disease. The frequencies of FcγRIIIa 158 V/V, V/F and F/F were 8.2%, 47.3% and 44.7%, respectively. Homozygous FcγRIIIa 158 F/F carriers had higher FLIPI index (chi square p=0.05), compared to V/F+V/V carriers. The treatment modalities (conventional versus ASCT) had the same distribution in V/V+V/F vs F/F patients (chi square p=0.38). The distribution of CRm rate was not significantly different in the subgroups of V/V+V/F vs F/F patients (chi-square, p=0.92). *Summary.* We found no difference in the quality of treatment response (molecular remission) after first-line immunochemotherapy among the FcγRIIIa subgroups. Risk-adapted intensive concomitant immunochemotherapy overcomes the negative prognostic impact of the FcγRIIIa genotype. The following question is whether patients with the F/F genotype will have the same benefit from rituximab maintenance treatment.

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0307

90Y-IBRITUMOMAB TREATMENT FOR RELAPSED AND/OR REFRACTORY B CELL TYPE NON-HODGKIN`S LYMPHOMA. MULTIINSTITUTIONAL ARGENTINIAN STUDY

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Abstract. The radionucleid conjugate with monoclonal antibodies (antiCD20/90Y-ibritumomab tiuxetan) have been approved for the treatment of relapsed, refractory and transformed (high grade) follicular lymphomas. Between September 2005 and February 2007, 27 patients with refractory/relapsed lymphoma were enrolled. Median age was 58 yrs old (45-76). Fourteen were women and 13 were men. Twenty-two were follicular and 5 were mantle cell lymphoma. Ten patients had bulky disease, 5 had bone marrow involvement and 16 had stage III-IV disease. Time from diagnosis was 0-3 years in 5 pts, 3-6 in 8 pts and over 6 years in 13 pts.. Eight pts had received 1-2 previous treatments, and 18 pts had received 3-5 previous treatments including 5 pts with autologous bone marrow transplantation. All had previously received anti-CD20 monoclonal antibody therapy. Two pts. received previous radiotherapy. 90Y-Ibritumomab (Zevamab TM Schering Argentina) was administered at 0,3 or 0,4 mCi, based on initial platelet count. Seven days before, and the same day of the inmunoconjugate administration, pts received rituximab 250 mg/m^2. Fifteen pts responded, (11 CR, 4 PR) and 10/11 CRs continued in remission between 3 and 15 months of follow-up. Eleven pts required filgrastim administration for neutropenia, 8 pts required platelet transfusions, 6 pts had neutropenia plus fever, 4 pts required red blood cells transfusion, and only 5 pts had to be admitted for complicated pancytopenia. One patient died 40 days after treatment with hypoplastic bone marrow complicated with septicemia. Five pts with previous bone marrow transplantation, required filgrastim, transfusions and 2/3 had febrile neutropenia. Our experience shows 41% CR. Even heavily treated pts, that had previous bone marrow transplantation were able to receive the radioimmunoconjugate, although they required extra support. Our experience favours the use of 90Y-Ibritumomab tiuxetan in relapsed and refractory lymphomas even if they had received previous autologous bone marrow transplantation.

0308

RITUXIMAB COMBINED WITH DEXABEAM SALVAGE THERAPY FOLLOWED BY HIGH DOSE THERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY B-NHL: FIRST RESULTS OF A PHASE II MULTICENTRE STUDY

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Background/Aims. HDT is an established treatment for relapsed aggressive B-NHL (aNHL) and its use in indolent lymphoma (iNHL) is supported by several trials. In a phase I study we could demonstrate the safety/feasibility of the combination of Rituximab with Dexa-BEAM followed by HDT with R-BEAM or R-TBI/CY for treatment of relapsed lymphoma. Here we present the results of a prospective multicentre phase II study testing the efficacy of this treatment. *Methods.* After informed consent, patients aged 18-65, ECOG 0-2 with relapsed/refractory B-NHL after at least 3 cycles of anthracycline-containing chemotherapy were eligible. Rituximab (R) pre-treatment, but no prior HDT was allowed. Treatment consisted of 2 cycles of R-Dexa-BEAM (dexamethasone 8 mg *3, day 1-10; BCNU 60 mg/m^2, day 2; etoposide 75 mg/m^2, day 4-7; cytarabinoside 200 2 mg/m^2, day 4-7; melphalan 20 mg/m^2, day 3; Rituximab 375 mg/m^2, day 1). Stem cell mobilization was performed after the second cycle. HDT-regimens were BEAM (BCNU: 300 mg/m^2, day -7, etoposide 100 mg/m^2 day -6 - -3, cytarabinoside 400 mg/m^2 in two doses, day -6 - -3; melphalan 140 mg/m^2 day -2) or TBI/CY (total body irradiation 12Gy, day -6 - -4; cyclophosphamide 60 mg/kgbw, day -3 - -2;) in combination with 2 doses of Rituximab 375 mg/m^2, day -7/-2. *Results.* After ethics approval the study started 2001 and finished 2005. Overall, 103/107 patients are evaluable for analysis. For 67 patients with aNHL (DLCL 55, MCL 7, FL °3: 5) median age was 54 years (21-65) and median number of pre-treatments was 1(1-2). A low/low intermediate IPI was present in 74% and ECOG was 0 or 1 in 90% of aNHL patients. The corresponding figures for 36 iNHL patients (FL °1-2: 29, MZL 6, IC 1) are: median age 55 (22-64), pre-treatments 1 (1-3), low/low intermediate IPI and ECOG < 2 in 86 and 94%. Premature discontinuation: 28 patients did not proceed to HDT: treatment related mortality (sepsis, cerebral hemorrhage) (3, aNHL), progression (12, aNHL), mobilization failure (10, 8 aNHL, 2 iNHL), refusal (2, iNHL) or secondary cancer (1, iNHL). Therefore, 66% of patients with aNHL and 86% with iNHL underwent HDT. HDT (R-BEAM in 84% of aggressive and 75% of indolent NHL) could be performed with manageable toxicity, 1 patient died due to MOF. Recovery occurred timely (ANC > 500 median 11 days (8-27). Restaging at day 60 revealed an ORR of 80% (59% CR, 21% PR) in patients with aNHL and 90% (81, 10%) with iNHL. With a median follow up of 2.2 years post HDT, PFS and OS for patients with aNHL are 63 and 83%. Corresponding figures for iNHL are: 63 and 100%. There were no statistical differences between patients with or without R-pretherapy. *Conclusions.* The inclusion of Rituximab in salvage therapy and HDT resulted in high response rates and sustained remissions. R-containing pre-therapy was not associated with inferior outcome, underlining the continuous importance of HDT for relapsed/refractory lymphoma, which seems to be further improved with the inclusion of R. With no comparative studies available R-DexaBEAM followed by HDT can be considered as an established relapse therapy for NHL.

Novel therapeutics and targeted therapies I

0309

FUNCTIONAL CHARACTERISTICS AND GENE EXPRESSION PROFILES OF PRIMARY ACUTE MYELOID LEUKAEMIA CELLS IDENTIFY PATIENT SUBGROUPS THAT DIFFER IN THE SUSCEPTIBILITY TO HISTONE DEACETYLASE INHIBITORS

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Modulation of gene expression through histone deacetylase (HDAC) inhibition is now considered a possible therapeutic strategy in several malignancies, including acute myeloid leukaemia (AML). In the present study we investigated the *in vitro* effects of various HDAC inhibitors on primary human AML cells. The relation between the biological response to HDAC inhibitors and basal gene expression profiles were assessed. AML cells were derived from 59 consecutive patients. Effects of structurally different HDAC inhibitors on cell proliferation, apoptosis induction, colony formation and CD34 cell subsets were studied. Basal gene expression of untreated cells was examined by microarray techniques. The HDAC inhibitors valproic acid, PXD101, trichostatin A and sodium butyrate were investigated. All drugs inhibited AML cell proliferation in a dose-dependent manner when tested at high concentrations. However, at lower concentrations, proliferation increased for a subset of patients. HDAC inhibitors also decreased proliferation of clonogenic and $CD34^+$ leukaemic cells and increased apoptosis. Again the inhibition was observed at high concentrations whereas enhanced proliferation and decreased apoptosis were observed for a subset of patients at lower concentrations. Based on basal expression of 100 genes it was possible to separate patients (a group of 17 FLT3-ITD+ patients with normal cytogenetics) with growth enhancement at intermediate HDAC inhibitor concentrations and those without this response into two mutually exclusive groups. Several of the genes have previously been used for classification of AML patients. E.g. high expression of Dynein light chain 1 (DLC1) and low expression of transmembrane 4 superfamily member 2 (TM4SF2) has been associated with bad prognosis; DLC1 showed high expression in patients with drug-induced growth enhancement, whereas high TM4SF2 expression was observed for our group without growth enhancement. Low expression of members of the apolipoprotein system has been associated with adverse prognosis, and our patients with growth enhancement also showed low levels of Apolipoprotein C1 (APOC1). The two groups could not be separated on the basis of expression of HDACs and histone acetyl transferases. In conclusion, functional characterisation and gene expression analyses identify AML patient subsets that differ in their response to HDAC inhibitors *in vitro*. These observations may explain why HDAC inhibitor therapy affects only a subset of AML patients.

0310

TARGETED THERAPY OF ADULT T CELL LEUKEMIA: FROM THE BENCH TO BEDSIDE

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Background. Adult T-cell leukemia/lymphoma (ATL) is an aggressive malignancy of mature activated T-cell caused by human T cell lymphotropic virus type I (HTLV-I). ATL carries a very bad prognosis because of intrinsic chemoresistance and severe immunosuppression. The viral transactivator protein Tax is responsible for several aspects of the malignant transformation such as proliferation, resistance to apoptosis, genetic instability, tumor dissemination, immnune escape and chemotherapy resistance. Through all of these activities, Tax acts as a powerful oncogene, and represents the most attractive viral protein for targeted therapy. Among Tax properties, activation of the NF-κB pathway plays a crucial role in the proliferation and transformation of the infected cells. Indeed, most of the activated cellular genes in HTLV-I infected cells are through this mechanism. *Results.* We have, using an *in vitro* model of ATL derived cells, identified several potential targeted therapies for ATL: 1) Arsenic trioxide (As) synergizes with interferon-α (IFN) to induce G1 arrest and apoptosis in ATL through shut-off of the NF-κB pathway and Tax degradation by the proteasome. This combination yielded promising clinical results in relapsed/refractory ATL patients. 2) Clinically achievable concentrations of bortezomib, a selective proteasome inhibitor, targeted multiple cellular pathways that resulted in dramatic inhibition of cell proliferation and apoptosis of ATL-derived cells. 3) ATL cells secrete high concentrations of functional angiogenic factors, establish functional gap junctions with endothelial cells, and extravasate through the endothelial barrier using mechanisms involved in angiogenesis. Angiogenesis inhibitors, such as anti-VEGF monoclonal antibodies (bevasizumab) or specific kinase inhibitors of the VEGF receptors (PTK-787) inhibit ATL-induced angiogenesis and impede ATL cell invasion. We are investigating the *in vivo* therapeutic potential of these targeted therapies identified in our *in vitro* studies, using as a preclinical model SCID mice injected with lymphomatous spleen cells from transgenic mice for the Tax gene of HTLV-I. Results of these ongoing studies, with respect to tumor regression, apoptosis induction and survival will be presented.

0311

HE TYROSINE KINASE INHIBITOR DASATINIB SUPPRESSES IGE-DEPENDENT ACTIVATION AND HISTAMINE RELEASE IN BASOPHILS

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Background. Dasatinib (BMS354825) is a small molecule-type inhibitory drug that blocks several tyrosine kinases including src kinases, PDGFR, KIT, and BCR/ABL. Recently, dasatinib has been introduced as a new effective antileukemic agent in patients with imatinib-resistant chronic myeloid leukemia (CML) and acute lymphocytic leukemia. Moreover, dasatinib may act on normal lymphocytes and other physiologic cells through diverse target structures. In particular, dasatinib has recently been examined for its immunosuppressive and anti-inflammatory effects. Methods and *Results.* We here report that dasatinib at 1 μM completely blocks the anti-IgE-induced release of histamine in human blood basophils in healthy subjects. The effects of dasatinib on histamine secretion in basophils were dose-dependent (IC_{50}: 50-100 nM) and were found to be specific for IgE-dependent activation of basophils in that no inhibition was seen in C5a-activated or Ca-ionophore-exposed basophils. Moreover, dasatinib was found to block recombinant allergen-induced release of histamine from human basophils in sensitized individuals. In addition, as assessed by flow cytometry, dasatinib (1 μM) was found to inhibit the IgE-dependent upregulation of several activation-linked antigens, including aminopeptidase N (CD13), LAMP-3 (CD63), endolyn (CD164), and the basophil-related ecto-enzyme E-NPP3 (CD203c) on human basophils. The effects of dasatinib on upregulation of basophil differentiation antigens were found to be dose-dependent and were seen in normal subjects as well as in allergic individuals. *Conclusions.* Dasatinib blocks IgE-mediated activation and histamine release in human blood basophils. These anti-allergic effects of dasatinib may have clinical implications and may point to additional indications for this multifunctional drug.

0312

MLN3897, A NOVEL CCR1 INHIBITOR, IMPAIRS OSTEOCLAST FORMATION AND FUNCTION ASSOCIATED WITH DOWNREGULATION OF C-FOS

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Background. Osteolytic lesions due to enhanced osteoclast (OC) activity are a hallmark of myeloma bone disease and other metastatic cancers. Chemokines play a critical role in enhancing OC formation and activity. *Aims.* Since CCR1 is one of the main chemokine receptors expressed on monocytes and OC, we here characterized the effects of MLN3897 (Millennium Pharmaceuticals), a novel specific antagonist of CCR1, on OC formation and function. *Methods.* In order to analyze the effects of MLN3897 on osteoclastogenesis, we generated OC from peripheral blood mononuclear cells of healthy donors stimulated with RANKL and M-CSF (50 ng/mL) for 3 weeks, in the absence or presence of MLN3897. Mature OC were multinucleated TRAP+ cells, and their

functional activity was confirmed by pit formation and collagen release ELISA assays. *Results.* CCR1 expression was induced early during OC formation, at one week 60% of the monocyte population expressed CCR1 by flow cytometric analysis. Macrophage inflammatory protein-1 α secretion was also stimulated, from 57 pg/mL at 24 hours to 156 pg/mL at one week. Treatment with MLN 3897 (10 nM) resulted in inhibition of both OC formation (by 40%) and function (by 70%) in a dose-dependent way ($p<0.05$). Our data demonstrate that MLN3897 induced a 60% reduction in the multinucleated cell number at one week (control 61±14 vs treated 35±9), without affecting cell viability. MLN3897 also inhibited the OC-specific protease cathepsin k activity and protein expression, suggesting an independent effect of CCR1 inhibition on OC formation and activity. We further characterized the mechanism of action of MLN3897, analyzing the expression levels of c-fos and ERK, important mediators of OC differentiation. MLN3897 (10 nM) resulted in reduced c-fos transcription levels and concomitant abrogation of ERK activation. *Conclusions.* Taken together, these data suggest that CCR1 inhibition by MLN3897 blocks ERK pathway activation and c-fos expression and results in impaired monocyte multinucleation process as well as cathepsin K expression. These studies delineate a novel mechanism of action of MLN3897 on osteoclastogenesis, and provide the therapeutic rationale for its clinical evaluation for treatment of osteolytic bone disease.

0313

LS104 IS A NOVEL SUBSTRATE INHIBITOR OF MUTANT JAK2 KINASE AND ACTS SYNERGISTICALLY WITH ATP-BINDING-SITE KINASE INHIBITORS IN JAK2 V617F POSITIVE CELLS

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Background. The JAK2 V617F mutation (V617F) is a novel molecular marker in Ph-negative myeloproliferative disease (MPD). The incidence of the V617F mutation in essential thrombocythemia and in idiopathic myelofibrosis is about 50%, whereas it is more than 90% in polycythemia vera (PV). *In vitro*, the V617F mutation confers cytokine independent growth of Ba/F3 cells expressing erythropoietin receptor (EpoR) and constitutive activation of the JAK2 kinase and of the JAK-STAT pathway. In a murine bone-marrow transplant model the V617F mutation alone is sufficient to induce a PV-like phenotype. Therefore, development of JAK2 kinase inhibitors is considered as an important step towards targeted therapy of MPDs. *Aims.* In this report, we characterize the small molecule kinase inhibitor LS104 as a novel non-ATP-competitive JAK2 kinase inhibitor. *Methods.* Ba/F3 cells transfected with EpoR and the V617F mutant (Ba/F3-EpoR-VF) were treated with LS104. Proliferation and apoptosis assays as well as immunoblotting of JAK2 and of downstream signalling pathways were performed. Results obtained were compared with those observed in cells transfected with EpoR and expressing endogenous wildtype JAK2. Target validation was performed by transfection of JAK2 siRNA. In addition, we performed a JAK2 kinase assay to evaluate the effect of LS104 on JAK2 kinase activity. ATP-doses in the kinase assay were escalated and the effects on kinase activity using various kinase inhibitors were measured. Apoptosis assays were performed employing a combination of LS104 with JAK-inhibitor I, which acts via the ATP-binding site. For this purpose we chose dose levels, where each inhibitor alone induced only low to moderate levels of apoptosis. Finally, the ability of LS104 to inhibit growth of endogenous erythroid colonies (EECs) obtained from patients with V617F positive MPD was investigated. *Results.* LS104 exhibited dose-dependent and selective growth inhibition and induction of apoptosis in Ba/F3-EpoR-VF cells in comparison to control cells. By immunoblotting we found inhibition of JAK2 autophosphorylation and of downstream targets as STAT5, AKT and ERK upon treatment with LS104. Activation of these targets by JAK2 was confirmed in experiments using JAK2 siRNA. In an *in vitro* kinase assay, LS104 inhibited JAK2 kinase activity in a dose dependent manner with an IC_{50} of <5 μM, and this effect was not reversible using escalated ATP-doses. Combination treatment of Ba/F3-EpoR-VF cells using LS104 plus JAK-inhibitor I lead to significantly increased apoptosis as compared to each substance alone. Furthermore, LS104 significantly inhibited *in vitro* formation of EECs: We observed 89% inhibition at 10 μM LS104 whereas growth of CFU-GM obtained from normal controls was virtually unaffected. *Conclusions.* Taken together, our data show that LS104 specifically inhibits JAK2 kinase activity and JAK2 downstream signals in V617F positive cells. LS104 also inhibits growth and induces apoptosis in Ba/F3-EpoR-VF cells. Our data demonstrate that LS104 is a substrate kinase inhibitor and may be combined with ATP-competitive JAK2 inhibitors to enhance treatment efficacy. Growth of EECs from patients with MPD is shown to be significantly suppressed by LS104. Based on these data, a phase I/II clinical trial of LS104 for patients with JAK2 V617F positive MPDs is currently in preparation.

0314

LENALIDOMIDE STRONGLY ENHANCES NATURAL KILLER CELL AND MONOCYTE MEDIATED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY OF RITUXIMAB TREATED CD20+VE CANCER CELLS *IN VITRO*

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Lenalidomide is an anti-angiogenic, anti-proliferative and immunomodulatory drug that is approved for the treatment of transfusion-dependent patients with anemia due to low- or intermediate-1-risk MDS associated with a del 5q cytogenetic abnormality with or without additional cytogenetic abnormalities, and in combination with dexamethasone is for the treatment of multiple myeloma patients who have received at least one prior therapy. Encouraging early results suggest a potential for clinical efficacy in B cell non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Lenalidomide has been shown to enhance Th1-type cytokines and T cell and NK cell activation markers in patients with advanced cancers. Furthermore, lenalidomide has been shown to enhance rituximab-mediated protection in a SCID mouse lymphoma model *in vivo*. We have utilized an *in vitro* ADCC system to assess the ability of lenalidomide to directly enhance human NK cell and monocyte function in response to rituximab (chimeric anti-CD20 mAb). Pre-treatment of NK cells with lenalidomide greatly enhanced IFN-g production by NK cells in a dose-dependent manner. In a functional ADCC assay, NHL cell lines (Namalwa, Farage, Raji and Jeko-1) were pre-coated with rituximab and exposed to either NK cells or monocytes pre-treated with lenalidomide. After 4 (NK cells) or 15 (monocytes) hours in culture the viability of the tumor cells was assessed. Lenalidomide consistently and synergistically increased the killing of tumor cells in a dose-dependent manner and up to 6-fold compared to rituximab alone. Rituximab alone had only a small effect and there was no killing of cells in the absence of rituximab. The presence of either exogenous IL-2 or IL-12 was required to see enhanced killing by lenalidomide-treated NK cells but was not required for killing by lenalidomide-treated monocytes. Enhanced ADCC is most likely due to increased signaling through NK and monocyte FCg receptors. Preliminary results suggest an inhibitory effect of lenalidomide on NK cell SHIP-1 activity, although other pathways are also being investigated. In conclusion, we have shown that lenalidomide strongly enhances the ability of rituximab to induce ADCC mediated killing of CD20+ve NHL cell lines and primary CLL cells *in vitro*. This provides a measure of the potential synergy of combining lenalidomide with current and investigational monoclonal antibodies in these and other diseases, and supports further study.

0315

DASATINIB IS AN EFFECTIVE TREATMENT OF CENTRAL NERVOUS SYSTEM PH-POSITIVE LEUKEMIA

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Central nervous system (CNS) involvement is a relatively common complication in Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) and chronic myeloid leukemia (CML) in blast crisis (BC). The current treatment options (intrathecal or systemic chemotherapy, radiotherapy) for CNS leukemia are limited. The BCR-ABL kinase

inhibitor imatinib, which is widely used in the treatment of Ph-positive leukemia, has a poor penetrance into cerebrospinal fluid (CSF) with inadequate concentrations for kinase inhibition. Animal model data indicate that dasatinib, a potent SRC/BCR-ABL inhibitor, may cross the blood-brain barrier (BBB) in clinically significant amounts. We assessed the clinical efficacy of dasatinib therapy in the treatment of CNS leukemia in patients with Ph+ALL and BC CML. A total of 11 patients were included in the study, most treated within clinical studies (Bristol-Myers Squibb, n=9) in Europe and the US. Nine patients received dasatinib as a front-line therapy for CNS disease, and 2 patients had a CNS relapse while on dasatinib. Some patients received intrathecal chemotherapy in combination with dasatinib. All patients showed significant clinical activity of dasatinib (Table 1). One of the two patients who had a CNS relapse while on dasatinib therapy had the T315I BCR-ABL mutation in blasts isolated from CSF. This mutation confers absolute resistance to dasatinib. The finding of a mutated clone in CSF is indicative of selection pressure exerted by dasatinib and thus access of the drug into CSF. Pharmacokinetic data are being collected. In conclusion, dasatinib penetrates the BBB and induces significant clinical responses even as monotherapy in patients with Ph-positive leukemia.

Table 1.

0316

EFFICACY OF APO010, A FAS-ACTIVATING MOLECULE, IN MULTIPLE MYELOMA

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Background and Aims. The Fas receptor is expressed on the surface of many malignant cells. Fas activation triggers strong apoptotic signals, and therefore represents an interesting anticancer target. APO010 is a recombinant form of Fas Ligand with a hexameric structure that is currently being evaluated as an anticancer agent in Phase 1 clinical trials. In order to identify possible target indications, we tested the *in vitro* efficacy of APO010 on multiple myeloma (MM) cells. *Material and methods.* Eight MM cell lines were used: MM1S, MM1R, U266, RPMI-LR5, RPMI-Dox40, MM144, RPMI-8226 and OPM-1. PBMCs from 3 healthy donors were isolated by Ficoll-Hypaque. Cytotoxicity induced by APO010 was analyzed by MTT and Annexin V staining. Cell cycle changes were studied by propidium iodide uptake by flow cytometry and changes in protein expression by Western-blotting. ELISA for BrdU was used to evaluate the proliferation induced by the presence of microenvironment. *Results.* The sensitivity of eight MM cell lines to cell killing by APO010 was determined in a cytotoxicity assay (MTT). Six cell lines were highly sensitive to APO010 with IC-50 at 24h of 0.5-20 ng/mL (2.5-100 pM), whereas two were resistant (RPMI-8226 and OPM-1). We found a clear correlation between the APO010 sensitivity and the expression of Fas by flow cytometry. Activation of apoptosis was rapid (within two hours of incubation) with maximum effect at 10 hours, as determined by Annexin V staining. In comparison, APO010 cytotoxicity against PBMCs (both resting and activated) from 3 healthy donors was not significant at doses effective against MM cell lines. APO010 was also able to overcome the proliferative advantage conferred to multiple myeloma cells by the presence of IL-6, IGF-1 and the coculture with BMSCs. The mechanism of action of APO010 was studied in the sensitive cell line MM1S. Apoptosis was, as expected, the main mechanism of cell death as assessed by Annexin-V staining by flow cytometry. Cell killing was independent of variations on the cell cycle profile. Treatment with APO-010 induced PARP, caspase-3, caspase-7, caspase-8 and caspase-9 cleavage, suggesting activation of both the intrinsic and extrinsic apoptotic pathways. The presence of the pan-caspase or caspase-8 inhibitors (Z-VAD-FMK and Z-IETD-FMK respectively) and not the caspase-9 inhibitor (Z-LEHD-FMK) were able to completely abrogate the APO010-induced cell death. APO010 also provoked cleavage of MCL-1 and BIM and a decrease of BID. An important downregulation of pAKT was also induced by APO010. Regarding activation of the MAPK pathway, although an initial upregulation of pERK was induced with the treatment (after 2h), this upregulation was transient and decreased even below baseline levels after 6h of exposure to APO010. Finally, the addition of Doxorrubicin and Bortezomib, and in a less extent of Melphalan and Lenalidomide, to APO010 potentiated the efficacy of the drugs alone. *Conclusions.* These data show that APO010 triggers efficient killing of multiple myeloma cells by apoptosis, and provide an initial rationale for the use of this compound for treatment of multiple myeloma patients. Future work will aim at determining the activity of APO010 in animal studies.

0317

SIGNIFICANT PRECLINICAL ANTILEUKEMIC ACTIVITY OF THE TYROSINE KINASE INHIBITOR BVT I IN ACUTE MYELOID LEUKEMIA

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Background. BVT I is a novel kinase inhibitor which has shown activity against several receptor tyrosine kinases in enzymatic assays. *Aims.* The major aim of this study was to further explore the antileukemic activity of BVT I in hematological malignancies *in vitro* as well as *in vivo*. *Methods.* The semiautomated fluorometric microculture cytotoxicity assay (FMCA) was used for *in vitro* evaluation of BVT I activity in a cell line panel as well as in primary tumor cells from 42 patients with hematological malignancies (acute myeloid leukemia (AML; n=16), acute lymphocytic leukemia (n = 12) and chronic lymphocytic leukemia (n=14)). For comparison, normal peripheral blood mononuclear cells (PBMC; n=7) were used. Assessing the *in vivo* activity, cells from 2 patients with AML and the flt-3-positive AML cell line MV-4-11 were cultured in semipermeable hollow fibers. The fibers were implanted subcutaneously into NMRI male mice, which were treated twice daily with either 20 mg/kg or 40 mg/kg of BVT I subcutaneously, or vehicle only. After 6 days the fibers were retrieved and cell density evaluated using the MTT-assay. *Results.* The most sensitive cell line in terms of IC_{50} was the flt-3-positive AML cell line MV-4-11. Furthermore, results from the cell line panel showed that BVT I was not influenced by any resistance mechanism studied. BVT I showed activity *in vitro* against all hematological malignancies tested, as well as against PBMC. AML tended to be the most sensitive cell type with IC_{50} below 10 μM. In the subsequent *in vivo* study, significant antileukemic effect was observed in primary AML cells as well as in MV-4-11 (Figure 1).

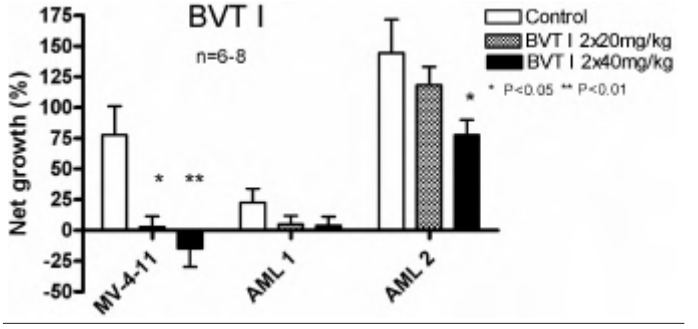

Figure 1. ***In vivo*** **activity of BVT I against AML cells (cell line MV-4-11 and primari AML cells from two individual patients) in the mouse hollow fiber assay. Results are presented as net growth, defined as the percent change in cell density in the fibers during the 6 days of** ***in vivo*** **experiment.**

No major toxicity, neither hematological nor non-hematological, was observed in the animals. *Conclusions.* The tyrosine kinase inhibitor BVT I has significant *in vitro* and *in vivo* effect in AML. Lack of toxicity in the hollow fiber study, suggests that BVT I is well tolerated in mice. Further preclinical studies in AML are ongoing.

0318

FACTORS DETERMINING CLINICAL ACTIVITY OF THE HISTONE DEACETYLASE INHIBITOR SODIUM VALPROATE IN PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKAEMIA

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Background. Histone deacetylases inhibitors (HDIs) represent an important new class of drugs in the treatment of acute myeloid leukaemia (AML). However the mechanism by which they induce tumour specific apoptosis *in vivo* and the factors regulating tumour cell sensitivity are not known. Aims; In order to define factors determining clinical sensitivity to the HDI valproic acid (VPA) we have correlated the impact of VPA exposure on gene expression *in vitro* and *in vivo* with clinical responses in patients with high risk AML. Methods; A panel of 14 haemato-lymphoid cell lines were tested for their sensitivity (IC_{50}) to apoptotic cell killing by VPA. Gene expression array analyses using HGMP chip 6500 gene arrays were performed on the same cell lines prior to HDI exposure. A bioinformatics approach combined the array and IC_{50} data to generate a score for each gene identifying those whose elevated expression correlated with resistance, or sensitivity to VPA *in vitro*. In a concurrent Phase I/II clinical trial 24 patients with high risk AML (relapsed n=11, newly diagnosed n=6, primary refractory n=3) with a median age of 64 yrs (41 -83 yrs) received combination treatment with VPA, all trans-retinoic acid (ATRA) and theophylline. *in vivo* histone acetylation and expression of HDI responsive genes was measured in leukaemic blasts before and after commencement of VPA therapy. Expression of genes associated with VPA sensitivity *in vitro* were re-analysed with respect to their expression in pre-treatment blasts from non-responding and responding trial patients. *Results.* By combining LC50 values to VPA and microarray data generated from pre-treatment RNA in 14 haematolymphoid cell lines we were able to identify candidate genes mediating sensitivity to VPA killing *in vitro*. These studies identified potential VPA-signalling networks containing sensitivity genes including IL-1β and genes associated with VPA resistance including PLOD2, cyclin B1 (CCNB1) and ACVR2A. In the clinical trial 5 patients, all with relapsed AML, achieved clinical responses (complete remission n=1, partial remission n=4). Treatment with VPA and ATRA for 28 days resulted in increased histone acetylation in peripheral blood mononuclear cells and increased expression of the pro-apoptotic genes p15, p16, p21 and TRAIL in patient myeloblasts. Combined upregulation of p21 and TRAIL was only observed in myeloblasts from the patient whom achieved a CR. We then specifically compared the pattern of gene expression, as defined by microarray studies, in responding and non-responding patients with the previously identified *in vitro* VPA signalling networks. The pattern of expression of VPA sensitivity and resistance genes in pre-treatment myeloblasts from 4 patients identified similar networks to those defined *in vitro* and appeared to be correlated with clinical response. *Conclusions.* This study demonstrates induction of pro-apoptotic gene expression by VPA in a clinical context and identifies potential mechanisms through which its anti-leukaemic effect may be mediated *in vivo*. Furthermore we have identified potential VPA-signalling networks containing novel sensitivity and resistance genes whose expression correlates with VPA-mediated tumour cell killing *in vitro* and may predict clinical activity.

0319

GOLD COMPOUND AURANOFIN INDUCES APOPTOSIS IN MYELOMA CELLS VIA TARGETING IL-6 SIGNALING PATHWAY

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[Background] Multiple myeloma is an incurable B-cell malignancy, requiring new therapeutic strategies. Recent understanding of the biology in multiple myeloma has led to the development of various novel agents, which target the myeloma cells and its microenvironment. These new agents have shown remarkable activity against refractory myeloma in early clinical trials, but prolonged drug exposure may result in the development of de novo drug resistance. In addition, unexpected pulmonary complications by bortezomib, a promising agent for multiple myeloma, have been reported. Therefore, identification and validation of novel agents with less toxicity to overcome drug resistance and to improve clinical outcome of multiple myeloma are necessary. *Aims.* Auranofin (AF) is a coordinated gold compound, which has been widely used for the treatment of rheumatoid arthritis. Recent studies have shown that AF induces apoptosis in leukemia cells. Therefore, assuming that AF has a potency to induce apoptosis in myeloma cells as well, it may become a candidate of a novel targeted therapeutic agent. *Methods.* To address our hypothesis, the effects of AF on inducing apoptosis of various myeloma cells were examined. Further, the molecular mechanism of AF-induced apoptosis in myeloma cells was investigated. *Results.* AF inhibited the growth of U266 myeloma cells in a time (0-24 h)-dependent manner with IC_{50} of 50 nM. AF significantly induced cell cycle arrest at G1 phase and subsequent apoptosis of U266 cells. AF upregulated the expression of cdk inhibitor p21 and caused dephosphorylation of Rb protein. Furthermore, AF-induced apoptosis in myeloma cells involved the activation of caspase-8 and the cleavage of Bid, a mediator that is known to connect the death receptor to the mitochondrial apoptosis pathway. We also found that AF induced the cleavage of Mcl-1 protein, but had no effect on the expression of Bax or Bcl-2 proteins. To clarify the importance of Mcl-1 in AF-induced apoptosis, the Mcl-1 expression vector was introduced into U266 cells. Induction of apoptosis by AF was almost abrogated in Mcl-1-overexpressed U266 cells. AF also inhibited IL-6-induced activation of JAK2 and phosphorylation of STAT3 in U266 cells, suggesting that AF could inhibit the IL-6-mediated signaling pathway in myeloma cells. Previous reports have demonstrated that STAT3 binds to the Mcl-1 gene promoter and activates its transcription, resulting in the upregulation of Mcl-1 expression. Therefore, we then examined the effect of AF on the DNA binding activity of STAT3. Electrophoretic mobility shift assays using U266 nuclear extracts demonstrated that IL-6-induced STAT3 binding activity was inhibited by the presence of AF. *Conclusion.* We report here for the first time that AF induced apoptosis in human multiple myeloma cells. Down-regulation of Mcl-1 with modulation of IL-6-mediated signaling pathway plays an important role in AF-induced apoptosis in myeloma cells. Low pharmacological concentration (50 nM) of AF is widely employed for the management of rheumatoid arthritis without any side effects, and may be used for treating myeloma without the risk of severe toxicity. We conclude that AF is one of the promising candidates for the new therapeutic agent as a signal transduction therapy of myeloma.

0320

DASATINIB INHIBITS T CELL ACTIVATION AND PROLIFERATION IN A DOSE-DEPENDENT MANNER

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Background. Dasatinib (Sprycel®, BMS-354825, Bristol-Myers Squibb) is a novel inhibitor of BCR-ABL, SRC kinases, c-kit, PDGF receptor and other kinases. It has been demonstrated to be safe and more effective in CML patients than Imatinib (Glivec®, STI571, Novartis) and has been approved in 2006. An increased infection rate, which has been described in patients undergoing dasatinib treatment, might be due to inhibitory effects on LCK and FYN, since dasatinib targets tyrosine kinases (TK) that play an important role in T cell development and function. A recent case report also demonstrated dasatinib's efficacy in the treatment of a patient with thymoma. *Aims.* We have now evaluated dasatinib's effects on human purified T cells from healthy blood donors and compared them with the effects of the promiscuous TK inhibitor staurosporine, which has been the base for several SRC kinase inhibitors in clinical development. Methods and *Results.* All assays described herein were performed at clinically relevant doses between 1nM and 100nM. CFSE labeled T cells were stimulated with the murine monoclonal CD3 antibody OKT-3 for four days. A dose-dependent inhibition of T cell proliferation was detected (EC50=7 nM) and almost complete inhibition (96% inhibition) occurred at a concentration of 20 nM. T cells incubated with dasatinib for 24h, and then removed from dasatinib, proliferated as well as untreated T cells (p=0.118). This argues for a reversible blockade of T cell proliferation, in contrast to staurosporine that led to a dose-dependent but irreversible inhibition of proliferation at a dose of 10 nM (on average 90% inhibition). We further investigated the functional effects of dasatinib on T cells. A statistically significant inhibition of OKT-3 induced up-regulation of the T cell activation marker CD69 was

observed at a concentration as low as 10 nM, and almost complete inhibition (99%) occurred after 24h at a concentration of 50nM (EC_{50}=11 nM). In line with these results, we also observed reduced IL-2 production in dasatinib treated purified T cells (EC_{50}=1.3nM, measured by ELISA), while 1nM staurosporine led to a 70% inhibition of IL-2 release, and 10nM almost to a complete inhibition (99.9%). $CD4^+$ T cells were more sensitive than $CD8^+$ T cells to the inhibitory effects of staurosporine on activation and proliferation (89% proliferation inhibition for $CD4^+$ cells vs. 81% for $CD8^+$ cells at 10 nM). The same held true for dasatinib (activation: EC_{50} 9 nM for $CD4^+$ cells and 15 nM for $CD8^+$ cells, proliferation: EC_{50} 5 nM for $CD4^+$ cells and 14 nM for $CD8^+$ cells). Since $CD8^+$ T cell-mediated immunity is essential for long-term control of persistent DNA viruses, we evaluated dasatinib's impact on antigen-specific T-cell responses to CMV and EBV. Proliferation inhibition of CFSE-labeled CMV- and EBV-specific $CD8^+$ T cells was observed, which might explain the increased frequency of viral infections in dasatinib treated patients besides the described induction of myelosuppression. *Conclusions.* Close monitoring of patients under TK inhibitor treatment seems warranted with respect to reactivation of persistent viral infections and newly acquired opportunistic infections. However, these findings also indicate dasatinib's potential as an immunosuppressant in the fields of transplantation and rheumatology.

0321

BOSUTINIB (SKI-606) EXHIBITS CLINICAL ACTIVITY IN PATIENTS WITH PHILADELPHIA CHROMOSOME POSITIVE CML OR ALL WHO FAILED IMATINIB

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Background. Bosutinib (SKI-606) is an orally available, dual Src/Abl kinase inhibitor. *Aims.* To assess safety and preliminary clinical activity of bosutinib, we conducted a phase 1/2 study in patients (pts) with Philadelphia chromosome positive (Ph^+) chronic myelogenous leukemia (CML) or acute lymphocytic leukemia (ALL) who were imatinib resistant/intolerant. *Methods.* In part 1, 18 pts with imatinib-relapsed/refractory chronic phase (CP) CML received bosutinib 400 mg/day (3 pts), 500 mg/day (3 pts), or 600 mg/day (12 pts). Part 2 was an expanded cohort of 51 pts with all phases of Ph^+ CML and ALL dosed at 500 mg daily. Timed blood samples were collected on days 1-3, 15 for PK analysis. *Results.* Of 69 pts, median age was 59 yrs; 48 were CP; 90% imatinib resistant. Drug-related grade 1/2 adverse events (AEs) occurring in ≥10% of CP pts: diarrhea (69%), nausea (44%), vomiting (19%), abdominal pain (13%), rash (13%). Grade 3/4 AEs occurring in ≥5% of CP pts: rash (6%), thrombocytopenia (6%). 17 pts required dose reductions. In evaluable imatinib-resistant CP-CML pts with no prior exposure to other Abl inhibitors, 16/19 (84%) had complete hematologic response (CHR); 4/21 had partial and 7/21 had complete cytogenetic responses for major cytogenetic response (MCyR) rate of 52%. Of 58 pts evaluable for mutations, 13 different imatinib-resistant mutations were found in 32 pts. 12/14 CP pts with non-P-loop mutations and 3/3 with P-loop mutations achieved CHR. 5/11 CP pts with non-P-loop mutations and 1/1 with P-loop mutation achieved MCyR. 4/9 evaluable advanced leukemia pts had CHR, 2 had MCyR. After oral administration, steady state exposure of bosutinib was nearly 2-fold higher than single-dose exposure. Mean elimination half-life was approximately 22-27 hours, supporting a once-daily dosing regimen. *Conclusions.* Bosutinib was well tolerated in pts with CML, with primarily low-grade gastrointestinal and dermatologic AEs. Bosutinib showed clinical activity in imatinib-resistant pts with cytogenetic responses and CHR across a range of mutations. Durability of response continues to be assessed.

0322

SULINDAC SULFIDE REVERTS THE ACTIVATION OF THE WNT-SIGNALING AND ABERRANT SELF RENEWAL INDUCED BY THE AML-ASSOCIATED FUSION PROTEINS PML/RAR AND PLZF/RAR

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Background. More than 60% of acute myeloid leukemias (AML) harbor specific chromosomal aberrations mainly translocations. Translocations such as t(15;17), t(11;17), or t(8;21) lead to the formation of chimeric genes encoding for fusion proteins such as PML/RAR, PLZF/RAR and AML-1/ETO (leukemia associated fusion proteins - LAFP). LAFP are able to induce and to maintain the leukemic phenotype by blocking terminal differentiation of early hematopoietic progenitors and by increasing the self renewal potential of the leukemic stem cells (LSC). The key mechanims by which LAFP increase LSC self renewal is the activation of the Wnt-signaling pathway. PML/RAR, PLZF/RAR or AML-1/ETO activate Wnt-signaling by up-regulating γ-catenin and β-catenin at a transcriptional level. Activation of the Wnt signaling augments self renewal of normal HSC and LSC. We and others have recently shown that the aberrant activation of Wnt-signaling by the LAFP decisively contributes to the pathogenesis of AML. *Aim.* To disclose whether a *leukemic stem cell therapy* is effective we targeted the Wnt-signaling by Sulindac Sulfid (SuSu) in PML/RAR- or PLZF/RAR- (X-RAR) positive stem cell models. SuSu represents the active metabolite of Sulindac, a nonsteroidal anti-inflammatory drug (NSAID), known to inactivate the Wnt-signaling. *Methods.* SuSu was used at a concentration of 50-100 μM which is achievable in patients at a dosage of 0.2-0.4g. As leukemia models we used U937 cells stably expressing PML/RAR or PLZF/RAR and the PML/RAR-positive cell line NB4. As stem cell models we used i.) the $CD34^+/CD38^-$ fraction of the KG-1 cells stably expressing PML/RAR or PLZF/RAR under serum free culture conditions; ii.) Sca-1+/lin- murine HSC retrovirally transduced with PML/RAR or PLZF/RAR and plated in methyl cellulose containing mIL-3, mSCF and mIL-6. The amount of total γ-catenin and β-catenin and activated β-catenin was determined by immunoblotting. *Results*. Here we report that SuSu i.) down-regulated not only β-catenin but also γ-catenin in X-RAR expressing U937 and KG-1 cells; ii.) reduced the active form of β-catenin in the presence of X-RAR; iii.) induced a high apoptosis rate in PML/RAR-positive NB4 cells; iv.) reduced the $CD34^+/CD38^-$ *stem cell fraction* of KG-1 cells expressing X-RAR but not of mock transfected controls; iv.) reduced the self renewal potential of X-RAR-positive HSC as revealed by a significantly reduced replating efficiency. *Conclusions*. Here we provide first evidence that it is possible to the exposure to therapeutically achievable dosages of a NSAID revert the aberrant activation of the Wnt-signaling by LAFP. The significant reduction of the aberrant self renewal potential of HSC in the presence of X-RAR further support that the inhibition of the aberrantly activated Wnt signaling in AML might be a valid molecular therapy approach which has to further validated in *in vivo* leukemia models and in a clinical setting.AML-1/ETO activate Wnt-signaling by up-regulating γ-catenin and β-catenin at a transcriptional level. Activation of the Wnt signaling augments self renewal of normal HSC and LSC. We and others have recently shown that the aberrant activation of Wnt-signaling by the LAFP decisively contributes to the pathogenesis of AML. *Aim*. To disclose whether a *leukemic stem cell therapy* is effective we targeted the Wnt-signaling by Sulindac Sulfid (SuSu) in PML/RAR- or PLZF/RAR- (X-RAR) positive stem cell models. SuSu represents the active metabolite of Sulindac, a nonsteroidal anti-inflammatory drug (NSAID), known to inactivate the Wnt-signaling. *Methods*. SuSu was used at a concentration of 50-100 μM which is achievable in patients at a dosage of 0.2-0.4g. As leukemia models we used U937 cells stably expressing PML/RAR or PLZF/RAR and the PML/RAR-positive cell line NB4. As stem cell models we used i.) the $CD34^+/CD38^-$ fraction of the KG-1 cells stably expressing PML/RAR or PLZF/RAR under serum free culture conditions; ii.) Sca-1+/lin- murine HSC retrovirally transduced with PML/RAR or PLZF/RAR and plated in methyl cellulose containing mIL-3, mSCF and mIL-6. The amount of total γ-catenin and β-catenin and activated β-catenin was determined by immunoblotting. *Results*. Here we report that SuSu i.) down-regulated not only β-catenin but also γ-catenin in X-RAR expressing U937 and KG-1 cells; ii.) reduced the active form of β-catenin in the presence of X-RAR; iii.) induced a high apoptosis rate in PML/RAR-positive NB4 cells; iv.) reduced the $CD34^+/CD38^-$ *stem cell fraction* of KG-1 cells expressing X-RAR but not of mock transfected controls; iv.) reduced the self

renewal potential of X-RAR-positive HSC as revealed by a significantly reduced replating efficiency. *Conclusions*. Here we provide first evidence that it is possible to the exposure to therapeutically achievable dosages of a NSAID revert the aberrant activation of the Wnt-signaling by LAFP. The significant reduction of the aberrant self renewal potential of HSC in the presence of X-RAR further support that the inhibition of the aberrantly activated Wnt signaling in AML might be a valid molecular therapy approach which has to further validated in *in vivo* leukemia models and in a clinical setting.

0323

MTORC1 INHIBITION ACTIVATES PI3K/AKT BY UP-REGULATING THE IGF-R SIGNALLING IN ACUTE MYELOID LEUKAEMIA: RATIONAL FOR INHIBITION OF BOTH PATHWAYS

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Background. The PI3K/Akt and mTORC1 pathways are frequently activated in acute myeloid leukaemia (AML) cells and represent potential therapeutic targets. *Aims.* In this report, we studied the interactions between PI3K and mTORC1 pathways in primary blast cells from the bone marrow of patients with AML at diagnosis after 4 hours of cytokine and serum starvation. *Methods.* Bone marrow samples were obtained from 22 newly diagnosed AML patients before induction of chemotherapy. Blast cells were starved 4 hours in cytokine and serum free media. Cells were then incubated with or without inhibitors: IC87114 (p110delta specific inhibitor), RAD001 (rapamycin derivative inhibitor of mTORC1), LY294002 (multiple kinases inhibitor including mTORC1 and PI3K), ?IR3 (inhibitory anti-IGF-1 receptor monoclonal antibody) and Western blot analysis and cell proliferation assays were performed. To purify the blast cell population, cells were sorted according to CD45 expression and side scatter. Immunofluorescence on cytocentrifuge preparations and quantitative RT-PCR for IGF-1 expression on CD45low blast cells were performed. *Results.* We observed that specific inhibition of mTORC1 activity with RAD001 upregulated PI3K activity, as evidenced by an increased phosphorylation of AKT (Ser 473) on Western blot analysis. The mean increase of AKT phosphorylation in the presence of RAD001 was 186% (130-500%). The increase was maintained after 24 hours of blast cell incubation with RAD001. This mTORC1-mediated Akt up-regulation was explained by an IGF-1/IGF-1 receptor autocrine loop: a/ blast cells expressed a functional IGF-1 receptor and IGF-1-induced Akt activation was increased by RAD001, b/ a neutralizing anti-IGF-1R α-IR3 monoclonal antibody reversed the RAD001-induced Akt phosphorylation, c/ autocrine production of IGF-1 was detected in highly purified CD45low blast cells from 8 patients by quantitative RT-PCR and immunofluorescence. d/ Activation of the IGF-1 receptor paralleled an up-regulation of the IRS2 adaptor protein. Finally, we observed, in AML blast cells, a dissociation of the mTORC1 and PI3K pathways: IC87114, a specific p110delta PI3K inhibitor, had no inhibitory effect on mTORC1 signalling. This was confirmed by the fact that RAD001 and IC87114 induced additive anti-proliferative effects on AML blast cells. *Conclusions.* Our results suggest that dual inhibition of mTORC1 complex and IGF1/IGF-1R/PI3K/Akt axis may enhance the efficacy of mTOR inhibitors in AML.

0324

DISCOVERY AND PRECLINICAL DEVELOPMENT OF SELECTIVE JAK INHIBITORS FOR THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES

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Background. Increased kinase activity is a common occurrence in malignant cells and is associated with the activation of multiple downstream pathways implicated in the pathobiology of various diseases. The identification of activating genetic mutations in multiple members of the Janus kinase (JAK) family of non-receptor tyrosine kinases has recently been described in numerous malignancies, including the majority of Philadelphia chromosome negative (Ph^-) myeloproliferative disorders (MPDs). Mouse experiments have suggested a causal role for these mutated JAKs implicating them in the pathogenesis of the human conditions. This suggests that JAK inhibition may be a promising therapeutic strategy for the treatment of diseases associated with elevated JAK kinase activity. *Aims.* To identify and characterize potent, selective, orally bioavailable inhibitors of the JAK kinases. *Methods.* A compound library was screened for inhibition of JAK kinase activity. Structure-activity relationship based lead optimization was conducted to identify novel, potent and selective JAK inhibitors. Further characterization of *in vitro* and *in vivo* pharmacokinetic, pharmacological and toxicological properties was performed to identify molecules suitable for further advancement into animal models of cancer and MPDs. *Results.* Incyte has identified multiple novel, potent, and selective inhibitors of the JAK kinases, including the recently described JAK2 V617F mutant common to the majority of the Ph- MPDs. These compounds are active against JAKs at nanomolar concentrations while demonstrating excellent selectivity against a broad panel of unrelated kinases. In cell-based assays, these compounds retain their nanomolar potency and selectivity and reduce JAK-mediated tumor cell growth and survival. In contrast, these compounds do not impact the growth or survival of cells dependent on alternative signaling pathways (e.g. Bcr-Abl), even at micromolar concentrations. *in vivo*, in a mouse model of MPD-associated mutant JAK2 driven organomegaly (BaF/3-JAK2V617F cells), Incyte JAK inhibitors markedly reduce the splenomegaly common to both the model and the human disease (Figure 1). A detailed characterization of the activity of these molecules will be presented. *Summary.* Increased JAK signaling has been associated with various malignancies, including the majority of Ph-MPDs. Incyte has identified multiple potent and selective inhibitors of the JAK kinases. These compounds are orally bioavailable and are efficacious *in vivo* at well tolerated doses. As such, these compounds may be promising new therapeutics for the treatment of MPDs and other disease states associated with elevated JAK activity.

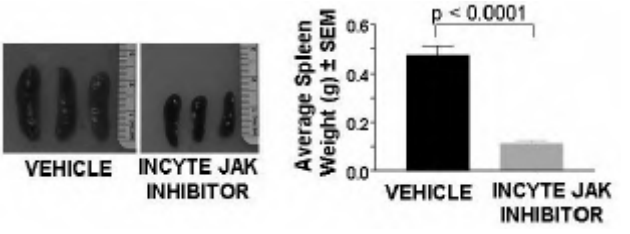

Figure 1. Normalization of MPD-related splenomegaly.

0325

GEMTUZUMAB OZOGAMICIN (MYLOTARG) AS MAINTENANCE THERAPY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. 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 fludarabine 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 directions include therapies targeted at immunomodulation, and, among 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 regimens for Stem Cell Transplantation (SCT). *Patients and Methods.* In this study we analyzed 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 mg/mq for four doses). Patients were evaluated for Continue Complete Remission (CCR) and therapy-related adverse events. *Results.* All patients are in CCR at +17, +13, +11 months, respectively. Neutropenia (between 500 and 1000/microl) and thrombocytopenia (between 50.000 and 100.000/microl) were observed in two (when GO was given at a dose of 6 mg/mq) and in three patients, respectively. No grade 3 or 4 liver toxicity was observed. *Conclusions.* GO administered in fractionated doses as maintenance treatment after ASCT in older patients with $CD33^+$ AML in first or second CR demonstrated a good efficacy and an acceptable safety profile.

Stem cell biology and microenvironment

0326

THE SIMILARITIES OF HUMAN MESENCHYMAL STEM CELLS AND PERICYTES

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Background. Multipotent mesenchymal stromal cells (MSC) can be isolated from a variety of tissues. Their relationship with pericytes and fibroblasts has not been established thus far, although they share many functional properties. Aims. To evaluate the relationship between MSC and pericytes on the basis of gene expression. Materials and Methods. We analyzed 14 samples of MSCs from adult or fetal tissues, two MSC cultures differentiated *in vitro*, and two cultures of retinal pericytes obtained either by immunological separation with anti-CD146 or adhesion, respectively. Cells were cultured under appropriate conditions to promote osteogenic, adipocytic or chondroblastic differentiation, evaluated both by morphology and by specific histochemical staining. Global gene expression was analyzed by SAGE for human retinal pericytes and MSC from BM and from umbilical cord vein. The expression of 39 selected genes was evaluated by serial dilutions of the cDNA in RT-PCR reactions for 31 different cell cultures including undifferentiated and differentiated MSC from adult and fetal sources, pericytes, fibroblasts, endothelial cells, and cells from bone marrow, liver, brain, skeletal muscle, skin and heart. Real time PCR for five genes confirmed the results obtained by serial dilution. Cluster analysis of gene expression data was performed using the Cluster 3.0 software, both for gene expression of 31 cell cultures evaluated by serial dilution and for 17 SAGE libraries from MSCs, endothelial cells, skeletal and heart muscles, $CD34^+$ cells, pericytes, skin fibroblasts, stellate hepatic cells and myofibroblasts differentiated from stellate cells. The use of human cells in these experiments has been approved by the institutional research review committee. *Results.* Cell morphology of MSCs and pericytes were very similar, both under light and transmission electron microscopy, as well as the phenotypes defined by 23 markers, especially negativity for CD34, CD33, CD45, CD14, HLA-DR, KDR and CD31, and positivity for CD73, CD90, CD29, CD44 and HLA-I. Osteogenic and adipocytic differentiation was documented for most MSC cultures and for pericytes; chondrocytic differentiation was positive for the MSCs that were tested. Cluster analysis of SAGE gene expression profiles showed that MSC, pericytes and stellate hepatic cells form a consistent group, separated from another consistent group formed by fibroblasts, smooth muscle cells and myofibroblasts differentiated from stellate cells. Cluster analysis of semi quantitative expression data of the 39 selected genes confirmed that all the MSC lines, pericytes and fibroblast share a common expression profile distinct from other normal cells. Despite the similarity, FSP-1 was more frequently expressed on fibroblasts and NG-2 on pericytes as compared with MSCs. *Conclusions.* We conclude that human MSC and pericytes, as operationally defined by culture methods, are similar cells located in the wall of the vasculature where they function as a source of precursor cells for repair and tissue maintenance. Additionally, the close relationship of fibroblasts and smooth muscle cells with myofibroblast differentiated from stellate cells further indicates that these functionally compromised cells may be closely related to these precursor cells, a proposition that has conceptual and practical implications.

0327

STUDY OF FUNCTIONAL AND MOLECULAR CHARACTERISTICS OF THREE DIFFERENT BONE MARROW CELL POPULATIONS FOR EXPANSION OF MESECHYMAL STEM CELLS

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Background. There is currently great interest in exploring the use of mesenchymal stem cells (MSCs) for clinical purposes. Identification of markers and sources for MSC isolation is, therefore, of particular importance. *Aims.* To compare the colony/cloning efficiency, expression of *stemness* markers and differentiation potential of three different bone marrow (BM) derived cell populations, namely the $CD105^+/CD45^-$, the Glycophorin A $(GlycoA)^-/CD45^-$ and BM mononuclear cells (BMMCs). *Methods.* Normal human BM cells were isolated from posterior iliac crest aspirates. A colony forming unit-fibroblast (CFU-F) assay was used for the determination of the colony efficiency of the BMMC or the immunomagnetically sorted $CD105^+/CD45^-$ and GlycoA-/CD45- BM cell fractions. CFU-F colonies derived from the three different cell subpopulations were isolated with clone-rings and further expanded using a standard MSC culture protocol. Clone efficiency was determined by evaluating the number of clones survived after full *in vitro* expansion versus the number of initially expanded clones. The adipogenic, osteogenic, and chondrogenic (AOC) differentiation potential of MSCs expanded from these clones and cultured under defined culture conditions, were assessed by using Oil red O, Alkaline phosphatase/Von Kossa and Alcian Blue immunohistochemical stains respectively, as well as by evaluating the expression of specific differentiation-associated gene expression by RT-PCR. Finally, the immunomagnetically sorted $CD105^+/CD45^-$ and $GlycoA^-/CD45^-$ cell populations were assessed at day-0 for the expression of Oct4 and Nanog *stemness* genes using real-time RT-PCR. *Results.* In the CFU-F assay, the colony efficiency was statistically significantly higher when $CD105^+/CD45^-$ cells were used in the culture (144.8±62.8) compared to $GlycoA^-/CD45^-$ cells (21.5±16.2) or BMMCs (10.9±11.6) ($p<0.0001$, one-way ANOVA; colony numbers are expressed as CFU-F per 100000 cells cultured). By determining clone efficiency as the number of clones survived after full *in vitro* expansion versus the number of initially expanded clones, we found that clone recovery by $CD105^+/CD45^-$ cells (47.0%±23.86%), although higher, was not statistically significant compared to the respective of BMMCs (40.2%±32.21%) and $GlycoA^-/CD45^-$ (39.61%±38.62%) derived clones ($p=0.9018$, one-way ANOVA). The AOC differentiation capacity of clones generated from the three different cell sources were similar as evaluated my the immunohistochemical staining and the expression of aP2 and PPAR-γ for adipogenic, CBFA1 and ALP for osteogenic, aggrecan and coll-II for chondrogenic differentiation. Expression of Oct4 and Nanog was found 16-fold and 47-fold higher, respectively, in $CD105^+/CD45^-$ cells compared to $GlycoA^-/CD45^-$ cells ($p<0.05$ and $p<0.05$, Mann Whitney test). *Summary and Conclusions.* The data presented above suggest that the $CD105^+/CD45^-$ BM cell fraction contains a more immature MSC population compared to $GlycoA^-CD45^-$ cells with higher colony-forming efficiency and higher Oct4 and Nanog expression. The $CD105^+/CD45^-$ BM cells display also the potential to differentiate towards the AOC lineages and accordingly, represent a better source for MSC studies compared to BMMCs or $GlycoA^-/CD45^-$ cells. Comparison between the molecular and proteomic profile of $CD105^+/CD45^-$ and $GlycoA^-/CD45^-$ cells is an interesting field for further investigation.

0328

COMPARATIVE ANALYSIS OF ANGIOGENIC CAPACITY OF MONOCYTES AND CD 133+ CELLS IN A MURINE MODEL OF HINDLIMB ISCHEMIA

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Background. The presence of endothelial progenitor cells (EPC) in adult humans has been recently described. Two cell sources, $CD133^+$ cells and monocytes have been shown to have angiogenic capacity. *Aims.* To perform a prospective study comparing the angiogenic capacity of monocytes and $CD133^+$ cells in a murine model of hindlimb ischemia. *Material and Methods.* Cells were obtained from voluntary healthy donors, monocytes by adherence from buffy-coats of blood donations and $CD133^+$ cells from leukapheresis products for allogeneic transplantation after immunomagnetic selection (Variomacs, Miltenyi). *In vivo* studies were performed in a mouse model of acute hindlimb ischemia. It was induced after ligation and excision of the left femoral artery in 42 Swiss-nu/nu 6-8 weeks old mice. The day of ischemia monocytes (10^5 or 10^6 cells; n=14) or $CD133^+$ cells (10^5 or 10^6 cells; n=14) or only saline serum (control group; n=14) were IV infused into the mice. Revascularization was analysed using two different approaches: blood flow by laser Doppler analysis (moor LAB server) and capillary density studies (number vessels/μm^2) (Visilog programme). In order to know where the infused cells were localized immunofluorescence and immunocytochemistry techniques were performed and analysed by light (Olympus CX40), fluorescence (Olympus Provis) and confocal (Leica TCS SP) microscopes. The following markers were used: human CD34 (Novocastra TM), human CD31 (Dakocytomation) and mouse CD31 (BD). *Results.* Monocytic and $CD133^+$ cell purity was 80% (73-85) and 82%

(50-99) respectively. Serial analysis showed a progressive recovery of limb perfusion in all animal groups analysed. From day +4 until day +28 a significant improvement in limb perfusion (p<0,05) was observed in both, mice treated with monocytes and CD133$^+$ cells when compared with non treated mice. However, no differences were observed between the two experimental groups of mice (treated with monocytes or CD133$^+$cells). Capillary density also increased in mice treated with both monocytes (48 (14-56) capillaries//∝m^2) and CD133$^+$ cells (51 (18-61) capillaries/∝m^2) versus non treated mice (20 (8-51) capillaries//∝m^2). With significant differences in mice receiving CD133$^+$ (p=0,02). By contrast, in ischemic muscles not receiving cells the number of capillaries was lower than in control mice (p=0,01). When we looked if the infused cells were or not incorporated in mice vessels we could observe that, when a low number of monocytes or CD133$^+$ cells was infused (10^5), no cells were observed into the vessels suggesting that cells could act throughout a paracrine mechanism. However, when higher doses were used (10^6 cells) few cells from both cell sources could be observed into the mice vessels. *Conclusions.* Our data show that treatment with both, monocytes or CD133$^+$ cells, improves blood perfusion in a murine model of hindlimb ischemia increasing the number of capillaries in a very similar way, probably mainly by a paracrine mechanism but some cells from both sources can incorporate into the endothelium.

0329

REGULATION BY TGFβ SUPERFAMILY OF HUMAN B LYMPHOPOIESIS IN A NEWLY ESTABLISHED LYMPHOCYTE CULTURE SYSTEM USING HUMAN MESENCHYMAL STEM CELLS AS A SUPPORTIVE MICROENVIRONMENT

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Background and Aims. Recently, the analysis of human B lymphocyte developmental biology using human origin hematopoietic cells has been progressing. However, in many of these studies, murine stromal cell lines were utilized as microenvironment to support B lymphopoiesis. Previous studies showed that there are some differencet mechanism between murine and human B cell differentiation. Hence, there is a great need for a more biologically relevant model system to determine the role and function of various mediators in human B lymphopoiesis. *Methods.* Using human mesenchymal stem cells (hMSCs) as stromas, we have developed a long-term culture system to produce B lymphocytes from human CD34$^+$ cells purified from umbilical cord blood (CB). To determine the effects of various regulators on the growth of B progenitor cells, we tested effects of several low molecular weight inhibitors, neutralizing antibodies, as well as recombinant proteins in this culture system. *Results.* HMSCs can support more efficiently the commitment and differentiation of CB CD34$^+$ cells into B lineage compared to the murine bone marrow stromal cells, MS-5 and human umbilical vascular endothelial cells (HUVEC). In various combinations of recombinant human growth factors, the addition of stem cell factor (SCF) and Flt-3 ligand (FL) efficiently enhanced the growth of surface μ$^+$ immature B cells. The addition of interleukin 7 (rhIL-7) is not required for this culture system, and the addition of rhIL-7 suppress the emergence of surface IgM expression from CD34$^+$ CB cells. Thus, our co-culture of 2,000 CB CD34$^+$ cells with hMSC in the presence of SCF and FL successfully produced approximately 5×10^5 CD10$^+$ B lymphocytes at 4 weeks. When various small molecules inhibitors were added to this culture system, NAC (ROS inhibitor) and DUP697 (COX2 inhibitor) exerted no effect on B lymphopoiesis with this culture. Addition of BIO (GSK-3 inhibitor) diminished the emergence of CD10$^+$ cells. SB431542 (ALK4/5/7 inhibitors) showed significant effect of the expansion of CD10$^+$ cells. With regard to the regulation of TGFβ superfamily, follistatin (physiological antagonist of Activin A) or a neutralizing antibody for Activin A enhanced B lymphocyte production approximately 2.5-fold and 3-fold, respectively. The neutralizing antibody for TGFβ1 had no effects. The B lymphocyte production tended to be suppressed by the addition of recombinant TGFβ1, but not Activin A. The differential effects of inhibitors andfactors on B lymphocyte production seemed to be related to the fact that much higher concentration of Activin A was contained in culture supernatant than TGFβ1. With regard to the expression of TGFβ superfamily, RT-PCR revealed that hMSC expressed TGFβ family, Activin family; CB CD34$^+$ cells expressed their cognate receptors. Immunohistocal analysis revealed that both Activin A and TGFβ1 were produced in bone marrow. *Conclusions.* We established a useful co-culture system of CB CD34$^+$ cells with hMSCs for analyzing regulatory mechanisms of human B lymphocytes. With this system, we showed that members of TGFβ superfamily, Activin A and TGFβ1, are negative regulators for the early onset of normal human B lymphopoiesis.

0330

MESENCHYMAL STEM CELLS (MSC) REGULATE IMATINIB RESISTANCE: NO EFFECT OF IMATINIB ON MSC CYTOKINE SECRETION

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Imatinib mesylate (IM) specifically blocks BCR/ABL tyrosine kinase and has become standard therapy of BCR/ABL positive leukemias such as chronic myeloid leukaemia (CML). However, occurrence of IM-resistance and persistence of minimal residual disease (MRD) are major obstacles to the issue of potential cure of CML. The hematopoietic microenvironment closely regulates normal and abnormal hematopoiesis. Mesenchymal stem cells (MSC) are an important component of the bone marrow microenvironment, and control hematopoiesis via cytokine secretion and adhesion. We and others have recently reported that cytokines can mediate BCR/ABL-independent resistance to IM and Nilotinib (NI), a novel more potent BCR/ABL-kinase inhibitor (Wang Y et al, Blood 2007). Here we ask, whether IM controls the growth and changes cytokine secretion pattern of CML MSC, thereby potentially contributing to IM resistance development or persistence. In an attempt to address these questions, we isolated MSC of 4 healthy donors and 7 CML patients before and 3-12 months after commencing IM. IM potently inhibited not only proliferation of healthy donor bone marrow-derived MSC, but also CML MSC in both dose-dependent and time-dependent manners *in vitro* and NI exposure had a similar effect. Growth arrest occurred in G1 phase of the cell cycle and was associated with accumulation of the cyclin-dependent kinase (CDK) inhibitor p27kip1 and decrease of p45skp2, a negative regulator of p27kip1. Knocking down p27kip1 with a p27kip1 specific siRNA could partially overcome growth arrest by IM, indicating p27kip1 is not the only effector for the growth inhibition. Next, by scanning 120 cytokines in conditioned media made from MSC of the same CML patients before and after IM therapy via a cytokine antibody array, we found no impact of IM on differential cytokine secretion in CML MSC. Additionally, BCR/ABL positive cell line LAMA or K562 exhibited no apoptotic difference in conditioned media from MSC pre- and post-IM therapy in response to IM. However, both the conditioned media and co-cultivation between MSC and K562 could significantly prevent IM induced apoptosis of leukaemia cells, respectively, as compared with fresh culture medium, indicating that the interaction of MSC and leukaemia cells has the potential to regulate IM resistance/persistence. In summary, our data suggest that even though IM can reduce MSC pool in bone marrow, leading to decreased overall cytokines secretion, individual MSC after long term of IM treatment *in vivo* still possesses the same protection of leukemic cells nearby as the one before IM therapy due to unchanged cytokine secretion pattern and ability.

0331

WNT/β-CATENIN PATHWAY MODULATES MULTIPOTENCY OF HEMATOPOIETIC STEM/PROGENITOR CELLS REGULATING THE ACTIVITY OF VARIOUS ESSENTIAL TRANSCRIPTION FACTORS

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Background. Wnt/β-catenin pathway has been well known as an important regulator of hematopoietic cell proliferation and differentiation. Purified Wnt3a that activates the canonical Wnt/β-catenin/TCF/LEF pathway was shown to expand murine and human hematopoietic stem/hematpoietic progenitor cells (HSC/HPCs) *ex vivo*. Also, it was reported that post transplantation treatment with an ATP-competitive GSK-3 inhibitor, which leads to activation of intrinsic β-catenin increases repopulation of HSCs *in vivo*. In this way, activation of Wnt/β-catenin pathway modulates the *in vitro* and *in vivo* self-renewal ability and mulitipotency of HSCs. However, the precise regulatory mechanisms have not been elucidated. *Aims.* In this study, we examined the effects of GSK-3 inhibitor 9 (6-bromoindirubin-3-oxime) (GI9) on the growth and differentiation of human HSC/ HPCs *in vitro*. *Methods*. Human umbilical cord blood (CB) was obtained at normal full-term deliveries after informed consent was given and CD34$^+$ HSC/HPCs were derived from CB using a MACS system. CD34$^+$ HSC/HPCs were cultured for 7 days in a serum-free medium containing with cytokines (SCF, FL, TPO, IL-6 and sIL-6R) and also with 2 μM ,10 μM of GI9, or vehicle (negative control; NC). After the culture, viable cell numbers, the distribution pattern of immunophenotype (CD34 and lineage specific markers), and the colony

forming ability were evaluated. *Results*. The expression and localization of β-catenin in CD34+ HSC/HPCs treated with GI9 was observed with confocal microscopy. After the treatment for 24 hrs, expression of β-catenin in NC cells was scarcely detected except for the membrane-bounded form, which constitutes the cytoskeleton. On the other hand, in GI9-treated cells, β-catenin accumulated in their nucleus in a dose dependent manner. These results suggested that GI9-treatment activates intrinsic β-catenin in human HSC/HPCs. After 7 days culture in a serum-free medium containing with cytokines, total viable cells and CD34+ cells were expanded 31.6±4.6 and 17.9±3.8 fold in NC cells, respectively (n=3). However, GI9-treatment could not maintain a proportion of CD34+ cells compared with NC and significantly caused the growth inhibition in a dose dependent manner. About 80% of expanded cells expressed myeloid marker, CD33 in our culture system, however, GI9-treatment perturbed myeloid differentiation of CD34+ HSC/HPCs but induced the differentiation toward to megakaryocyte and erythroid lineages. Furthermore, in methylcellulose assay, although expanded cells with GI9-treatment generated all types of progenitors, GI9-treatment was inferior significantly in terms of expansion rate of myeloid progenitor, CFU-GM and superior in formation of erythroid progenitor, BFU/CFU-E compared with NC (No. of CFU-GM/1000 cells 151±65.8 vs. 284±17.0, No. of BFU/CFU-E/1000 cells 132±18.5 vs. 32.7.±7.0, respectively) (p<0.05, n=3). As for this mechanism, we found that activated β-catenin suppresses the transcriptional activity of C/EBPα, which is essential transcription factor for granulocyte development, while it promotes the function of GATA1, essential transcription factor for megakaryocyte and erythrocyte development during the differentiation of HSC/HPCs. *Summary and Conclusions*. Wnt/β-catenin pathway was supposed to play an important role in multipotency of HSC/HPCs and control the balance of lineage commitment of HSC/HPCs, presumably by regulating the interaction with essential transcription factors.

0332

ACUTE MYELOGENOUS LEUKEMIA CELLS CHANGE INTO FIBROBLAST CELLS MORPHOLOGICALLY AND FUNCTIONALLY DURING *IN VITRO* LONG-TERM CULTURE

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Introduction. Recently controversial results are reported on stromal cells generated from MDS patients' bone marrow, in which stromal fibroblasts are originated from normal clone or the abnormal MDS clone. We observed various kinds of de novo acute leukemia cells which were cultured long-term in liquid, and the generated stromal cells were characterized biologically and molecularly. *Materials and Methods.* Bone marrow cells or blood from patients with acute leukemia whose abnormal clones were identified with RT-PCR products for the translocated chimeric molecules, were separated with gravity sedimentation, and the obtained non-adherent mononuclear cells were cultured in DMEM with 10% FCS in the humidified 5% CO2 incubator. Morphological changes of the cultured cells were observed, and when cells were converted into stromal fibroblastoid cells, they were treated with tripsin, and re-cultured until whole cells became stromal cells. Then, cells were divided into sub clones in a 96-well plate and after culturing to be confluent, RNAs from each sub clone were extracted, cDNAs were synthesized, and RT-PCR was performed to select clones. When the abnormal translocation products, which were identified in the original leukemia blasts, were observed, the clones were examined on DNA levels with FISH or southern blotting, and when the clones were identified on DNA level, cells were characterized on their expression of cell-surface molecules, the activities on cytokine production and growth-promotion activities for the normal bone marrow-derived cells or leukemia blasts. *Results and Discussions.* Morphological changes into stromal fibroblastoid cells were observed in acute myelogenous leukemia cells except AML (M3), and acute biphenotypic leukemia cells, but not in acute lymphocytic leukemia cells. The fibroblastoid cells expressed the abnormal molecular translocation markers which were identified in the parental leukemia cells on RNA and DNA levels. The probability to identify the abnormal leukemia clones in the whole fibroblastoid sub clones was 2-56%. These fibroblastoid cells with leukemia markers expressed CD 106, fibronectin, smooth muscle actin and FSP1 which is expressed specifically in myofibroblasts not in macrophages. CD13, 33 and myeloperoxidase were also expressed which are the myeloid markers. CD34 and CD133 were expressed in these fibroblastoid cells, which are stem cell markers. These cells produced VEGF, IL-6 and G-CSF more than that produced by the normal bone marrow-derived fibroblast cells, and when leukemia blast cells were cultured onto the generated fibroblastoid cells, the proliferation activities were elevated significantly with 3H-thymidine incorporation assays. These data indicate that acute myeloid leukemia cells have the capacity to be changed into stromal fibroblastoid cells and create the microenvironment for the proliferation by themselves. Cancer stem cell hypothesis is that leukemia stem cells can have the activity for self-replication and abnormal differentiation as is observed in normal hematopoietic stem cells. Our observation suggests that leukemia stem cell may also produce the stromal microenvironment for the growth of leukemia stem cells.

0333

INVOLVEMENT OF TNF-ALPHA IN LEUKAEMIA ONSET AND BONE MARROW TURNOVER

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Leukaemia is characterized by abnormal ratios of bone marrow cells from several hematopoietic lineages, meaning that normal tissue turnover is impaired in this situation. Bone marrow diseases have also been associated with an increase in bone marrow angiogenesis. It is known that changes in bone marrow microenvironment may lead to a loss of homeostasis; important players in this process may be cytokines and extracellular matrix (ECM) molecules present in the stroma of this organ. TNF-α is involved in bone marrow cell turnover, in normalcy and in disease, thus our study is focused on the functions of this cytokine in regulating cell apoptosis within the bone marrow microenvironment and also in regulating extracellular matrix (ECM) turnover. Whilst studying bone marrow recovery following sublethal irradiation, we observed that TNF-α undergoes significant variations, acutely increasing following irradiation, and decreasing to normal values 5 days after the stimulus. To understand the possible role of TNF-α in leukaemia development, we irradiated TNF-α knock-out (KO) and wild-type (wt) mice thrice (each irradiation was separated 1 month apart), a model that has been shown to result in leukaemia induction. From the five mice we irradiated from each genotype, four wt mice died 6-7 months after the last irradiation as a result of leukaemia, while in the KO group only one mouse succumbed to a possible bone marrow deficiency (although not overt leukaemia). FACS analysis of peripheral blood and bone marrows from the different mice, revealed that wt mice had increased endothelial progenitors and endothelial cells, suggesting that the vascular lineage was increased in these mice, following the irradiation schedules. In addition, the bone marrows of TNF-α KO mice were smaller in diameter, but after irradiation, while wt bone marrows undergo a notorious reduction, the KO bone marrows remain with more or less the same size, meaning that probably, the altered mice are more resistant to irradiation. KO bone marrow (irradiated and control) also exhibited increased megakaryocyte numbers, accompanied by a decrease in fibronectin and laminin levels, in irradiated KO mice. Another important observation is that irradiated wt mice, which developed a bone marrow disease phenotype, have increased bone marrow angiogenesis, which consisted of dilated blood vessels. Taken together, these results suggest that TNF-α plays a role in bone marrow homeostasis, which is more clearly linked with angiogenesis and ECM turnover. In KO mice, selective apoptosis may contribute to select leukaemia clones; in parallel, TNF-α is also crucial in modulating MMP activity and, as a result, its absence may contribute towards a global reduction in bone marrow MMP activity. Reduced MMPs may in turn lead to a reduced availability of ECM-bound VEGF, thus regulating bone marrow angiogenesis. Globally, our data point out for a crucial role of TNF-α in modulating bone marrow turnover and angiogenesis, which may contribute to leukaemia onset.

0334

EXPRESSION OF SURFACE MARKERS INVOLVED IN CELL-CELL-INTERACTIONS, ADHESION AND ACTIVATION ON ENDOTHELIAL PROGENITOR CELLS

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Background. Human peripheral blood contains bone-marrow derived progenitor cells which differentiate into mature endothelial cells (EC) and are thought to promote tumour neoangiogenesis as well as vascular homeostasis in adults. These endothelial progenitor cells (EPC) are characterized as CD34, CD133 and CD309 positive. However, phenotyping of circulating EPC *in vivo* still remains unclear and information about EPC function and factors that determine their cell-cell-interactions, homing and tissue distribution are scarce. *Aims.* We analysed the expression of ten surface markers on EPC involved in endothelial cell-

cell-interaction, adhesion and activation. To study EPC phenotype in health *in vivo*, two sources of cells were compared: naïve cells from umbilical cord blood and circulating cells out of peripheral blood from young, healthy adults. *Methods.* Classic Ficoll isolation was used to enrich mononuclear cells (MNC) from peripheral blood of twenty young healthy donors (between 20 and 30 years) and cord blood of twenty newborns. To identify EPC MNC were stained with monoclonal antibodies (mAb) against CD34, CD133 and CD309 and analysed by fluorescence activated cells sorting (FACS). In the same analysis tubes MNC and thereby EPC were stained with additional mAb against surface molecules involved in activation, adhesion, rolling and migration of leucocytes and mature EC. Therefore, MAbs used were: CD9, CD29, CD31, CD44, CD47, CD49, CD51/CD61, CD54, CD58, CD62e and CD63. *Results.* Expression of the β-1 integrin (CD29), Pgp-1 (CD44) and gp42 (CD47) on EPC did not differ between naïve cord blood from newborns and young healthy donors. PECAM-1 (CD31), which has been reported to be upregulated to nearly 100% in cardiovascular disease was only expressed of 53.7% on all EPC (donors and naïve did not differ), suggesting a non-activation state. Expression of MRP-1 (CD9) and LFA-3 (CD58) did significantly differ between the two cohorts: MRP-1: 81% versus 68%, and LFA-3: 34% versus 10% (donors versus naïve). Molecules reported to be expressed on mature EC and involved in activation, adhesion, rolling and migration like α-V-β-3 integrin (CD51/61), ICAM-1 (CD54), E-selectin (CD62e) and LIMP(CD63) could only be weakly detected on EPC (0-10%). *Summary and conclusions.* Significant difference of CD58 expression between donors and naïve cells might be attributed to an ongoing differentiation of circulating EPC as mature EC show an expression of CD58 up to 80%. Likewise, the upregulation of CD9 in donors versus naïve might resemble an increased proliferation potency of EPC since CD9 is likely under the regulation of the LIF/STAT3 pathway, which is critical for self-renewal of ES cells. The high expression of CD29, CD44 and CD47 may reflect the important role of these molecules in recruitment of EPC, which will be investigated in a future study. Low to not existing expression of CD51/61, CD54, CD62e and CD63 might lead to upregulation during e.g. acute ischemia. However, this cannot be proved from this data. Limitations of our study are the small sample size. Nevertheless, to our knowledge, this is a first report of EPC phenotyping in health *in vivo*.

0335

IN VITRO CHARACTERIZATION OF CORD BLOOD MESENCHYMAL STROMAL CELLS (CB-MSC): IMMUNOMODULATORY PROPERTIES AND HAEMATOPOIESIS SUPPORTING ACTIVITY

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Introduction. In vitro studies and some encouraging clinical results have demonstrated that bone marrow derived MSC (BM-MSC) might play an important role in haematopoietic stem cells transplantation (HSCT), reducing Graft Versus Host Disease (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 immuno-selection (RosetteSep) and density gradient separation (Ficoll) respectively. Both sets of cells were cultured in aMEM with 20%FCS and 2 mM L-glutamine. CB derived CD34$^+$ haematopoietic progenitor cells (HPC) were isolated by positive immunoselection using the MidiMACS system. CD 34$^+$ cells were seeded on the MSC feeder layers at 40.000 cells/ml in RPMI medium+10%FCS and co-cultured for 12 days. Cytokine production in the culture supernatants was quantified by enzyme linked immunoadsorbent assay (ELISA) when cells achieved confluence after 3 passages, corresponding to the initial phase of their exponential growth. *Ex vivo* expanded CB and BM MSC were co-cultered with allogeneic lymphocytes (PBLs) to assess their capacity to elicit an immunoresponse. Moreover, CB and BM-MSC were added at different doses (105, 5×10^4, 104) to PBLs stimulated with Phytohaemagglutinin (PHA) and to Mixed Lymphocyte Cultures (MLC). Lymphocytes proliferation was measured by 3H-Thymidine incorporation. *Results*. CB and BM-MSC produced substantial amounts of SCF, IL-3, IL-6, TPO but almost undetectable Flt-3L. IL-6 production was significantly higher in BM-MSC cultures than in CB-MSC (p<0.05), whereas there was no statistical difference for SCF, IL-3 and TPO. Moreover, CB and BM-MSC supported ex-vivo expansion of HPC (2,0±0,3 and 2,3±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 T cell allo-response and they did inhibit mitogenic lymphocyte proliferation by PHA (48-88%) in a dose dependent manner (Figure 1a). Suppression of T cell proliferation was observed after the addition of different CB-MSC doses to MLC but, as shown in Figure 1b, CB-MSC seemed to have a less consistent inhibitory effect. *Conclusions.* 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.

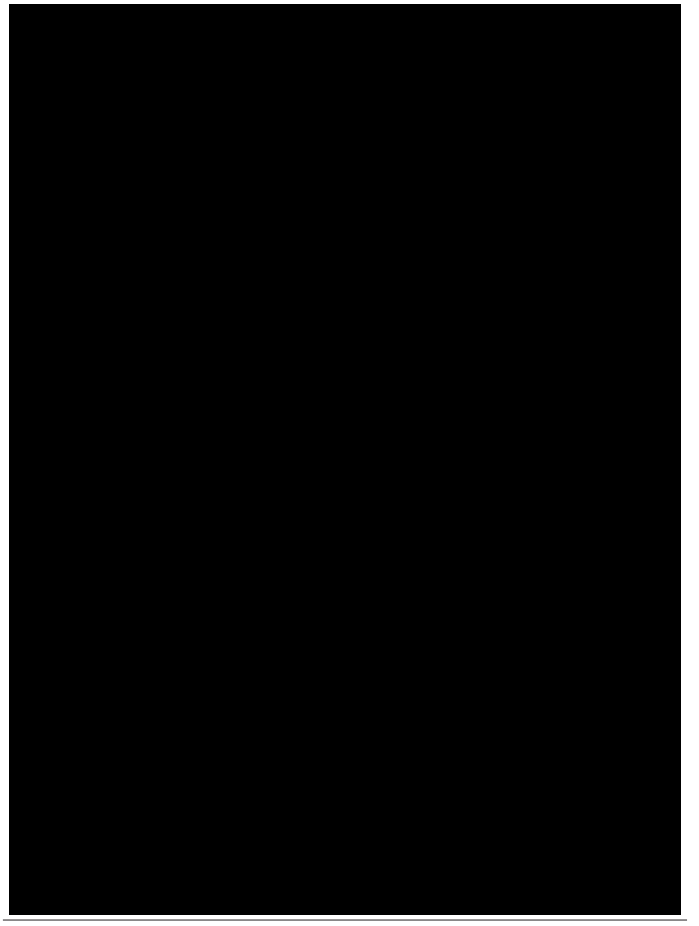

Figure 1. a) Effects of various amounts of CB and BM-MSC on PBL mitogenic stimulation. Data were expressed as percentage of the maximum proliferative response (A+PHA), mean±SEM.b) Effects of various amounts of CB and BM-MSC on MLCs. Data were expressed as percentage of the maximum proliferative response (A+B), mean±SEM.

0336

CXCR4$^+$ CIRCULATING PROGENITOR CELLS COEXPRESSING MONOCYTIC AND ENDOTHELIAL MARKERS MAY BE INVOLVED IN THE PATHOGENESIS OF FIBROSIS IN SYSTEMIC SCLEROSIS

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Background. Although high level of circulating endothelial progenitor cells has been reported in patients affected by systemic sclerosis (SSc), there are ongoing controversies regarding the role played by circulating endothelial mature and progenitor cells (CECs/CEPs) in the pathogenesis of this disease. *Aims.* To better characterize the immunophenotype and the clonogenic potential of circulating progenitor cells (CPCs), CECs and CEPs in SSc. The presence of the various cell subsets are correlated to the disease activity score and the severity of pulmonary dysfunction. *Methods.* A multiparametric 4-color flow cytometric protocol, in combination with different progenitor cell replating assays, were used to

analyse peripheral blood specimens obtained from 40 patients (pts) with SSc, and 10 healthy subjects. Data were compared with patient's clinical features and serum levels of angiogenic factors (VEGF, PDGF, EGF, IGF and SDF-1 chemokine). *Results.* An increased number of viable (7-AADneg) $CD34^+/CD45^+/CD184^+$ (CXCR4) and $CD34^+/CD45^+/CD117^+$ (c-kit-R) CPCs subsets coexpressing endothelial and monocytic markers were detected in SSc, as compared with normal subjects. No circulating $CD45^-$ CEPs and CECs were observed. Clonogenic assays confirmed the differentiation of the CPCs towards hematopoietic-erythroid lineage (BFU-E) but not endothelial cells even after extensive culture expansion. Interestingly, in 27% of SSc patients, the *in vitro* presence of adherent endothelial-like cells, having a reduced adherent expansion capacity, with a spindle shaped morphology (SELC) that resulted positive for endothelial, and myelomonocytic markers, was observed. The presence of $CXCR4^+$CPC resulted correlated either to SDF-1 and VEGF serum level or to some clinical features related to a more fibrotic clinical subset of the disease, thus supporting a possible role of these cells in fibrosis. *Conclusions.* In SSc patients, the mobilization of endothelial-like $CXCR4^+$CPCs suggests an unreported contribution of the hematopoietic progenitor cells in the pathogenesis of the disease. Angiogenic factors could play a role in facilitating their organ homing and their perivascular positioning and retention. These findings could have clinical and biological implication, and may help in refining the sequence of the pathogenetic events in SSc .

0337

THE ANABOLIC EFFECTS OF STRONTIUM INCREASE THE NUMBER OF PROGENITOR BUT NOT PRIMITIVE HAEMOPOIETIC STEM CELLS

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The regulation of haemopoietic stem cells (HSC) fate requires a specialized microenvironment in the bone marrow (BM) cavity called the HSC niche. Recent evidence indicates that osteoblasts (OB) are a key component of this niche, modulating HSC quiescent and proliferation. In this study the role of OB in the niche was further investigated by manipulating their activity with strontium (Sr); a substance that enhances osteoblast function and inhibits osteoclast activity *in vitro* and likewise stimulates bone formation and decreases bone resorption *in vivo*. Alizarin red S staining revealed that Sr promotes the mineralization of bone nodules in primary OB cultures. RT-PCR analysis demonstrated increased production of runx2 mRNA in primary OB cultures treated with Sr. Administration of Sr to mice resulted in elevated levels of serum osteocalcin measured by ELISA. Sr-treated mice showed increased bone volume, trabecular separation and trabecular thickness defined by micro-CT analysis. In regard to haemopoiesis CFU-C assay revealed increased numbers of haemopoietic progenitor cells in Sr-treated mice as compared to untreated control mice. However, no difference in the primitive HSC numbers was detected between the two groups evaluated by LTC-IC assay and by FACS analysis. When Sr-treated mice were used as donors in HSC transplantation experiments no difference in the HSC engraftment ability was observed. These results verify that Sr increases OB function and number both *in vitro* and *in vivo*. They also indicate that Sr affects an OB subset which interacts with more differentiated but no primitive HSC and that its effect on OB is not sufficient to increase the stem cell pool size or alter HSC function. In summary, this study underlines that although OB are indeed a key component of the niche, they cannot solely account for the regulation of primitive HSC numbers. In fact, a simple increase in OB number/activity is not enough to expand the stem cell pool size and other cell types may be required.

Thrombosis I

0338

ANTI- PLASMINOGEN MONOCLONAL ANTIBODY(MC2B8) INHIBITES ANGIOGENESIS

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*Abstract. Background.*Angiogenesis is a complex process during which of new blood vessels are produced from the pre-existing blood vessels. Formation and growth of new vessels play an important role in the physiologic process (embryonic growth, tissue repair) and pathologic process (tumor growth, inflammation) for surviving of the tissues. In fact, the development of tumors is depended upon new vessel formation through which the tumor is provided with nutrient and oxygen. *Aims.* In this research, the role of plasminogen conformation with MC2B8 mAb (an antibody directed against C-terminal part of plasminogen) in clot lysis and angiogenesis is observed. *Methods.* In experimental model of angiogenesis, beads, covered with endothelial cells of bone marrow capillaries, are the source of endothelial cells. It coated in three-dimensional structure to be provided through fibrin gel. Different titers of monoclonal antibody (30-480 μg/mL) MC2B8 were added in fibrin gel. *Results*. 3-5 days after culturing of endothelial cells, growth and migration was seen as the result of capillary formation MC2B8 mAb delayed clot lysis and inhibited angiogenesis at the concentration of 240 μg/mL.*Conclusions.*Our findings suggest that these effects on capillary tube formation and clot lysis caused blockage or conformational changes in plasminogen epitopes involved in angiogenesis and fibrinolysis.

0339

OUTCOMES OF THROMBOLYSIS FOR REPEATED COURSES OF SYSTEMIC TISSUE PLASMINOGEN ACTIVATOR FOR INTRAVASCULAR THROMBOSIS IN CHILDREN

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Background. Systemic tissue plasminogen activator (tPA) has been used for treatment of life or limb-threatening intravascular thrombosis. Previous reports have varied in the rates of hemorrhagic complications. *Aims.* To summarize the outcome and safety of repeated courses of tPA thrombolysis for intravascular thrombus in the critical care unit at a single institution. *Methods.* Retrospective analysis of hospital records was completed for patients who received systemic tPA, according to institutional protocol over a 7 year period (1999-2006) at the Hospital for Sick Children, Toronto. *Results.* 51 patients received systemic tPA in doses of 0.1-0.6 mg/kg/hr for up to 6 hours per course. There were 82 courses of systemic tPA in 51 patients. Patients received from 1 to 4 courses of tPA. The indications for tPA were venous thrombi (n=63) and arterial thrombi (n=19). After the first course of tPA (n=51), there was complete resolution in (13/51) 25%, partial resolution in (17/51) 33%, no change in (20/51) 39% and progression in (1/51) 2%. After the second course of tPA (n=23), there was complete resolution in (11/23) 48%, partial resolution in (5/23) 22%, no change in (7/23) 30% and progression in (0/23) 0%. After the third course of tPA (n=6), there was complete resolution in (3/6) 50%, partial resolution in (2/6) 33%, no change in (0/6) 0% and progression in (1/6)17%. After the fourth course of tPA (n=2), there was complete resolution in (0/2) 0%, partial resolution in (1/2) 50%, no change in (0/2) 0% and progression in (1/2) 50%. Overall, major bleeding occurred in (4/82) 5%, minor bleeding in (11/82) 13% of the tPA courses. Major bleeding and minor bleeding occurred in 3/51 (6%) and 4/51 (8%) at first course, 1/23 (4%) and 6/23 (26%) at second course, 0/6 (0%) and 1/6 (17%) at third course respectively. No bleeds occurred with the fourth course. Bleeding complications were more frequent with arterial [(7/19), 37%] than venous clots [(8/63), 13%]. *Conclusions.* Repeated courses of systemic tPA therapy may improve complete resolution rates, and increase the risk of minor but not major bleeding. Bleeding complications may be more common with arterial thrombi than venous thrombi in children treated with tPA thrombolysis.

0340

ALTERED FIBRIN CLOT STRUCTURE IN PATIENTS WITH ISCHEMIC STROKE ASSOCIATED WITH FORAMEN OVALE

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Background. A patent foramen ovale (PFO) has been implicated with embolic stroke. The mechanism underlying this association remains elusive. Dense fibrin clot structure, resistant to fibrinolysis, has been found to be related to thromboembolic complications of coronary artery disease. *Aims.* to test the hypothesis that altered fibrin clot architecture might characterize individuals with stroke associated with PFO. *Materials and Methods.* 62 patients (22 M, 40 F; mean age 40.5 [SD 13.7] years) with documented PFO, divided into two groups based on the presence of documented ischemic stroke or not (S^+ and S^-). Forty-two age- and sex-matched apparently healthy individuals served as controls (n=42). Plasma fibrin clot permeability (expressed as Ks, a measure of the pore size), turbidity (lag phase and maximum absorbancy [max Abs]) and tissue plasminogen activator (tPA)-induced fibrinolysis efficiency (expressed as lysis time in a turbidimetric assay and maximum D-dimer levels, along with their maximum rate of increase in a pressure-driven clot system) were determined. Genotyping for the factor (F)XIII Val34Leu and fibrinogen α chain Thr312Ala polymorphisms was performed. *Results.* In the S^+ group, clot permeability was lower compared with the S- group (median [IQR], Ks, 9.3 [1.4] vs 10.5 [1.3]$\times 10^{-9}$ cm^2; $p<0.0001$), while clot lysis was prolonged (8.45 [3] vs 7.1 [1] min;$p<0.0001$). Maximum absorbancy was higher and the lag phase shorter in the S+ group than in the S^- group (0.865 [0.07] vs 0.7 [0.065]; $p<0.0001$, and 35.5 [2.6] vs 47 [5] seconds; $p<0.0001$, respectively). Maximum D-dimer levels, measured at 120 min before the clot collapse in the S^+ group, were elevated compared with those in the S^- group (3.67 [0.14] vs 3.36 [0.13] mg/L; $p<0.0001$, respectively). Maximum rate of increase in D-dimer levels were also higher in the former group (0.081[0.008] vs 0.069 [0.0085] mg/L/min;$p<0.0001$, respectively). Variables describing clot properties in the S^- group were similar to those obtained in healthy controls with the exception of decreased rate of D-dimer increase in the former group ($p=0.01$). Genetic analysis showed no difference in the genotype frequencies among all the groups. After adjustment for fibrinogen levels, Ks, turbidity variables and lysis time remained significantly different between the S^+ group and both S^- group and healthy controls. *Conclusions.*decreased permeability of fibrin clots, composed of thicker fibers, and their relative resistance to fibrinolysis observed in subjects with PFO who experienced stroke, may represent a novel mechanism that might explain why only a small percentage of individuals with PFO suffer from thromboembolic complications.

0341

THE RISK OF PREGNANCY-RELATED VENOUS THROMBOEMBOLISM IN DOUBLE CARRIERS OF FACTOR V LEIDEN AND PROTHROMBIN G20210A

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Background. The rate of venous thromboembolism (VTE) during pregnancy in double heterozygotes for factor V Leiden (FVL) and prothrombin G20210A (PT) is not established. The optimal management of these women in terms of antithrombotic prophylaxis is unknown. *Aims.* To assess the rate of VTE in double heterozygotes for FVL and PT during pregnancy. *Methods.* The source population were relatives of probands with VTE, who remained pregnant at least once before diagnosis of thrombophilia. Fifty-two double heterozygotes were compared to 104 single heterozygotes for FVL and 104 for PT. The rate of VTE during pregnancy and puerperium was recorded. *Results.* Double heterozygotes had 135 pregnancies, FVL 208 and PT 216. Double heterozygotes had a median age (range) at blood sampling of 50y (22-78), at first pregnancy of 24y (12-36); the median number (range) of pregnancies was 3 (1-8) with a rate of pregnancy loss of 20%. Single heterozygotes had similar characteristics. No VTE occurred in pregnancy. VTE was observed in 2% of puerperia (4% of women) in double heterozygotes, 3% (7%) in single FVL and 4% (9%) in single PT (p=ns). No double heterozygote, 43% of single FVL and 78% of single PT were primiparous at the time of thrombosis. *Conclusions.* The risk of first VTE during pregnancy and puerperium in double heterozygotes of FVL and PT is not different from that of single heterozygotes. As for single heterozygotes, antithrombotic prophylaxis during pregnancy does not appear to be justified in double heterozygotes.

0342

INTRACARDIAC THROMBOSIS IN CHILDREN

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Background. Intracardiac thrombosis (ICT) in children is rare. It usually associates with congenital heart disorders (CHD), central venous catheters (CVC), surgery, and infections. *Aims.*We aimed to investigate clinical and laboratory findings, risk factors, and treatment of children with ICT. *Methods.*Clinical and laboratory characteristics of 35 children with ICT who followed in our hospital from January 1997 to May 2006 were retrospectively investigated from their files. *Results.* During study period, a total of 122 patients diagnosed with thrombosis. Among them, 35 (28,7%; mean age 5.4) had intracardiac thrombosis. Most of the patients (n=23) were above 1 year of age (p=0,04, OR=4,8). 31 patients had ICT on one anatomic site and 4 patients on more than one site. Twelve patients also had extracardiac site thrombosis. Congenital heart disease (CHD) was the most frequent associated factor (19 patients;10 cyanotic and 9 acyanotic; p=0.008, OR=8.8). Acquired risk factors were present in 30 patients; CVC in 21 patients (p=0.023, OR=5.4); infection in 16 patients (p=0.042, OR=3.1), corrective surgery in 10 patients (p=0.027, OR=5.8) and cardiac catheterization in 8 patients (p=0.022, OR=6.8). When we investigated the presence of some thrombophilic mutations [Factor V Leiden (FVL), methylene tetrahydrofolate reductase 677C-T, and prothrombin 2021 G-A], 13 patients had one and 2 patients had 2 mutations. Among them, 8 patients had FVL mutation (25%) and this percentage was higher than those obtained in the normal Turkish population (7-9%). 16 patients were treated with thrombolitic agents (11 with t-PA, 5 with streptokinase) and 7 with surgical embolectomy. *Conclusions.* In our study, we found close relation between ICT and congenital (FVL) and acquired risk factors. We suggest close monitoring and effective anticoagulation in these patients in order to prevent ICT.

0343

COMPARISON OF HEMOSIL HOMOCYSTEINE ASSAY WITH ABBOTT IMX IMMUNOASSAY FOR MEASUREMENT OF PLASMA TOTAL HOMOCYSTEINE

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Background. The demonstration of an association of hyper homocysteinemia with heightened risk of thrombosis lead to increasing demand for measurement of plasma total homocysteine (tHcy) in clinical laboratories. Since patients at risk of thrombosis are often screened for hemostasis abnormalities that are associated with thrombophilic states, it would be very useful to measure tHcy using the same blood sample and the same instrument that are used for hemostasis screening. *Aims.* The aim of our study was to compare the performance of HemosIL™ Homocysteine assay of tHcy on the ACL 9000 coagulometer (Instrumentation Laboratory) to that of an established immunoassay for plasma tHcy measurement (Abbott IMx Immunoassay). *Methods.* Blood samples from 64 fasting patients with previous venous and/or arterial thrombosis and 12 fasting healthy controls were collected in 10.9 mM sodium citrate (HemosIL™ Homocysteine), and in EDTA (Abbott IMx immunoassay). All samples were immediately placed on ice and centrifuged at 2200xg at 4°C for 20 min; the supernatant plasma was stored at -80° C until assay. Plasma samples were reduced with dithiothreitol and incubated with S-adenosyl homocysteine (SAH) hydrolase in the presence of excess adenosine, to convert Hcy into SAH. In both assays, sample and SAH tracer interact with a specific monoclonal antibody. Detection of SAH is based on fluorescence polarization immunoassay (Abbott IMx Immunoassay) or agglutination of SAH/latex particles (HemosIL™ Homocysteine): the concentration of tHcy in plasma samples is inversely related to the intensity of polarized light or the intensity of light transmission. *Results.* The inter-run CV of HemosIL™ Homocysteine was 3.83 for a 18.8 μM tHcy sample and 3.95 for a 9.9 μM tHcy sample (n=10). The concentration of tHcy in tested samples ranged between 4.8 and 60 μM. The correlation between measurements obtained by Abbott IMx Immunoassay and HemosIL™ Homocysteine was very good (r=0.9915). Values of plasma tHcy tended to be slightly lower when

tested with HemosIL™ Homocysteine (y=0.8292x+0.3503). Since blood samples for HemosIL™ Homocysteine assay are collected in 1/10 (v/v) sodium citrate, the observed discrepancy could be due to dilution of blood sample by citrate. After correction for dilution factor, the regression line between the two measurements was almost superimposable to the identity line (y=0.9711x + 0.4135). *Summary and Conclusions.* The HemosIL™ Homocysteine assay for tHcy on ACL 9000 compares well with the established Abbott IMx immunoassay and can be used for routine measurement of plasma tHcy, especially in subjects who undergo screening for abnormalities of hemostasis that are associated with thrombophilic states. As expected, a proportional bias, due to different plasma dilutions between the EDTA and citrated samples (pendent 0.8292), was corrected applying the appropriate volume correction factor (pendent 0.9708).

0344

ROLE OF THE PAI-1 GENE 4G/5G POLYMORPHISM ON PAI-1 LEVEL AND RELATIONS AMONG PAI-1, TNFα AND TGFβ LEVELS IN OBESE CHILDREN

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Objective. Obesity is a metabolic disorder that is associated with changes of plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNFα) and transforming growth factor beta (TGFβ) levels. These changes are related to insulin resistance, dyslipidemia and cardiovascular disease. However the precise effect of the PAI-1 gene 4G allele is still contradictory. *Aims.* In this study we aimed to elucidate the role of the 4G/5G polymorphism of PAI-1 gene on PAI-1 level and determine the associations between cytokines α (TNFα and TGFβ), glucose and lipid metabolism parameters in Turkish obese children. *Methods.* Thirty nine obese children [12 male and 27 female; mean age of 11.4±3.3 (5.9-17.4) years] and 38 age-matched healthy control group [(19 male and 19 female; mean age of 10.3±3,5 (4.7-17.0) years] were included in the study. In all cases, serum levels of glucose, lipid and insulin were measured, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated, and 4G/5G polymorphism of PAI-1 gene, plasma PAI-1 level and serum TNFα and TGFβ levels were studied. *Results.* The mean of relative body mass index (BMI) and HOMA-IR score VLDL, TG, insulin, PAI-1, TNFα levels were higher and HDL and TGFβ levels were lower in obese group. The frequency of the PAI-1 gene 4G/4G genotype was considerably higher in obese children than in controls. There was a significant interaction between groups and genotypes with regard to PAI-1 levels in obese children (p<0.001). However, PAI-1 genotype had no effect on PAI-1 level in healthy-lean children. Also, a positive correlation was found between PAI-1 and TNFα levels, and relative BMI, HOMA-IR score, insulin, TG, HDL levels. TGFβ was inversely correlated only with relative BMI. There was no correlation among three cytokines. *Conclusions.* From these results, we hypothesized that genetic background might play an important role having adiposity. Besides genetic background, cytokine disturbances such as elevated PAI-1 and TNFα originated from increased adipose tissue may be associated with metabolic disorders such as insulin resistance and dyslipidemia. Since these are not true for lean children, we conclude obesity should be treated as early as possible.

0345

THROMBOSIS IN CHILDREN WITH CARDIAC PATHOLOGY: ANALYSIS OF ACQUIRED AND INHERITED RISK FACTORS

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Background. Thrombosis is one of the most frequent secondary complications seen in children treated for major primary illnesses. Several cardiac disorders are associated with thrombosis in children, including congenital heart diseases (CHD), cardiomyopathy (CMP), intracardiac defects, cardiac procedures, and acquired heart disease. *Aims.* In this retrospective study, we aimed to investigate the association between thrombosis and genetic and acquired risk factors in children with CHD or CMP during January 1997-May 2006. *Methods.* Clinical and laboratory characteristics of children with cardiac pathology (CHD or CMP) and thrombosis were retrospectively investigated from their files. *Results.* There were 58 children who had CHD (n=54) or CMP (n=5) and thrombosis (mean age 3.5 years). During study period, thrombosis incidence in patients with cardiac pathology (CHD or CMP) was calculated at 205/10,000 whereas the incidence of thrombosis in all hospitalized patients aged 0-18 years was 88.6/10,000. Thirty-two patients had cyanotic CHD, 22 patients had acyanotic CHD. Thirty-one (52.5%) children had venous system, twenty-one (35.6%) children had arterial system thrombosis, and 7 (11.9%) children had both. Eighty-one major anatomic thrombosis sites were detected in the enrolled study. Fourty children (67.8%) had one and 22 children had more than one anatomic site involved in thrombosis. The presence of CHD was significant risk factor for the development of intracardiac thrombosis (p=0.022, OR=6.3), and central nervous system thrombosis (p=0.020, OR=8.9). The presence of pulmonary stenosis (p=0.047, OR=8.3) and aort coarctation (p=0.004, OR=11) were significant risk factors for the development of peripheral arterial system thrombosis. Acquired risk factors were identified in 49 of the 59 patients (p=0.000, OR=20), including major surgery (p=0.000, OR=13 for all surgical interventions; p=0.045, OR=3.4 for corrective surgery of Tetralogy of Fallot; p=0.007, OR=6.7 for Blalock Taussig shunt operation), systemic infection (p=0.04, OR=1.9), cardiac catheterization (p=0.000, OR=60), central venous catheter, and hypoxia. Many patients had more than one of these acquired risk factor (n=33, p=0.000, OR=4.7). Also, polycytemia was identified significant risk factor for thrombosis in patients with Tetralogy of Fallot (p=0.001, OR=21.8). One patient had protein C and another had combined PC and PS deficiency. Fifty-two of 59 patients were analyzed for 3 thrombophilic mutations; Factor V Leiden, methylene tetrahydrofolate reductase 677C-T, and prothrombin 20210G-A. Twenty-three of these patients had at least one of these thrombophilic mutations. *Conclusions.* In summary, the data presented here underline the multifactorial cause of symptomatic thrombotic events in children with cardiac pathology. Further multicenter studies are needed to clarify in an evidence-based model the unanswered questions, eg, rate of recurrence, thrombosis locations, involvement of prothrombotic risk factors, underlying clinical conditions, and the preventive use of different treatment modalities.

0346

HEMOSTATIC FACTORS AND MORTALITY IN SEVERE SEPSIS

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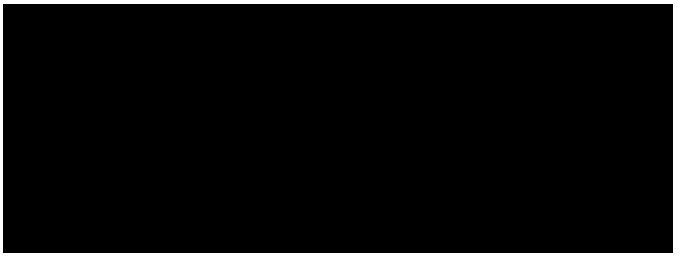
Background. Sepsis often is complicated by disseminated intravascular coagulation, related to severity of the disease. The aim of this study is to evaluate the dynamics and magnitude of hypercoagulability and fibrinolysis plasma markers in severe sepsis and its relation to global mortality. *Methods.* 2619 patients in 14 intensive care units were studied at admission for severe sepsis. 324 episodes in 311 septic patients were included during a 6 months period, according to definition of ACCP Consensus Conference 1992. The coagulation state was assessed by measurements of thrombin-antithrombin complexes (TAT) and Protein C (PC). The fibrinolytic system was assessed by the measurement of plasmin-antiplasmin complexes (PAP) and plasminogen-activator inhibitor type 1 (PAI-1). All the assays were performed using an enzyme linked immuno-assay method (ELISA). Samples were collected at diagnosis, and on 3rd and 7th days since the first episode of severe sepsis in a sample of 145 patients. A control group of 100 normal plasma samples was used. Statistics were analyzed using the non-parametric Mann-Whitney test. *Results.* There was an increased procoagulant response, with high values of TAT and an increased fibrinolytic response measured as PAP related to controls (p<0,001). We also found at diagnosis very low levels of anticoagulant Protein C and very high plasma concentration of fibrinolytic inhibitor PAI-1 (p<0,001). The severity of the disseminated intravascular coagulation decreased during 3rd and 7th days after treatment. Non survivors had higher values of PAI-1 (p=0,018) and lower levels of protein C (p=0,024) related to survivors. (Table 1).

Table 1. Median values at diagnosis.



Conclusions. This study demonstrates a marked prothrombotic status in severe sepsis, with hypercoagulability and impaired fibrinolytic response. The prognosis in severe sepsis is related with the magnitude of PAI-1 and Protein C at diagnosis.

0347

THE ANGIOTENSIN-CONVERTING ENZYME INSERTION/DELETION POLYMORPHISM AND SERUM LEVELS OF ANGIOTENSIN-CONVERTING ENZYME IN VENOUS THROMBOEMBOLISM: DATA FROM A CASE-CONTROL STUDY

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Background. The angiotensin-converting enzyme (ACE) has been suggested to affect blood coagulation and fibrinolysis. Results from literature on the role of the frequent insertion/deletion (I/D) polymorphism in the ACE gene in venous thromboembolism (VTE) are controversial. Only limited data on ACE serum levels in VTE exist. *Aims.* The purpose of our present study was to investigate the association of the ACE I/D polymorphism and of ACE serum levels in a population of selected patients with VTE. *Methods.* We determined the ACE I/D polymorphism by genotyping and ACE serum levels by an enzymatic assay in 100 high risk patients (45 female/55 male; mean age ±SD: 55 ±12 years) with objectively confirmed recurrent VTE and at least one event of an unprovoked deep venous thrombosis or pulmonary embolism. One-hundred-twenty-five age- and sex-matched healthy individuals (64 female/ 61 male; mean age ±SD: 53 ±11 years) served as controls. *Results.* ACE genotype frequencies were not significantly different between patients (DD: 26.0%, ID: 52.0%, II: 22.0%) and controls (DD: 29.6%, ID: 44.8%, II: 25.6%; p=0.56). Neither individuals with ACE DD genotype nor those with ACE ID genotype had a higher risk for VTE in comparison to those with ACE II genotype (odds ratio and [95% confidence interval]: 1.0 [0.5-2.1] and 1.4 [0.7-2.6], respectively). Serum ACE levels (U/l) did not differ between patients (median [25th -75th percentile]: 25.25 [20.20-33.70]) and controls (24.20 [17.85-34.50], p=0.49). In the total population involved in the study the ACE DD genotype (n=63: 36.00 [26.40-43.00]) was associated with higher ACE levels than the ACE ID genotype (n=108: 24.10 [19.80-31.48], p<0.001) and the ACE II genotype (n=54: 19.35 [15.00-22.95], p<0.001). *Summary and Conclusions.* We found a significant association of the ACE I/D polymorphism with ACE serum levels. However, neither the serum levels nor the I/D genotype were associated with VTE.

0348

NON ADHERENCE TO DOSING GUIDELINES FOR LMWH IN RENAL IMPAIRMENT LEADS TO A HIGH INCIDENCE OF SIGNIFICANT BLEEDING EVENTS - RESULTS OF A PROSPECTIVE AUDIT

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Background. Recently Guidelines have been developed for dose reduction in LMWHs in the treatment of patients with renal impairment. Following a number of significant bleeding events related to LMWH (Enoxaparin) usage in Medical admissions to this hospital we prospectively audited Enoxaparin usage in patients with renal impairment and the incidence of adverse events over a three month period. All patients included were receiving therapeutic doses of Enoxaparin for the treatment of thromboembolic events or ischaemic heart disease. *Aims.* To determine adherence or otherwise to LMWH prescribing guidelines in patients with renal impairment. *Methods*. Estimated GFR (eGFR) was calculated using the 4 variable MDRD formula. 20 patients with an eGFR <30 mL/min/1.73m^2 who were commenced on a therapeutic dose of Enoxaparin over the three month period were included. Patients were assessed as to whether they were weighed prior to commencing treatment and if Enoxaparin dose was reduced appropriately as per established guidelines. The number of adverse bleeding events was recorded. Antifactor Xa levels were measured on each patient. Concurrent anitplatelet drug usage was also recorded. *Results.* The mean age of the 20 patients included (9 male, 11 female) was 75. 15/20 (75%) patients were incorrectly prescribed a higher initial dose than appropriate based on their weight and renal function. Of these patients 5/15 (33%) developed significant GI haemorrhage. 1 patient developed a haematoma at the injection site. Of the 6 patients who developed bleeding complications 5 had therapeutic Antifactor Xa levels and only one had an elevated level > 1u/mL. 11/20 (55%) patients were commenced on initial treatment without a recorded weight. *Conclusions*. Failure to record weight and to appreciate renal impairment can lead to excessively high dosing of LMWHs in patients admitted to general medical and cardiology wards. Renal function frequently changes over the duration of medical admissions and the dose of LMWHs should be reviewed daily and altered accordingly in order to avoid serious complications. Routine measurement of Antifactor Xa does not identify patients at risk of bleeding and should not be routinely measured.

0349

PREGNANCY OUTCOME IN WOMEN WITH THE LUPUS ANTICOAGULANT AND A HISTORY OF THROMBOEMBOLISM

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Background. Scarce data are available on the pregnancy outcome in patients with the lupus anticoagulant (LA) and a history of TE. The aim of this study was to evaluate the outcome of pregnancy in a cohort of women with persistent LA and previous TE. *Aims.* To evaluate the pregnancy outcome in women with the lupus anticoagulant and a history of thromboembolism (TE). *Methods*.This is a report from an ongoing observational study in which patients with LA are prospectively followed. Criteria for inclusion into the present evaluation was persistent LA before onset of pregnancy and previous TE. *Results*. Nine women (age m=32 years) had 12 pregnancies, five of these had a history of pregnancy complications (3 abortion, 1 stillbirth and 1 HELLP). Patients received a combination of low molecular weight heparin (LMWH, median 71 U/kg/day, range 41-114 antiXa U) and aspirin 100 mg) during 8 pregnancies, only LMWH (82 and 75 U/kg/day) and danaparoid (26 antiXa U) and aspirin during 2 pregnancies, respectively. Pregnancy outcome was favourable with a viable infant in 10 pregnancies born at a median of 34 weeks of gestation (range 28-40), 7/10 pregnancies ended prematurely. There was 1 abortion (6th week), 1 preeclampsia and stillbirth (27th week) and 1 HELLP-syndrome (34th week). The mean weight of viable infants was 2665g (range 700-3950 g). In one woman a post-partum pulmonary embolism occurred. *Conclusions*. The outcome of pregnancy was favourable in most pregnancies, the risk for pregnancy-associated recurrent TE events seems to be low with the applied treatment regimen. However, despite anticoagulant treatment pregnancy ended prematurely in most patients. Women should be closely followed for signs of fetal growth retardation, preeclampsia and HELLP-syndrome.

Table 1. Table of positive JAK2-V617F DNA samples.

0350

JAK2-V617F MUTATION IS PRESENT IN PATIENTS WITH IDIOPATHIC DVT

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Background. In 30% of patients with venous thrombo-embolism (VTE), no environmental or genetic risk factors can be identified. A mutation in the JAK2 gene is present in 60-97% of patients with polycythemia vera (PV) and is considered the cause for the disease phenotype. The muta-

tion is probably absent in the healthy population. *Aims.* Since PV-patients are at increased risk for VTE, we set out to investigate whether the JAK2-V617F mutation constitutes a risk factor for VTE in non-PV patients. *Methods.* The presence of the JAK2-V617F mutation was retrospectively assessed in 188 consecutive patients with objectively diagnosed deep venous thrombosis (DVT), using a quantitative ASO Taqman PCR. Clinical parameters of mutation-positive patients at the time of DVT were analyzed. *Results.* Of the 188 samples, 180 (96%) were evaluable. A positive signal was observed in 6 VTE patients (3.3%). Quantitative analysis revealed that between 0.12 and 1.0% of cells were JAK2-V617F positive. Sensitive measurement of samples from age-matched healthy blood bank donors is currently being performed and will be presented at the meeting. *Conclusions.* We found the JAK2-V617F mutation in 3.3% (95%CI 1.5-7.0) of DVT patients, who did not fulfill diagnostic PV criteria. It is possible that in these patients DVT is a first symptom of a myeloproliferative syndrome. Investigation in an appropriate control group will determine the strength of this relationship.

0351

A PROPOSAL OF SCORING SYSTEM IN ASSESSMENT OF THROMBOTIC RISK IN CANCER PATIENT. A MONOCENTRIC STUDY

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Background. Deep venous thrombosis (DVT) is present in about 3-15% of cancer patient. Paraneoplastic thrombosis pathogenesis is multifactorial. Antithrombotic prophylaxis frequently is not oriented to real thrombotic or haemorragic 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 recognize 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 92 patients with solid neoplasm (52 colon, 20 lung, 10 gastric, 10 others) and without anticoagulant prophilaxis. Of these only 76 were evaluable because in these complete data were available. Median age was 67.5 years (R 57-83). M/F ratio was 55/37. The threshold value of third quartile was chosen as risk cut-off (C3: 130 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), realtive risk (RR). *Results.* 15 patients (16%) showed DVT. Of these 12(80%) had a score inferior/equal to 3 and 3 superior/equal to 4. 77 patients (84%) did not show DVT. Of these 35 (46%) had a score superior/equal to 4 and 26 inferior/equal to 3. Yates corrected chi square test is 5.1 (p 0.02), with an OR of 5.4 (95%CI 1.4-21) and a RR of 4 (95%CI 1.2-13). Negative predictive value is 0.57 (95%CI 0.52-0.60) and positive predictive value is 0.80 (95%CI 57-93). Sensitivity was 0.32 (95%CI 0.23-0.37) and specificity was 0.92 (95%CI 0.83-0.97). *Summary and conclusions*. Neoplastic patient frequently shows haemorragic risk (eg in chemotherapy induced thrombocytopenia). Therefore antithrombotic prophylaxis should be used considering the effective thrombotic and haemorragic risk of neoplastic patient. Our scoring system is useful in distinguishing cancer patient with low thrombotic risk and consent to avoid antithrombotic prophylaxis in patient with higher bleeding and lower thombotic risk. Nevertheless these data need confirmation on a larger cohort of patients.

0352

PLASMA THROMBOMODULIN: A POTENTIAL MARKER FOR PRE-ECLAMPSIA

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Background. Thrombomodulin (TM) is an endothelial cell membrane glycoprotein which functions as a thrombin receptor. The thrombin-thrombomodulin complex initiates the protein C anticoagulant pathway. It activates protein C rapidly which together with protein S can inactivate factor Va and factor VIIIa. Pre-eclampsia (P-Ec) is a complex multisystem disorder characterized by hypertension, proteinuria and edema. It occurs after the 20th week of pregnancy. P-Ec has no cure, except by pregnancy interruption. In more severe cases, conditions such as eclampsia and HELLP syndrome (hemolysis, elevated liver enzyme, low platelets) or DIC (disseminated intravascular coagulation) may develop. *Aims.* To quantify plasma TM levels in pre-eclamptic women and compare these to the plasma TM levels in non-pregnant women and healthy pregnant women. *Methods.* Plasma TM levels were measured using an enzyme-linked immunosorbent assay (ELISA). A total of 57 subjects were studied. These include non-pregnant women (n=22), healthy pregnant women (n=15), and pre-eclamptic women (n=20), at the third trimester. *Results.* The mean and standard deviation (mean±SD) for the three groups were: non pregnant women (0.609±0.311), healthy pregnant women (0.692±0.267) and pre-eclamptic women (0.917±0.324). Plasma TM levels showed a statistically significant difference when women with pre-eclampsia were compared to the non-pregnant women group ($p<0.05$). However, we observed no significant difference when the pre-eclamptic women group was compared with the pregnant women group. *Conclusions.* Plasma TM levels are significantly elevated in women with pre-eclampsia. Endothelial cell injury and/or inflammatory reaction could have resulted in the increased plasma TM levels seen in our study. This finding may have a significant clinical ramification in the management of such patients. Detailed studies are required to address such an important relationship further.

0353

TISSUE FACTOR AND TOTAL TISSUE FACTOR PATHWAY INHIBITOR IN CORONARY ARTERY DISEASE: CORRELATION WITH THE SEVERITY OF ATHEROMATOSIS

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Background. Atherosclerosis and its subsequent thrombotic complications are a major cause of morbidity and mortality in the western world. Tissue factor (TF) may contribute to the atherothrombosis process. *in vivo* TF-procoagulant activity is restrained by its major physiological inhibitor commonly known as tissue factor pathway inhibitor (TFPI). *Aims.* To determine plasma TF and total TFPI levels in subjects undergoing coronary angiography and to assess the relationship between these and the severity of coronary artery disease (CAD). Materials and *Methods.* Using an enzyme-linked immunosorbent assay (ELISA) assays, plasma TF and total TFPI levels were measured in subjects with normal coronary arteries (n=20; controls), mild/moderate atheromatosis (n=18) and severe atheromatosis (n=30). In addition, plasma lipoprotein(a) and D-Dimer (D-D) were analysed in all samples using ELFA and turbidimetric assays, respectively. The extent of CAD was assessed using coronary angiography. *Results.* The severe atheromatosis group showed significantly high TF levels compared to controls ($p<0.01$). Increased TF levels were associated with coronary stenosis of more than 70% of the luminal diameter. Plasma lipoprotein(a) levels were also significantly increased in subjects with severe atheromatosis compared to those with mild/moderate atheromatosis ($p<0.001$) or controls ($p<0.0001$). The difference in lipoprotein(a) levels between controls and mild/moderate atheromatosis group was also significant ($p<0.0001$). However, no such a significant difference was observed for TFPI and D-D. The presence of CAD was associated with increased TF (r=0.42, $p<0.0001$) and raised lipoprotein(a) levels (r=0.63, $p<0.0001$). For all groups there was a positive and significant association between TF and TFPI (r=0.34; $p<0.01$), TF and D-D (r=0.28, $p<0.05$) and TF and lipoprotein(a) (r=0.33, $p<0.01$). *Conclusions.* Increased plasma TF levels but not plasma TFPI levels in subjects with coronary artery disease are associated with the severity of atheromatosis. We observed an association between increased plasma TF levels and coronary stenosis of more than 70% of the luminal diameter. The advanced atherosclerotic injuries, the fatty core of the disrupted plaque and the increase in the macrophages and smooth muscle cells may have resulted in the observed rise in plasma TF levels.

0354

FACTOR V LEIDEN HOMOZYGOUS GENOTYPE IS ASSOCIATED WITH LATE PREGNANCY LOSS IN THE PROCARE-GEHT COHORT

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Background. A role for inherited maternal thrombophilia in the occurrence of pregnancy loss has been suggested by prevalence studies. However, limited data are available about the impact of the Factor V Leiden (FVL) homozygous genotype on early and late foetal losses. *Objectives.* We performed a retrospective multicentre study to evaluate the relationship between FVL homozygous genotype and foetal loss. *Aims.* The obstetrical history of 133 women homozygous for the FVL, included in the PROCARE-GEHT cohort, and who had initiated at least one pregnancy was carefully recorded. The frequencies of early (first trimester) and late (second/third trimester) foetal losses were analysed. All data obtained were compared with those obtained in 256 women heterozygous for the FVL recruited in Marseille centre and who also had been pregnant at least once. *Results.* 306 pregnancies were initiated in the 133 FVL homozygous women vs. 686 in the heterozygous patients. One late foetal loss occurred more frequently in homozygous FVL women than in heterozygotes (17/306 (6%) vs. 6/686 (1%); OR=6.7, 95% CI 2.6-17.1; $p<0.001$). On the other hand, the rate of early foetal loss i.e. occurring in the first trimester of pregnancy, was similar in both groups of women (38/306 (12%) vs. 73/686 (11%); OR=1.2, 95% CI 0.78-1.80; $p=0.41$). *Summary and conclusions.* The observed rates of foetal losses were similar with those previously reported1. This study supports that the homozygous status for the FVL increases the risk of foetal loss in the second-third trimesters of pregnancy. Therefore, further studies are mandatory to evaluate whether a specific monitoring of these women is useful together with anticoagulant prophylaxis.

Reference

1 De Stefano V et al. Inherited thrombophilia and obstetric complications. Haematologica 2005;1:18-21.

0355

SPONTANEOUS DEEP VEIN THROMBOSIS OF THE UPPER EXTREMITIES: RISK FACTORS AND LONG TERM FOLLOW UP

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Background. Risk factors of lower limb deep vein thrombosis (DVT) have been well established in large clinical studies. *Aims.* (1) To investigate whether these factors also confer an increased risk of upper limb DVT. (2) To evaluate the risk of recurrence of upper limb DVT. *Methods.* We followed 52 patients with upper limb DVT and 875 patients with lower limb DVT after discontinuation of secondary thromboprophylaxis. Exclusion criteria were previous or secondary venous thromboembolism, isolated pulmonary embolism, need for long term antithrombotic therapy, cancer, pregnancy, lupus anticoagulant and antithrombin, protein C, or protein S deficiencies. Symptomatic recurrent DVT was prospectively recorded over a mean follow-up of 44 months. *Results.* Patients with upper limb DVT were younger (38 vs. 49 years, $p<0.001$) and had a lower BMI (24 vs. 27, $p<0.001$) than those with lower limb DVT. Pulmonary embolism occurred in only 10% of patients with an upper limb DVT (lower limb DVT: 34%; $p<0.001$). No difference was found regarding sex distribution (male: 45% vs. 48%, $p=0.7$). The prevalence of Factor V Leiden was lower among patients with upper limb DVT than among those with lower limb DVT (12% vs. 30%, $p=0.005$). In addition, patients with upper limb DVT had lower levels of factor VIII (143 vs. 167 IU/mL, $p<0.001$) and factor IX (109 vs. 117 IU/mL, $p=0.015$). Low D-dimer (<250 ng/mL) was more frequent among patients with upper limb DVT than among those with lower limb DVT (60% vs. 32%, $p=0.001$). After 5 years, the probability of recurrence was 2.0% (95% CI 0%-6.0%) among patients with upper limb DVT compared with 19.6% (95% CI 16.4%-23.0%) among patients with lower limb DVT ($p<0.001$). *Conclusions.* Prevalence of thrombophilia among patients with a first upper limb DVT is low. The risk of recurrence among these patients is very low.

SIMULTANEOUS SESSIONS

Chronic myeloid leukemia - Clinical I

0356

EFFICACY OF DASATINIB IN CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA PATIENTS AFTER IMATINIB FAILURE ACCORDING TO BASELINE BCR-ABL MUTATIONS

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Background. Dasatinib is an oral, multi-targeted BCR-ABL and SRC kinase inhibitor with preclinical activity against 20/21 imatinib resistant BCR-ABL mutations. Clinical efficacy was demonstrated in phase I, II, and III studies in patients (pts) with chronic myelogenous leukemia (CML) in all phases of the disease and with BCR-ABL positive acute lymphoblastic leukemia (ALL). *Aims.* We sought to establish a relationship between the type of BCR-ABL mutations associated with imatinib resistance and efficacy of dasatinib in chronic phase (CP) CML pts. *Methods.* Between 10/03 and 03/06, dasatinib was commenced in 1,093 CP-CML pts recruited for three consecutive trials and administered for a median period of 8.7 months (range <1-25.9). BCR-ABL mRNA was screened for mutations of amino acids 207-517 by D-HPLC and regular sequencing and data are available from 961 cases (88%). ABL polymorphisms K247R and E499E were excluded from analysis. *Results.* Prior to dasatinib, 75 different BCR-ABL mutations involving 56 amino acids were detected in 18/240 imatinib intolerant (7.5%) and 324/721 (45%) imatinib resistant pts. 267 pts showed one, 53 pts two, 16 pts three, and six pts four mutations. In imatinib resistant pts, response was not different between 370 pts with and 351 pts without baseline mutations: Complete hematologic response (CHR) was achieved in 89% vs 92%; major cytogenetic response (MCR) in 48% vs 52% being complete (CCR) in 38 vs 36%, respectively. Response dynamics was associated with preclinical activity of dasatinib: Classifying mutations for IC_{50} values <2, 2-20 and >1,000nM (T315I), CHR was achieved in 93, 85 and 28%; MCR in 48, 42 and 0%; and CCR in 37, 35 and 0% of cases, respectively. During follow up, new mutations were detected in 30 cases, predominantly T315I (n=10), Y253H/F (n=4), and F317L (n=3). *Conclusions.* We conclude that dasatinib is capable of inducing hematologic and cytogenetic remissions in a significant proportion of imatinib resistant pts associated with BCR-ABL mutations, except T315I, but also in pts with BCR-ABL independent causes of resistance. Quality of response depends on the individual type of mutation which is consistent with preclinical observations.

0357

CYTOGENETIC RESPONSE AND DISCONTINUATIONS FROM IRIS STUDY WITH LONG-TERM IMATINIB THERAPY IN NEWLY DIAGNOSED PH+ CHRONIC MYELOGENOUS LEUKEMIA

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Background. The 5-year follow-up analysis of the International Randomized Interferon versus STI571 (IRIS) study was recently published. *Aims.* Here we focus on response rates for patients (pts) in long-term follow-up and provide details regarding those who discontinued prior to 31 Jan 2006 (analysis data cutoff). *Results.* Survival: In the IRIS study, routine follow-up data were collected for all randomized pts, including overall survival. For those who discontinued study treatment, quarterly assessments included whether pts were alive, deceased, lost to follow-up, or had received transplant. By the median 5-year cutoff (range: 5-5.5 years), after the last patient had been recruited into the study, 57 (10%) of the 553 pts initially randomized to imatinib (IM) were documented to have died. Of the remaining 496 pts, 333 (67%) had follow-up data of greater than 5 years, 126 (25%) pts had follow-up data between 4.5 and 5 years, 11 (2%) pts had follow-up data between 4 and 4.5 years, and only 26 (5%) pts were prematurely lost to follow-up with available survival data of less than 4 years. Using the Kaplan-Meier method, in which available data for each pt are considered, and survival is censored

at the last follow-up when pts are known to be alive, the probability of overall survival at 5 years was 89%. *Response.* Of all 553 pts, 454 (82%) achieved a complete cytogenetic response (CCyR) during IM study treatment. Of these CCyR pts, 86 discontinued IM treatment for reasons indicated in Table 1. Of the remaining 368 pts, 11 had a documented loss of CCyR while on study, but were still in MCyR at last follow-up. *Discontinuations*. At data cutoff, 382 (69%) of 553 pts remained on IM study; a total of 171 (31%) of the 553 pts had discontinued. Ten pts died for reasons other than CML while on IM treatment. Of the remaining 161 pts who discontinued IM on the IRIS protocol, 47 had died by the current follow-up (Table 1). Routine follow-up of the 79 pts who had achieved a CCyR while on IM and then discontinued due to reasons other than death revealed that 19 pts subsequently died (4 after BMT, 7 due to CML, and 8 due to other causes). *Summary and conclusions.* More detailed data from the 5-year IRIS follow-up will be presented including long-term MCyR and CCyR rates.

Table 1. Discontinuations from IM in the IRIS study (5-year analysis)

Reason	All pts (N=553) n (%)	CCyR during treatment, n	Deaths after discontinuation, n
Unsatisfactory therapeutic effect	59 (10.7)	31	20
Adverse event	23 (4.2)	15	10
Death	10 (1.8)	7	-
BMT	16 (2.9)	4	6
Withdrew consent	25 (4.5)	15	3
Lost to follow-up	5 (0.9)	3	1
Protocol violation	15 (2.7)	5	1
Other reasons	4 (0.7)	3	1
Discontinuations from first-line IM	157 (28.4)	83	42
Crossed over to IFN-α + Ara-C	14 (2.6)	3	5
Total discontinuations	171 (31)	86	47

0358

DASATINIB INDUCES DURABLE CYTOGENETIC RESPONSES IN PATIENTS WITH CHRONIC-PHASE CML WITH RESISTANCE OR INTOLERANCE TO IMATINIB: UPDATED RESULTS OF THE CA180013 (START-C) TRIAL

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Background. Dasatinib is a potent inhibitor of BCR-ABL (325-times more potent than imatinib and 16-20-times more potent than nilotinib *in vitro*) and other tyrosine kinases, and is approved in both the EU and US for the treatment of all phases of chronic myelogenous leukemia (CML) with resistance to or intolerance of prior therapy, including imatinib. *Aims.* This study was designed to establish the efficacy and safety of dasatinib; the longer-term follow-up presented here allow for a more definitive assessment. *Methods.* In this Phase-II, open-label study, patients with CP-CML with resistance or intolerance to imatinib received treatment with dasatinib 70 mg BID. Dose escalation to 90 mg BID was allowed for patients with an inadequate response, and dose reduction to 50 mg or 40 mg BID for toxicity or intolerance. All patients provided written informed consent. *Results.* From February through July 2005, 387 patients (median age 58 years; 49% male) were enrolled and treated at 75 centers worldwide. Median time from CML diagnosis was 61 months (range 3-250). Prior therapy included: interferon-a in 65% of patients; stem-cell transplantation in 10%; 55% had received prior imatinib therapy at >600 mg/day; and 53% had received imatinib for durations in excess of 3 years. Best response prior to imatinib failure was CHR in 82%, and major (MCyR) and complete (CCyR) cytogenetic response in 37% and 19%, respectively. With median follow-up now extending to 15.2 months, CHR was seen in 91% of patients. MCyRs were attained by 59% of patients, these were CCyRs in 49%. Cytogenetic response rates were higher for patients with intolerance to imatinib (MCyR 80%, CCyR 75%) than for those with resistance (MCyR 52%, CCyR 40%). MCyRs were consistently reported across subgroups: imatinib duration (″3 years 65%, >3 years 55%), prior imatinib dose (″600 mg 63%, >600 mg 57%), prior CHR (yes 63%, no 59%), prior CyR (yes 71%, no 42%), BCR-ABL mutation (yes 57%, no 48%). MCyRs were durable, with only 7 of the 230 patients (3%) with a CyR progressing. The 15-month progression-free survival rate was 88%, where progression was defined as confirmed accelerated- or blast-phase disease, loss of CHR/MCyR, or increasing WBC count. Grade 3-4 neutropenia or thrombocytopenia were both reported in 48% of patients. Dose interruptions occurred for 87% of patients, and reductions for 73%, with a median daily dose of 101 mg (range 11-171 mg). Non-hematologic toxicity consisted mainly of grade 1-2 diarrhea, headache, fatigue, dyspnea, rash, and pleural effusion (with 6% grade 3-4 pleural effusion). *Summary and conclusions.* Longer'term data confirm the durability of cytogenetic response with dasatinib 70 mg BID. Consistent responses were observed across subgroups.

0359

DASATINIB DOSE AND SCHEDULE OPTIMIZATION IN CHRONIC-PHASE CML RESISTANT OR INTOLERANT TO IMATINIB: RESULTS FROM A RANDOMIZED PHASE-III TRIAL (CA180034)

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Background. Dasatinib, a potent inhibitor of BCR-ABL (325-times more potent than imatinib and 16-20-times more potent than nilotinib *in vitro*) and other tyrosine kinases, has been shown to be both safe and effective at doses of 70 mg BID in patients with chronic-phase CML resistant or intolerant to imatinib. Nonetheless, notable major cytogenetic responses (CyRs) were also observed in the phase-I program for patients with CP-CML at 100-mg and 140-mg total daily doses when administered as either a QD or BID schedule; this was despite the achievement of only transient inhibition of phospho-CRKL by dasatinib in patients receiving QD therapy. Long-term phase-I follow-up data also demonstrated that pleural effusions were less frequent with the QD schedule than with BID dosing. *Aims.* This study was designed to compare the CyR rates associated with QD and BID regimens of dasatinib. Secondary objectives included estimating differences in CyR between the 100-mg and 140-mg dose regimens and an ongoing evaluation of safety to optimize the dose and schedule for this drug. *Methods.* In this phase-III, randomized, prospective, open-label study, patients with imatinib-resistant or 'intolerant CP-CML were randomized to one of four dasatinib treatment groups: 100 mg QD, 50 mg BID, 140 mg QD, or 70 mg BID. Dose escalation to 180 mg QD or 90 mg BID and reduction to 80 mg QD or 40 mg BID were allowed for inadequate response or adverse events (AEs), respectively. All patients provided written informed consent.

Table 1.

Results. From July 2005 through March 2006, 662 patients (median age 55 years; 47% male) were randomized and received treatment. Median time from CML diagnosis to randomization was 54 months. With a median duration of treatment of 8 months, marked and similar hematologic and cytogenetic response rates were seen across all four treatment groups (Table 1). Duration of CyR and progression-free survival were also similar across all treatment groups. Toxicities identified as being of special interest were fewer for the 100-mg QD regimen (Table 1); pleural effusions (p=0.024) and thrombocytopenia (p=0.004) were both markedly reduced in the 100-mg QD group compared with the 70-mg BID arm. There were fewer dose interruptions and reductions and the lowest number of patients discontinuing treatment for drug-related toxicity in the 100-mg QD treatment group. *Summary and Conclusions.* Dasatinib 100 mg QD offers the most favorable overall benefit-risk assessment in this CP-CML patient population.

0360

DASATINIB IS SAFE AND EFFECTIVE IN PATIENTS WITH PREVIOUSLY UNTREATED CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE

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Background. Dasatinib is a multi-targeted kinase inhibitor of BCR-ABL and SRC. Based on its high level of activity in patients with imatinib-resistant or -intolerant CML-CP, the present phase-II trial was designed to assess the efficacy and safety of dasatinib in previously untreated CML-CP patients. The primary objective was to estimate the proportion of patients attaining major molecular response (BCR-ABL/ABL ratio <0.05% by qPCR) at 12 months. *Methods.* All patients received dasatinib orally at 100 mg/day, and were randomized to either a 50-mg-twice-daily or a 100-mg-once-daily schedule. Dose escalation to 140 mg/day and 180 mg/day for poor response or dose reduction to 80 mg/day and 40 mg/day for toxicity, maintaining the same schedule, was allowed. *Results.* Of the 34 patients enrolled between 11/05 and 2/07, 50% were female; median age was 41 years (range 18-76). At 3 months, complete hematologic response (CHR) and major cytogenetic response both occurred in 23 (88%) of 26 evaluable patients and complete cytogenetic response (CCyR) in 20 (77%) patients. After 6 months of therapy, 24/26 (92%) evaluable patients had achieved CCyR; this compares favorably with a CCyR at 6 months of 54% with imatinib 400 mg/day and 85% with imatinib 800 mg/day, for historical data of similar patients treated in studies at MD Anderson. At 12 months, 5/11 (45%) evaluable patients achieved a major molecular response in the present study (1 of which was complete). The most common non-hematologic adverse events (AE) included dyspnea (8 patients), fatigue (7), muscle pain (6), and headache (5) and were predominantly grade (gr) 1-2. Pleural effusion occurred in 3 patients (gr 1-2 in all). Grade 3-4 hematologic toxicity (transient) included anemia in 4 patients, neutropenia in 7 patients, and thrombocytopenia in 4 patients. With a median duration of therapy of 10 months, 13 patients required transient treatment interruption, 9 due to non-hematologic toxicities, 3 due to hematologic toxicities, and 1 as a result of both. The actual median daily dose for all pts is 100 mg. No difference in AEs has been observed between dose schedules. *Conclusions.* Rapid, complete cytogenetic responses to dasatinib 100 mg/day have been observed in a high percentage of patients with previously untreated CML-CP. Accrual to this trial continues.

Chronic lymphocytic leukemia and related disorders - Clinical

0361

MAINTENANCE IMMUNOTHERAPY WITH LOW-DOSE RITUXIMAB IMPROVES OUTCOME IN 'HIGH RISK' CHRONIC LYMPHOCYTIC LEUKEMIA

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Monoclonal antibodies combined with chemotherapy have allowed us to achieve both more complete responses and longer response duration in B-cell chronic lymphocytic leukemia (CLL), reducing disease burden to levels detectable only by immunophenotypic methods. Moreover, low-dose rituximab may decrease CD20 antigen loss via shaving and promote enhanced targeting in CLL (Williams ME et al, 2006). We performed a phase II study that added rituximab to fludarabine (Flu) as therapy for symptomatic, untreated CLL. Remission status was assessed by a multiparametric flow cytometric method based on the detection of CD19+CD5+CD79b- residual B-CLL lymphocytes. VH mutational status, CD38, ZAP-70 and cytogenetics were obtained in all patients before treatment. We defined as *high risk* patients having at least two of the following markers: unmutated IgVH, CD38 >30%, ZAP-70 >20%, unfavourable cytogenetics (trisomy 12 or del11q or del17p). Seventy-nine CLL patients, median age 60 years (range 37-74) received six monthly courses of Flu (25 mg/m^2 for 5 days) and four weekly doses of rituximab (375 mg/m^2) starting after completion of Flu therapy. According to modified Rai stages, 9 patients had a low stage, 67 an intermediate stage and 3 a high stage. Based on NCI criteria, 63/79 (80%) patients achieved a complete remission (CR), 12/79 (15%) a partial remission (PR) and 4/79 (5%) a stable disease (SD) or no response or progression. Three patients presented grade 3 (WHO) infective lung toxicity and 1 patient acute fatal B hepatitis. Hematologic toxicity included mainly neutropenia (grade 3 and/or 4 in 41 pts) and thrombocytopenia (grade 3 and/or 4 in 4 pts). Thirty-five patients in clinical CR or PR, either with CD5$^+$CD19$^+$CD79b$^-$ (MRD) bone marrow (BM) cells >1% (n=20 pts) or with MRD peripheral blood lymphocytes (PBL) >1000/mL (n=15 pts) within 1 year after completion of the induction treatment, underwent consolidation/maintenance therapy with four monthly cycles of rituximab at 375 mg/m^2 followed by twelve monthly low doses of rituximab at 150 mg/m^2. The median follow-up duration was 38 months. Noteworthy, all B-CLL pts experienced a long progression-free survival (PFS) from treatment (69% at 6 years). Nevertheless, CLL patients that underwent consolidation therapy (n=35) showed a significant longer duration of response in comparison with the subset of not consolidated and BM or PBL MRD positive (n=13) patients (85% vs 20% at 5 years, p=0.00001, Figure 1).

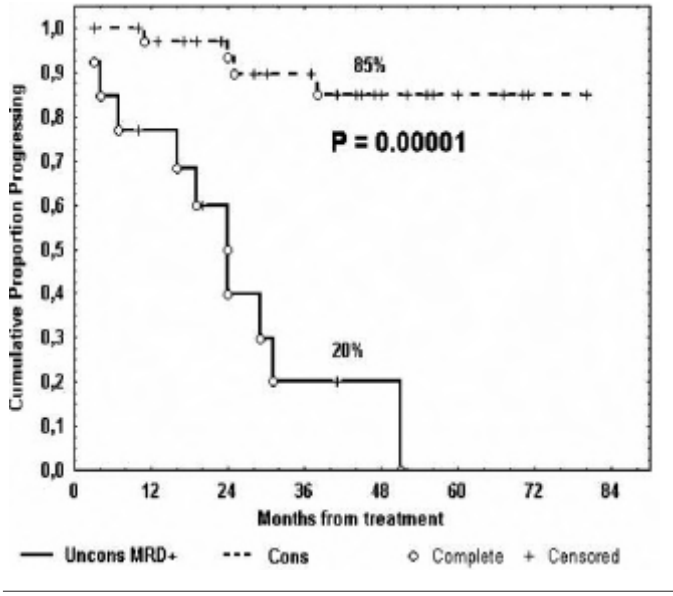

Figure 1. Duration of response in consolidated vs unconsolidated pts.

Of note, BM and PBL MRD negative patients (n=27) showed a duration of response similar to that of the consolidated pts. Moreover, a significant shorter PFS was observed within CD38+ pts (43% vs 79% at 5 years, p=0.005), unmutated pts (45% vs 94% at 2.5 years, p=0.001) and ZAP-70+ pts (39% vs 88% at 5 years; p=0.00004). Interestingly, within the *high risk* subset (n=30), the consolidated patients (n=11) showed a significant longer duration of response (64% vs 13% at 2 years, p=0.006) in comparison with MRD+ unconsolidated patients (n=9). Therefore, the addition of maintenance therapy with low dose rituximab prolongs significantly the duration of response also improving the outcome of *high risk* B-CLL pts subset.

0362

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION CURES CLL: A RETROSPECTIVE ANALYSIS FROM THE SFGM-TC REGISTRY

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This retrospective study concerned 471 B-CLL patients registered in the SFGM-TC registry from 1984 to 2005: 313 patients underwent autologous transplantation, 138 females (F) and 175 males (M) with a median age of 54 years, 236 received peripheral blood stem cell (PBSC) and 77 bone marrow (BM); 158 patients underwent allogeneic transplantation, 78 F and 80 M, median age = 49 years, 77 PBSC, 81 BM from 17 related and 141 unrelated donors. Before conditioning, 302 autoT and 143 alloT were evaluated for disease status: 100 and 26 patients were in CR, 170 and 55 in PR, 4 and 13 in stable disease, 28 and 49 in progressive disease for autoT and alloT respectively. Among alloT patients, 73 received reduced intensity conditioning and 85 standard conditioning (72 Cyt+TBI, 33 Fluda+TBI, 23 Fluda+Bu+ATG, 8 Cyt+Bu and 21 other). Before autoT the conditioning consisted of 224 Cyt+TBI, 45 BEAM and 44 other. After alloT, 71 patients developed an acute GVHD ≥grade II and 60 developed a chronic GVHD. The non-relapse mortality at 1 year was 29%. With a mean follow-up of 28 months for autoT and 40 months for alloT, the probabilities of 3-year, 5-year and 8-year overall survival were 80%, 66%, 45.5% after autoT and 52%, 48% and 35% after alloT respectively. An analysis aimed to determine the percentage of long-term survivors, or patients focused on the final plateau of survival curves was performed on alloT and autoT groups. A mixture model, gfcure with Splus statistical package determined the percentages of long-term survivors and its adequacy was verified graphically.

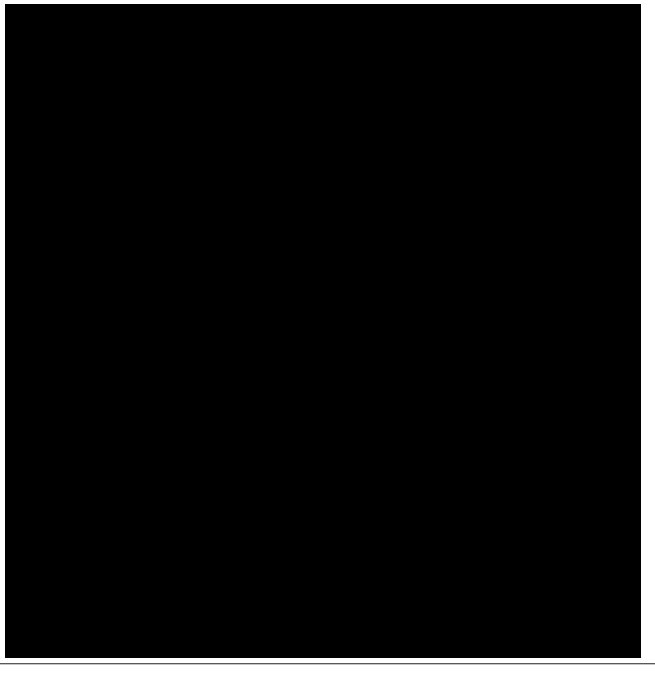

Figure 1-2. Autograft Long-term survivors prediction model [Cure Rate (Peng-Stat Med 1998)].

The percentage of long-term survivors for the autoT group was 1.2%, with a mean survival length for uncured population of 160 months, both curves were close and consequently show good adequacy and the absence of a final plateau (Figure 1). The percentage of long-term survivors for alloT was 34% (Figure 2) showing rather good adequacy. The study of the impact of usual prognosis factors (age, time diagnosis-transplant, sex match, HLA match, CMV status, type of conditioning, BM or PBSC, ABO compatibility and disease status before transplantation) on the percentage of long-term survivors showed that only the status of disease at transplant had a significant impact: (CR vs SD or PD, HR: 0.11 [0.02-0.5] p=0.01 and PR vs SD or PD, HR: 0.30 [0.09-0.96] p=0.04). This study pointed out the possibility of curing B-CLL patients who responded to conventional chemotherapy with allogeneic transplantation.

0363

LENALIDOMIDE IS AN ACTIVE AGENT IN RELAPSED AND TREATMENT-REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Lenalidomide is an immunomodulatory agent that stimulates T-cells, inhibits the production of TNF-α and VEGF, and impairs the interaction between bone marrow stroma and hematopoietic cells. These properties prompted us to initiate a phase II clinical trial of lenalidomide in patients with advanced CLL. In this study lenalidomide was given orally at 10 mg/day for the first four weeks and its dose was escalated by 5 mg every 28 days to a maximum of 25 mg/day. Thus far, 45 patients have been enrolled and data of the first 35 patients with a median follow up of 9 months was analyzed. The remaining 10 patients have been on treatment for < 3 months. All patients had received at least one purine-analogue-based regimen. The median age of the patients was 64 years (range 49-86), the median number of prior treatments was 4 (range 1-15), the median b2M was 4.1 mg/dL (range 1.6-10), 60% of the patients carried unfavorable genomic abnormalities (deletion of 17p or 11q23) and 71% had unmutated IgVH. Responses were evaluated according to the NCI-WG criteria: 13 patients (38%) responded [3 CR (9%), 1nPR (3%) and 9 PR (26%)]. The median response duration is 11+ months. Eight patients with stable disease continue on treatment. Twelve patients failed and 2 died early of infectious complications (on days 11 and 22). Minimal residual disease (MRD), as assessed by flow cytometry, was not detected in 2 of the 3 patients who attained CR. The most common toxicity was myelosuppression with grade >3 neutropenia and thrombocytopenia in 17% and 14% of the patients, respectively. Remarkably, our patients did not develop lymphopenia and the T cell count remained stable or increased during treatment. Grade >3 fatigue, diarrhea and tumor flare were observed in 9%, 6% and 6% of the patients, respectively. Twelve serious infectious episodes occurred: 8 pneumonias (PCP in 2), 2 FUOs, 1 mucormycosis (fatal) and 1 enteric cryptosporidiosis. None of the patients received routine antibiotic prophylaxis. The median dose of lenalidomide tolerated was 10 mg/day. Plasma levels of angiogenic factors and inflammatory cytokines were measured at baseline, day 8, day 28, and at 3 months in 18 patients. A significant increase in the levels of TNF-α and its soluble receptor, TNFR1, was noted on day 8, followed by a decline to basal level at 3 months. Similarly, a transient increase in the levels of IL-6, IL-10, IFN-γ, and soluble IL-2 receptor was observed on day 8, suggesting that lenalidomide activates the immune system in CLL, as previously described in multiple myeloma. We also showed that VEGF and β-FGF levels were significantly reduced at three months and the measurement of bone marrow microvessel density is ongoing. We conclude that lenalidomide, given in a low-dose continuous schedule, induces complete (MRD-negative) and partial responses in patients with advanced CLL. Ongoing correlative studies suggest a unique mechanism of action of this agent in CLL.

0364

CLINICAL EFFICACY OF OXALIPLATIN, FLUDARABINE, CYTARABINE, AND RITUXIMAB COMBINATION THERAPY IN PATIENTS WITH RICHTERS SYNDROME OR FLUDARABINE-REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A PHASE I-II CLINICAL TRIAL

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Background. Oxaliplatin is a platinum compound that covalently binds DNA, inducing DNA intra- and interstrand cross-links. Fludarabine and cytarabine act synergistically to inhibit excision repair of DNA cross-links. The clinical efficacy of oxaliplatin in patients with Richter's syndrome (RS) and fludarabine-refractory chronic lymphocytic leukemia (CLL) has not been investigated previously. We conducted a phase 1-2 trial of oxaliplatin, fludarabine, cytarabine, and rituximab (OFAR) in these diseases. *Aims.* The objectives of this trial were to identify a safe, tolerated dose and any dose-limiting toxicities (DLT) of oxaliplatin, to assess pharmacodynamic endpoints (phase 1), and to assess the complete (CR) and partial remission (PR) rates for the OFAR regimen (phase 2). *Methods.* The OFAR regimen consisted of increasing doses of oxaliplatin (17.5, 20, or 25 mg/m^2/d), days (D) 1-4 (phase 1); 30 mg/m^2 of fludarabine, D2-3; 1 g/m2 of cytarabine, D2-3; 375 mg/m^2 of rituximab, D3 on course 1 and D1 on subsequent courses; and 6 mg of pegfilgrastim, D6, every 4 weeks for a maximum of 6 courses. Prophylaxis for PCP pneumonia and DNA virus were given. DLT was defined as any non-hematologic, treatment-related toxicity > grade (G) 3. *Results.* From November 2004 to January 2007, 64 patients (26 RS, 38 fludarabine-refractory CLL) were treated with OFAR (phase 1, 19 pts). The highest tolerated oxaliplatin dose identified was 25 mg/m^2. No DLT was observed. Pharmacodynamic analyses demonstrated enhanced leukemia cell killing by oxaliplatin in the presence of fludarabine and cytarabine. For patients with RS and CLL, the median ages were 66 yrs (range, 41-78) and 62 yrs (range, 34-78) and the median numbers of prior therapies were 3 (range, 0-10) and 4 (range, 1-11), respectively. Among patients with fludarabine-refractory CLL, 24 (63%) had Rai stage 3-4. The median number of OFAR cycles was 2 (range, 1-6). Sixty patients are currently evaluable for response. The overall response rates were 44% (11 of 25; CR= 3, PR= 7) in RS and 43% (15 of 35; CR=1, nodular CR= 2, PR=12) in fludarabine-refractory CLL. Responses included 9 of 22 (41%) patients with 17p deletion, 5 of 9 (56%) patients with 11q deletions, 5 of 5 (100%) patients with trisomy 12, 2 of 5 (40%) patients with 13q deletions, and 4 of 12 (33%) patients with no genomic aberrations. Fifteen of 31 (48%) patients with tumors greater than 5 cm compared to 9 of 27 (33%) patients with tumors *less than or equal to* 5 cm (tumor size was not recorded in 2 patients) responded to OFAR therapy (p=0.16). Toxicities were mainly hematologic, but no prolonged myelosuppression was observed. Grade 3-4 infections occurred in 14 patients. *Conclusions.* OFAR is clinically active in patients with Richter's syndrome and fludarabine-refractory CLL. These results are encouraging and warrant further investigation of the OFAR regimen in the treatment of these disorders.

0365

ERADICATON OF MINIMAL RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA IS ASSOCIATED WITH PROLONGED SURVIVAL. LONG TERM FOLLOW UP OF PATIENTS TREATED WITH ALEMTUZUMAB

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Eradication of minimal residual disease (MRD) in chronic lymphocytic leukemia (CLL) is increasingly emerging as a desirable therapeutic end point predicting for better outcome. The monoclonal antibody alemtuzumab (Mabcampath) is licensed for the treatment of patients with CLL refractory to fludarabine-based therapies and results in the eradication of MRD in a proportion of patients. We previously published a series of ninety one (74 men and 17 women, median age 58 years [range, 32 to 75 years]) relapsed CLL patients who received a median of 9 weeks (range 1 to 16 weeks) of alemtuzumab treatment between 1996 and 2003 (Moreton et al, J Clin Oncol 2005 May 1;23(13):2971-9). 44 were refractory to purine analogues. Responses to alemtuzumab according to National Cancer Institute sponsored working group response criteria were complete remission (CR) in 32 patients (36%), partial remission (PR) in 17 patients (19%), and no response (NR) in 42 patients (46%). Detectable CLL to a level of less than a single CLL cell in 10,000 leucocytes, assessed by four-colour MRD flow cytometry, was eradicated from the blood and marrow in 18 patients (20%). 8/18 of these patients were refractory to prior therapy with purine analogues (fludarabine refractory). We report here the results of long term follow up of this cohort of patients after a median follow up of 7.3 years (range 4 to 10.6 years). Median survival was significantly longer in those patients achieving MRD negative responses compared with those who had detectable CLL at the end of therapy (MRD positive CR, PR, or NR). The median survival for all 18 MRD negative responders has not been reached but was 87 months for the 8 fludarabine-refractory patients who achieved MRD negativity following alemtuzumab. Overall survival for the 18 patients with MRD-negative remissions was 72% at 79 months (see Figure 1). Patients achieving an MRD positive complete response had a median survival of 41 months, an MRD positive partial response a median survival of 30 months and non-responders a median survival of 15 months. The median treatment-free interval prior to alemtuzumab for the 18 MRD negative patients was 6 months (range 1 to 28 months). We conclude that alemtuzumab can induce MRD negative remissions in CLL resulting in a clear survival advantage with 72% patients alive 10 years after alemtuzumab therapy.

Figure 1. Over survival of subgroups by response.

Stem cell transplantation I

0366

ALLOGENEIC STEM CELL TRANSPLANTATION FROM MATCHED RELATED AND UNRELATED DONORS IN THALASSEMIA MAJOR PATIENTS USING A REDUCED TOXICITY FLUDARABINE BASED REGIMEN

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Introduction. The only radical cure for thalassemia major patients today is the replacement of the defective hematopoietic system by allogeneic stem cell transplantation (allo-SCT). The major obstacles for the application of allo-SCT even from matched family members have been the transplant related morbidity and mortality and graft failure that is usually associated with the recurrence of the thalassemia hematopoiesis. The outcome of allo-SCT from HLA-identical family donors is largely dependent on the age of the recipient as well as on pre-transplant parameters reflecting the degree of organ damage from iron overload. We hereby report our experience of allo-SCT from matched related and unrelated donors, using a reduced toxicity conditioning. *Methods*. We analyzed a cohort of 20 patients with thalassemia major. Reduced-intensity conditioning consisted of fludarabine 30 mg/m^2/day from day -9 to day -4, p. o. busulfan 4 mg/kg/day or i. v. busulfex 3.2 mg/kg/day from day -7 to day -4, and antithymocyte globulin (ATG, Fresenius) 10 mg/kg/day from day -4 to day -1. The first two patients received a decreased total dose of busulfan 8 mg/kg, and one of them had a late rejection. Donors were fully HLA-matched siblings in 17 cases, a fully matched grandfather in one case, and the other two were matched unrelated donors. Unmanipulated PBSC and BM served as the source of stem cells in 8 and 12 patients respectively. *Results*. The regimen related toxicity was minimal. Engraftment of neutrophils was observed at median 15 days (range 10-27 days); engraftment of platelets was achieved in a median 15 days (range 9-35). Six patients never required any platelet transfusion support. The incidence of acute grade II-IV, and chronic GVHD was 25%, and 25%, respectively. With a median follow-up period of 39 months (range 5-112 months) the overall survival was 100%, while thalassemia free survival was 80% (Figure 1). At two months after BMT, 12 patients had complete donor chimerism; 7 mixed chimerism and one patient suffered prime graft failure. One out of 12 patients with complete donor chimerism and 1 out of 7 with mixed chimerism experienced recovery of host-type hematopoiesis. Three patients remained in a state of stable mixed chimerism. *Conclusions.* It appears that substitution of high dose cyclophosphamide with fludarabine and ATG is effective and results in minimal regimen-related toxicity without compromising the rate of engraftment.

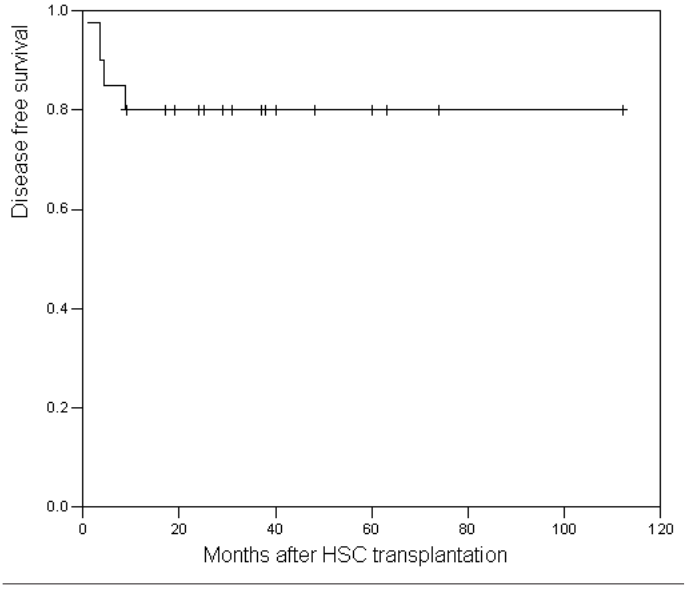

Figure 1. Disease-free survival (Kaplan-Meier Curve).

0367

THE EUROCHIMERISM CONCEPT FOR A STANDARDIZED APPROACH TO CHIMERISM ANALYSIS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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We present the results of a collaborative study supported by a grant from the European Commission, the EuroChimerism Concerted Action. The aim of this Concerted Action was the development of a standardized diagnostic methodology for the detection and monitoring of chimerism in patients undergoing allogeneic stem cell transplantation. A set of microsatellite markers, selected on the basis of their excellent performance in chimerism analysis at the reference centers involved, have been carefully evaluated and optimized for quantitative chimerism testing under standardized experimental conditions. A EuroChimerism panel designed to optimally meet the specific requirements of quantitative chimerism analysis was established utilizing 13 markers (D2S1360, D7S1517, D8S1132, D9S1118, D10S2325, D11S554, D12S1064, D12S391, D17S1290, D19S253, MYCL1, P450CYP19 and SE-33), which best satisfied the criteria of the EuroChimerism consortium. Individual microsatellites from the panel can be analyzed in multiplex reactions to facilitate the identification of one or more markers optimally suited for the monitoring of chimerism during the post-transplant period. Extensive testing of related individuals (>500 pairs) revealed that the EuroChimerism marker panel will provide at least two informative markers which meet the stringent criteria of eligibility defined by the EuroChimerism consortium (Watzinger *et al.*, Leukemia 2006) in >99% of all patient/donor constellations. In addition to the outstanding informativeness of the marker panel, chimerism testing by singleplex assays permitted sensitive detection of residual cells of patient or donor origin at levels ranging between 0.8-1.6% in about 90% of instances. The assay also facilitates accurate and reproducible quantification of donor and recipient hematopoietic cells in peripheral blood or bone marrow specimens and in specific cell lineages isolated by flow-sorting. The precision in determining the relative contribution of donor/recipient cell populations to mixed chimerism was high within the range between 10-90% donor- or recipient-derived cells, while there was a tendency to overestimate the percentage of the subdominant cell population within the range between 1-10%. Wide use of the EuroChimerism assay will provide a basis for international standardization of chimerism testing, which will ultimately contribute to improved clinical management of patients undergoing allogeneic stem cell transplantation.

0368

LONGTERM FOLLOWUP AND SAFETY OF BONE MARROW AND PERIPHERAL BLOOD STEM CELL DONORS: A SINGLE INSTITUTION / DONOR REGISTRY REPORT

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Background. The safety of bone marrow and peripheral blood stem cell donation is an issue of utmost importance to ensure continuous motivation of volunteer donors for hematopoietic stem cells and the high standing of this procedure in modern civilisation. Procedures have been standardised to guarantee minimal risk during both donor preparation and stem cell harvest. However, recent single reports of leukemias emerging in related donors several years after G-CSF stimulated peripheral blood stem cell mobilisation have raised concern about the long-term safety of this approach. *Subjects and Methods.* Here we report our twelve years experience (March 1994 - January 2007) of 596 consecutive

bone marrow and peripheral blood stem cell donors who were recruited, screened, harvested and followed at our institution and the associated donor registry. 40% were females and 60% males, with a median age of 37 years (range: 1-71 years). 318 were family donors and 278 unrelated volunteer donors. 110 donated bone marrow, 472 peripheral blood stem cells after mobilisation with G-CSF, and 14 both. 33 donors underwent two harvests, four donors even three. Our preparative procedure consisted of a medical work-up including history, examination, blood and clinical chemistry tests, infectious disease markers, electrocardiogram, chest X-ray, lung function test, and abdominal ultrasound. Follow-up was scheduled at 1 and 6 months, as well as 1, 2, 5, and 10 years after donation of hematopoietic stem cells. *Results.* Within the twelve year period of evaluation, no donor death was recorded, no single case of leukemia has been reported, and no splenic rupture occurred in any of our donors. Five donors experienced severe adverse events: In one female bone marrow donor, post-harvesting pain persisted for more than twelve months. A 42 year old female donor developed breast cancer 3 years after G-CSF mobilised stem cell apheresis; however, this was not considered to be of causal relationship. In two donors of peripheral blood stem cells, vascular complications occurred: one donor had to be admitted to hospital during G-CSF mobilisation for symptoms and signs of pulmonary embolus (which, however, could be ruled out), and subsequently was able to donate bone marrow. Another donor had recurrence of a deep vein thrombosis eleven days after stem cell apheresis, despite sufficient anticoagulation with low molecular weight heparin during G-CSF stimulation. Finally, a donor with sporadic epilepsy, who donated twice, had a generalised seizure few days after each G-CSF mobilised harvest. This is particularly interesting in view of recent *in vitro* and animal findings of G-CSF receptors on neural tissues and pleiotrophic neurostimulatory functions of G-CSF. *Conclusions.* In our experience, bone marrow and peripheral blood stem cell donations are safe and generally well tolerated, with very few early and virtually no late complications.

0369

ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH WALDENSTRMS MACROGLOBULINAEMIA. ANALYSIS OF 106 CASES FROM THE EUROPEAN BONE MARROW REGISTRY (EBMT)

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Background. Despite effectiveness of standard chemotherapy regimens, complete response is infrequent in Waldenström's Macroglobulinaemia (WM) patients and there is no cure. The role of allogeneic stem cell transplantation (Allo-SCT) has not been explored extensively and the available data are limited. *Aims.* We retrospectively analyzed the results and long-term outcome of a group of 106 WM patients from 65 European centres who underwent an Allo-SCT between 1989 and 2005 and were reported to the database of the Lymphoma WP of the EBMT. *Patients and Methods.* There were 69 males and median age at transplantation was of 49 years (range, 21-65). Time interval between diagnosis and Allo-SCT was 34 months (5-310) and the median number of lines of therapy prior to Allo-SCT was 3. Nineteen patients (18%) had failed a prior autograft. At Allo-SCT, 10 patients (10%) were in CR≥2, 35 patients (33%) in PR1, 29 patients (27%) in PR≥2 and 32 patients (30%) had relapsed or refractory disease. Seventy-nine patients (74%) were allografted from an HLA-identical sibling donor, 18 (17%) from a matched unrelated donor and the remaining 9 patients from other donors. Conventional conditioning protocols (CT) were used in 44 (41%) patients and reduced intensity conditioning (RIC) regimens in 62 (59%) patients. *Results.* Forty-eight (45%) patients developed acute graft versus host disease [Grade III-IV (n=14)] with no statistically significant differences between CT and RIC regimens. After a median follow up of 31 months (3 to 169), 17 (16%) patients had relapsed at a median time of 8 (1-89) months post Allo-SCT. The incidence of relapse at 3 years was 18%, 12% after CT and 25% after RIC. Thirty-five (33%) patients died, 5 (5%) from disease progression and 30 (28%) from non-relapse mortality (NRM), with an incidence of NRM of 27% and 31% at 1 and 3 years. The progression free survival (PFS) rates were 61%, 50% and 48% at 1, 3 and 5 years and the overall survival rates were of 69%, 63% and 63%, at 1, 3 and 5 years, respectively. In a multivariate analysis, conditioning regimen had no impact either on NRM or on relapse rate. Refractory patients presented with a higher risk of relapse (p=0.03). The use of TBI in the conditioning regimen was associated with a lower relapse risk (p=0.02) and a trend to a better PFS (p=0.1). *Conclusions.* Allogeneic stem cell transplantation is a feasible and well tolerated procedure in this heavily pre-treated group of patients. Relapse rate is low and long-term outcome seems promising, with half of the patients being alive and disease-free 5 years after the procedure.

0370

HEMATOPOIETIC STEM CELL TRANSPLANTATON FOR OSTEOPETROSIS

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Autosomal recessive osteopetrosis is a disorder of osteoclast function, generally lethal in the first decade of life without allogeneic hematopoietic stem cell transplantation (HCT). To determine the outcomes of patients with this disorder undergoing transplantation, we examined long-term survival after HCT in 124 children with infantile osteopetrosis reported to the Center for International Blood and Marrow Transplant. The data reflected outcomes of transplantation for patients with osteopetrosis from 1978-1999. The median age at transplantation was 8 months and median time from diagnosis to transplantation 4 months. Forty percent of allografts were from HLA-matched siblings, 34% from unrelated donors and 26% from alternative related donors. Busulfan and cyclophosphamide was the most commonly used conditioning regimen (74%) and bone marrow the predominant graft source (87%). The cumulative incidence of neutrophil recovery at day +100 was 75% (93/124) with similar rates across the three donor sources. Fifty-four children are alive with a median follow up of 7.5 years and an 8-year probability of survival of 43%. The 8-year probabilities of survival were 54%, 39% and 35% after HLA-matched sibling, alternative related and unrelated donor transplantation, respectively. Early mortality rates were high after HCT regardless of donor type; 34% at day +100 and 50% at 1- year after transplantation. Common causes of mortality were graft failure (33%), interstitial pneumonitis or adult respiratory distress syndrome (34%), infections (12%) and graft-versus-host disease (10%). Nineteen patients underwent a second transplant for either primary or late graft failure. Six of these patients are alive. Most second transplants occurred within 6 months from the first transplant (n=15) and the remaining 4 occurred at 4, 4.5, 5 and 9 years after the first transplant. We conclude that long term survival for autosomal recessive osteopetrosis can be obtained with HCT. Peri-transplant mortality and graft failure remain significant obstacles in this population. Second transplantation is a viable option if engraftment is not initially achieved.

Acute lymphoblastic leukemia - Clinical

0371

HIGH INTERLEUKIN-15 EXPRESSION LEVELS IN INITIAL DIAGNOSTIC LEUKEMIC CELLS OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA ARE ASSOCIATED WITH SUBSEQUENT RELAPSE INVOLVING THE CENTRAL NERVOUS SYSTEM

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Background. Central nervous system (CNS)-targeted therapy as part of treatment for childhood acute lymphoblastic leukemia (ALL) is highly effective in the prevention of CNS relapse, but is associated with significant toxicity. The intensity of CNS-targeted treatment is adjusted according to the risk of ALL relapse originating from the CNS, the most important risk factor being overt CNS involvement at diagnosis. However, most ALL relapses involving the CNS occur in patients classified as CNS-negative at initial diagnosis. Although there are some known risk factors for CNS involvement of ALL such as T-cell-precursor immunophenotype and high initial white blood cell (WBC) count, the underlying biology leading to CNS disease is poorly understood. *Aims.* To improve patient characterization and to explore potential distinct biological properties of leukemic cells that migrate into the CNS by comparing gene expression profiles of childhood ALL patients with initial CNS involvement to CNS-negative patients. *Methods.* Leukemic gene expression profiles from bone marrow of 17 CNS-positive patients and 26 CNS-negative patients frequency-matched for risk factors associated with CNS involvement were analyzed using microarrays containing more than 39,000 distinct cDNA clones (SFGF, Stanford CA). Data were analyzed applying Significance Analysis of Microarrays (PNAS 2001, 98, 5116-5121). Results were confirmed by real-time quantitative polymerase chain reaction analysis and validated using independent patient samples. *Results.* Our analytical approach identified several differentially expressed genes. To control for possible T-cell contamination of the samples, we purified leukemic cells from four samples and repeated the analysis. Interleukin 15 (IL-15) was one of the genes for which differential expression could be confirmed with an up to 10-fold higher expression in CNS-positive patients. The high expression of IL-15 in patients with CNS involvement was validated in an independent set of 13 CNS-positive and 26 CNS-negative patients by quantitative RT-PCR in a similar range as described above (P Mann-Whitney U-Test "0.001). In multivariate analysis, IL-15 expression levels above the median were associated with CNS involvement compared to expression equal to or below the median (odds ratio (OR) = 10.70, 95% confidence interval (CI) 2.95-38.81). Diagnostic likelihood ratios for CNS-positivity were 0.09 (95% CI 0.01-0.65) for the first and 6.93 (95% CI 2.55-18.83) for the fourth IL-15 expression quartile. Next, we compared IL-15 expression at initial diagnosis of CNS-negative patients subsequently relapsing with CNS involvement (n=22) to those without CNS disease and being in long-term remission (n=44). In patients CNS-negative at diagnosis, IL-15 levels above the median were associated with subsequent CNS relapse compared to expression equal to or below the median (OR=13.80, 95% CI 3.38-56.31). *Conclusions.* We conclude that measurement of IL-15 expression levels could serve as an additional tool to further tailor CNS-directed therapy in children newly diagnosed with ALL. Additional studies are needed to finally elucidate the role of IL-15 in the pathogenesis of CNS disease in childhood ALL.

0372

FINAL RESULTS OF THE TREATMENT OF ADOLESCENTS AND YOUNG ADULTS WITH STANDARD-RISK ALL WITH THE PEDIATRIC-BASED PROTOCOL PETHEMA ALL-96

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Background. Recent retrospective comparative studies have shown that adolescents and young adults with standard-risk (SR) ALL are better managed with pediatric-based than adult-based chemotherapeutic schedules. *Aims.* To report the final results of the PETHEMA ALL-96, a pediatric-based protocol, in adolescents and young adults with SR ALL. *Patients and therapy.* Criteria for SR-ALL: age 15-30 yr., WBC count $<25\times10^9$/L and absence of t(9;22)(or BCR/ABL), t(1;19) or 11q23 rearrangements. Induction: VCR, DNR, PDN, ASP and CPM over 5 weeks. Consolidation-1 (C1): MP, HD-MTX, VM26 and ARA-C. Consolidation-2/reinduction (C2): VCR, DNR, DXM, ASP and CPM. Maintenance-1 (M1): MP+MTX with monthly reinduction cycles (VCR, PDN, ASP) up to 1 yr. Maintenance-2 (M2): MP+MTX up to 2 yr. in continuous CR. CNS prophylaxis consisted of triple i.t. therapy (MTX+ARA-C+DXM), 14 doses over the 1st yr. *Results.* 86 patients included, mean (SD) age 20 (4) yr. 54 males, WBC count 7.6 (8.0)$\times10^9$/L. Phenotype: pro-B 6, Common+pre-B: 61, T: 19. Cytogenetics (61 evaluable cases, after review): normal 37, hyperdiploid 13, hypodiploid 1, other 10. Evaluable for response: 84 pts. CR 80 (95%), slow response (>10% of BM blast cells on day 14) 10 pts, early death 2, resistance 2, relapse 18 (1 CNS, 17 BM isolated or combined, 6 during therapy), death 16 pts (2 induction, 1 C2, 13 relapse/progression). With a median follow-up of 35 mo. 5-yr (95%CI) OS and DFS probabilities were 74% (63-85) and 66% (54-78). No pretreatment variables influenced CR, DFS and OS. The slow response to therapy was the only prognostic factor for CR (80% vs. 99%, p=0.04) and survival (40% vs. 85%, p=0.001). Overall toxicity was acceptable, although there were dose modifications or delay in therapy in 10% pts in induction, 26% pts in C1, 15% in C2, 22% in M1 and 21% in M2. *Conclusions.* The results of the pediatric-based PETHEMA ALL-96 study for adolescents and young adults with SR ALL treated in adult hematology units are identical to those reported in national series from pediatric centers. Slow response to therapy was the only prognostic factor for remission and survival.

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0373

TWO DECADES OF PROGRESS IN ADOLESCENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIAS TREATED IN THE FRALLE PROTOCOLS: ADOLESCENCE IS NO MORE A BAD PROGNOSTIC FEATURE IF AN INTENSIVE CHEMOTHERAPY IS APPLIED

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Adolescence has been claimed since the seventies to be associated to a a bad prognosis in childhood ALL. Out of 4658 patients with ALL, 258 adolescents (15-20 year old)(5.5%) were treated in the successive FRALLE 83, FRALLE 87-89, FRALLE 92 (pilot phase), FRALLE 93 and FRALLE 2000 protocols. The main characteristics were: a sex ratio of 1.8 (M/F), a B-lineage in 71% of the cases vs T lineage in 29%, and a median WBC of 12 G/L (9-1000). Translocation and fusion transcripts were searched for in 120 evaluable BCP-ALL: t(9;22)/BCR-ABL, 8 pts (6%); t(1;19)/E2A-PBX1, 12 pts (10%); t(4;11)/MLL-AF4, 4 pts (3%). Out of 75 evaluable pts t(12;21)/TEL-AML1 was found in only 4 pts (3%). 242 out of 258 adolescents were in CR at the end of induction therapy (EOI) (94%) without any significant difference according to the era. Nevertheless a major difference in the 3y and 5y EFS was found ans is shown in Table 1. The main modification introduced in the nineties was the adoption of a double delayed intensification for the good early responders. Autologous BMT or allogenic BMT were indicated in bad early responders (D8 poor prednisone response, D21 marrow M3 response) and/or unfavourable cytogenetics. The better results of the 2000 protocol can mainly be explained by the intensification of chemotherapy in most phases and especially between induction and delayed Intensification 1 and before delayed Intensification 2. These better results were obtained despite decreasing the indications of allo BMT (6 performed vs 20 in the nineties) and of CNS irradiation (100% in the nineties vs 35% in the current era, including the TBI for BMTs). ABMT was no more indicated. *Conclusions.* 1) excellent results can now be achieved in adolescents with ALL 2) this works emphasizes again the need to treat adolescents according to pediatric intensive protocols and not *adult type* protocols, as we

recently suggested (Boissel *et al.,* J Clin Oncol 2003). 3) Whether this could also be applied to young adults is currently under testing.

Table 1.

	Number of pts	CR at end of induction	3y EFS (%)	5y EFS (%)	10y EFS (%)
Eighties (F83, 87-89)	100	93	42 ± 5	35 ± 5	35 ± 5
Nineties (F92, F93)	84	93	71 ± 5*	67 ± 5**	67 ± 5
2000s (F2000)	74	96	86 ± 5*	86 ± 10	NYA

*: p=.04, **: p=.04

0374

RISK ADAPTED TREATMENT OF ADOLESCENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) ACCORDING THE GERMAN MULTICENTER STUDY GROUP (GMALL) STUDIES 06/99 AND 07/03 YIELDS SIGNIFICANTLY DIFFERENT OUTCOME FOR SUBGROUPS

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In the recent years several groups have reported treatment results in adolescents with ALL. Outcome with protocols for adults (EFS 34-71%) were generally inferior compared to pediatric protocols (EFS 64-80%) (S.Sallan, ASH Education 2006). The conclusion was that pediatric trials include higher doses of VCR, ASP and HDMTX and a higher time-dose intensity. However the poor outcomes of most compared adult protocols are probably also due to suboptimal approaches in these specific studies e.g. stem cell transplantation (SCT) in all pts with donor. The GMALL started in 1999 the study 06/99 followed by 07/03 with minor changes. Induction is based on DEXA, DNR, VCR, PEG-ASP and standard phase II. All pts received HDMTX/HDAC based consolidation1. High risk (HR) pts (B-lineage ALL with WBC>30.000 or late CR, pro-B, earlyT, mature T, t(4;11), t(9;22)) were allocated to allogeneic SCT. Standard risk (SR) pts received consolidation with HDMTX/ASP (3x), VM26/ARAC, CYCLO/ARAC and reinduction. Since 2003 most pts with Ph+ received Imatinib. Between 9/99 and 12/06 1514 pts were included. 417 (28%) were adolescents aged 15-25 yrs (18% 15-17, 41% 18-20 and 41% 21-25 yrs). Of those 58% were SR, 34% HR and 8% Ph+. Immunophenotypes were c/preB(63%), proB(5%), earlyT(7%) matureT(7%) and thymicT(18%). Entry criteria showed no differences for the three age groups. The CR rate was 90% (87% after phase I; 93%, 92% and 88% for the 3 age groups). 2% died in induction; 7% failed. The overall survival (OS) was 64%, 67% in CR pts and the relapse free survival 54%. OS according to age was 68%, 62% and 65%. CR rates and OS differed significantly for risk groups and subtypes. OS was 74% for SR, 49% for HR and 55% for Ph^+ ($p<.05$). For subtypes OS ranged from 36% for HR c/pre-B-ALL to 70% for thymic and 74% for SR c/pre-B-ALL ($p<.05$). SCT in CR1 was realised in 73% of HR/VHR pts. OS was 57% with significant differences for subtypes (range from 46% for HR B-lin to 76% for early T). TRM for allo SCT (sibling, MUD) was 25%. We present here the results of the largest cohort of adolescent ALL pts treated according to adult protocols so far. CR rate and OS are superior to most of those reported from adult protocols but still at the lower end of range for pediatric trials. There are two major problems: The high relapse rate in SR and the TRM in HR. Further chemo intensification is planned for SR pts. Imatinib is added for Ph^+ and Rituximab for $CD20^+$ ALL. SCT leads to improvement of OS in subgroups of HR ALL, although not in all subtypes. The indication fo SCT should therefore even in young pts be defined carefully considering the TRM and late effects. Several studies with pediatric protocols used in adults are ongoing. The CR rates (82-92%) and EFS (66-72% (ASH 2006, #147, #1858, #1875) are promising but considerable toxicity e.g. neuropathies, thrombosis was observed indicating that these protocols may probably be not be transferred to all adults.

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0375

DIFFERENT OUTGROWTH KINETICS OF CLONES WITH BCR-ABL KINASE DOMAIN MUTATIONS IN DE NOVO VERSUS RELAPSED PHILADELPHIA-POSITIVE ALL IMPLY AN ADDITIONAL ROLE OF NON-MUTATIONAL RESISTANCE MECHANISMS

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Background. Approximately 80% of patients with newly diagnosed Ph+ALL who relapse during imatinib-based therapy express a BCR-ABL Tyrosine Kinase Domain TKD mutation in the dominant cell population. Nearly all of these mutations are associated with high-level imatinib resistance. We recently showed that the same mutation found at relapse can already be detected prior to first imatinib exposure in 30% to 40% of patients with de novo Ph+ALL but that it is not associated with an inferior response: approximately 95% of patients achieve a complete remission (CR) with imatinib alone. In contrast, the CR rate with imatinib salvage therapy in patients who failed previous chemotherapy is only 20-30% and the median time to progression only 2.2 months. Possible reasons for this difference are a higher frequency of pre-existing mutations, a preponderance of mutations with greater transforming activity, a larger mutant clone or a contribution of non-mutational resistance mechanisms. *Aims.* We therefore examined the frequency and pattern of up-front mutations, the level of mutant clones, and the outgrowth kinetics of mutations during the first 4 weeks of imatinib monotherapy in de novo Ph+ALL patients and in patients who had failed prior chemotherapy. *Patients and Methods.* By denaturing high-performance liquid chromatography (D-HPLC) and cDNA sequencing, we examined bone marrow and/or peripheral blood samples collected pre-treatment, during therapy and at relapse from 48 pts. with newly diagnosed Ph+ALL (>55 yrs.) enrolled in a GMALL study of combined IM and chemotherapy, and from 67 Ph+ALL pts. who were enrolled in the initial phase II studies of single-agent imatinib as salvage treatment after prior chemotherapy. *Results.* The frequency of TKD mutations pre-IM was 38% (23/59) in pts. with advanced Ph+ALL and 37,5% (12/32) in newly diagnosed Ph+ALL. The incidence of mutations observed at relapse was substantially higher but did not differ significantly between the two cohorts: 70% in pts. with advanced disease who relapsed on IM monotherapy (36 of 51 pts.; P-loop 76%, T315I 21%, A-loop 2%) and 84% in de novo ALL pts. (n=26), treated with combination therapy (P-loop 47%, T315I 15%, A-loop 9,5%). In contrast, the outgrowth of cells expressing mutated bcr-abl during the first four weeks of imatinib therapy was considerably more rapid in Ph+ALL patients who had failed prior chemotherapy: mutations were detected after 2 and/or 4 weeks imatinib in only 34% of patients (6/18 evaluable) with newly diagnosed Ph+ALL, whereas 69% of patients (27/39) with advanced Ph+ALL expressed mutant BCR-ABL on at least one of these timepoints. Moreover, the percentage of mutated BCR-ABL was below 1% in all 6 pts. with a mutation in the former cohort (de novo Ph+ALL), but higher than 5% (range: 5-100%) in 16 of the 27 advanced disease patients (59%) who had a TKD mutation. *Conclusions.* In Ph+ALL, prior treatment with chemotherapy is not associated with a higher incidence, different spectrum or higher level of TKD mutations, either pre-imatinib or at relapse, when compared to patients with newly diagnosed leukemia. However, outgrowth kinetics of leukemic cells expressing BCR-ABL mutations are considerably more rapid in patients with advanced disease, providing clinical-translational evidence for a cooperative effect between mutational and non-mutational resistance mechanisms.

Anemia and PNH - Clinical trials

0376

RITUXIMAB THERAPY FOR IMMUNE ANEMIA AND THROMBOCYTOPENIA: A BELGIAN REGISTRY RUN BY THE RED CELL SUBCOMITTEE OF THE BELGIAN SOCIETY FOR HEMATOLOGY

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Background. Immune thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AHA) may respond to the chimeric anti-CD20 monoclonal antibody rituximab, even when refractory to conventional therapy. Aims. To collect data on Belgian patients given rituximab in the setting of ITP or AHA in order to assess the response rate and the factors predictive for response in a multicenter study. Method. Belgian hematology centers were invited to fill a questionnaire specifying the major characteristics and quality of response of ITP and AHA patients given rituximab. For ITP, complete response (CR) was defined as a platelet count >100,000/µL without immunosuppressive therapy, and a partial response as a platelet count 50-100,000/µL without immunosuppressive drugs. For AHA, CR was defined as a normal hemoglobin in the absence of hemolysis, and a PR as a 2g increase of the hemoglobin concentration. *Results.* Patient characteristics and response to therapy are presented in the attached Table 1. In practically all the patients reported here, rituximab was given at the dose of 375 mg/m^2 weekly for 4 weeks. In both ITP and AHA patients we could find no significant correlation between response and sex, age, prior splenectomy, platelet count or hemoglobin concentration when rituximab was started. *Conclusions.* In this still open registry, we could confirm that rituximab induces responses in a majority of previously treated patients with ITP or AHA. Responses could not be predicted from pre-treatment patient characteristics. As data are accumulating in the registry, more information on the duration of response will be made available.

Table 1.

	ITP	AHA
n	23	44 episodes in 35 patients
Male/Female	14/9	18/17
Median age (range)	44 (9-86)	57 (1-82)
Underlying lymphoma/leukemia	3	15
Median n of previous therapies	3 (1-4)	2 (0-5)
Previous splenectomy	14	10
Median platelet/hemoglobin at start of rituximab	24,000/µL (3-250)	8.4 g/dL (4-14.2)
CR/PR/Failure	13/2/8	15/20/9

0377

RESULTS FROM A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF DARBEPOETIN ALFA FOR THE TREATMENT OF ANEMIA IN CANCER PATIENTS NOT RECEIVING CHEMOTHERAPY OR RADIOTHERAPY

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Background. Patients with cancer, not receiving chemotherapy or radiation, often develop anemia as a result of the disease itself. *Aims.* This phase 3 study was designed to evaluate the efficacy and safety of darbepoetin alfa (DA) 6.75 mcg/kg administered every 4 weeks (Q4W) for the treatment of anemia of cancer (AoC). *Methods.* Pt eligibility included: ≥18 years, non-myeloid malignancy (with active disease), hemoglobin ″11 g/dL, ECOG status score of 0-2, receiving neither chemotherapy nor radiotherapy within 4 weeks of screening or during the study, and providing informed consent. Patients (n=985) were randomized to DA 6.75 mcg/kg or placebo Q4W for 16 weeks, with an end of study visit at week 19, and 2 years of follow up to evaluate survival. Patients were stratified by screening hemoglobin (<10 vs≥10 g/dL), geographic region (Europe vs rest of world), red blood cell transfusion in the prior 12 weeks (yes vs no), tumor type/treatment (chronic lymphocytic leukemia or low grade lymphoma, ongoing hormonal or antibody therapy, or all other eligible patients), and ECOG status (0-1 vs 2). DA dosing was withheld if hemoglobin increased >13 g/dL and reinstated at 25% dose reduction once hemoglobin <12 g/dL. The primary endpoint was all occurrences of transfusions from weeks 5 to 17. Secondary endpoints were: incidence of transfusions from week 5 to 17 (including a prespecified sensitivity analysis of incidence of either transfusion or hemoglobin ″8 g/dL), change in hemoglobin from baseline (BL) to end of treatment period, and safety. *Results.* Demographics were broadly similar between the groups: mean (SD) age was 64.1 (11.6) years; the most common cancers were non-small cell lung (18%), breast (13%), and prostate (11%); most patients had disease stage III/IV (82%), most had an ECOG score of 0 or 1 (72%); BL hemoglobin was 9.5 g/dL in each group. However, there were more men in the DA group (56% vs 47% in placebo), and more patients received prior chemotherapy in the DA group (73% vs 66% in placebo). The mean (SD) number of days between prior chemotherapy and first study drug dose was 262 (572) days for the DA group vs 315 (660) for placebo. The Table 1 shows efficacy endpoints and safety data. *Summary and conclusions.* This study did not meet its primary endpoint of reducing transfusions in the DA treatment group. More deaths occurred in the DA vs. placebo group. Based on the observed results and additional analyses, the risk/benefit profile of DA in anemic patients with active cancer not receiving chemotherapy is at best neutral and possibly negative. Results of long-term follow up are awaited.

Table 1. Summary of results.

0378

SAFETY AND EFFICACY OF THE TERMINAL COMPLEMENT INHIBITOR ECULIZUMAB IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: SHEPHERD PHASE III CLINICAL STUDY RESULTS

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Background. In paroxysmal nocturnal hemoglobinuria (PNH), lack of the GPI-anchored terminal complement inhibitor CD59 from erythrocytes renders these cells susceptible to chronic hemolysis resulting in anemia, fatigue, thrombosis, poor quality of life (QoL), and transfusion dependency. Eculizumab, a terminal complement inhibitor, reduced hemolysis and transfusion requirements in transfusion dependent patients with normal or near-normal platelet counts in a randomized placebo-controlled phase III study (TRIUMPH). *Aims.* To evaluate the safety and efficacy of eculizumab in an open-label non-placebo controlled global phase III study (SHEPHERD) in a broad PNH population including patients with significant thrombocytopenia (platelet counts as low as 30×10^9/L) and/or minimal transfusion requirements (at least 1 transfusion in the preceding 2 years). *Methods.* Eculizumab was dosed as follows: 600 mg IV every 7±2 days × 4; 900 mg 7±2 days later; and then 900mg every 14±2 days for a total of 52 weeks of therapy. *Results.* Eculizumab was administered to 97 patients at 33 international sites. The most frequent adverse events were headache (53%), nasopharyngitis (32%), and upper respiratory tract infection (30%). Most adverse events were mild to moderate in severity and not considered related to eculizumab. No serious adverse events were reported as probably or definitely related to drug. Eculizumab did not increase infections relative to the incidence of infections in placebo-treated patients from the TRIUMPH study. Intravascular hemolysis, the central clinical manifestation in PNH and the primary efficacy endpoint of the trial, was rapidly and significantly reduced in eculizumab patients as assessed by change in lactate dehydrogenase (LDH) area under the curve ($p<0.001$) from baseline. LDH levels decreased 87% from a median of 2,051 U/L at baseline to 269 U/L at 52 weeks ($p<0.001$; normal range 103-223 U/L). Eight of 97 patients had incomplete complement blockade and return of hemolysis during the 14-day dosing interval; shortening the dosing interval to 12 days per protocol led to sustained inhibition of complement and hemolysis in 6 of 6 patients. Control of hemolysis resulted in improvement in anemia as transfusion requirements decreased from a median of 8.0 (mean, 12.3±1.25) PRBC units/patient during the 12-month pre-treatment period to 0.0 (mean, 5.9±1.06) during 12 months of eculizumab treatment ($p<0.001$). Reduction in units transfused was demonstrated in each of four 12-month pre-treatment transfusion strata, reaching statistical significance in 3 strata. 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-Fatigue instrument and the fatigue scale of the EORTC QLQ-C30 instrument, were rapidly and significantly improved with eculizumab treatment ($p<0.001$ for each). Other EORTC-QLQ-C30 patient reported outcomes demonstrating improvement included global health status ($p<0.001$), all 5 patient functioning subscales ($p<0.001$) and 7 of 9 symptom/single item subscales ($p<0.003$). *Summary and conclusions.* Eculizumab appears to be safe and well tolerated. Beneficial effects of eculizumab in PNH are observed in a broader patient population than previously studied including those with thrombocytopenia and/or minimal transfusion requirements. This further underscores that eculizumab markedly reduces hemolysis, thereby providing significant clinical benefit to treated patients.

0379

THE CLINICAL BENEFIT OF ECULIZUMAB IS DEMONSTRABLE IN ALL SUBPOPULATIONS OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) WITH HEMOLYSIS

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a rare and life-threatening blood disease characterized by complement-mediated destruction of red blood cells (RBCs). Overall results from two Phase 3 clinical studies enrolling a heterogeneous group of PNH patients with hemolysis show that the terminal complement inhibitor eculizumab (Soliris™) significantly reduces hemolysis resulting in improved anemia, fatigue and other patient reported quality of life outcomes. *Aims.* Evaluate the efficacy of eculizumab across diverse subpopulations of patients with PNH. *Methods.* Efficacy outcomes with eculizumab treatment in various patient subpopulations were evaluated including hemolysis (lactate dehydrogenase, LDH, change from baseline at 6 months), transfusion requirements (median units transfused per patient 6 months before versus 6 months during eculizumab treatment), and fatigue (FACIT-Fatigue score change from baseline at 6 months) using the combined datasets from the Phase 3 clinical studies TRIUMPH and SHEPHERD.

Table 1. Effect of Eculizumab on 6-month outcomes in patients with PNH.

Sub-populations	Strata	N	Median change in LDH, U/L	N	Median units transfused pre- vs post-eculizumab	N	Median change in FACIT-Fatigue*
History of AA/MDS	Yes	38	-1379	39	7.0 vs 0.0	38	8.8
	No	99	-1879	101	7.0 vs 0.0	97	7.0
Platelets† (10^9/L)	≥151	69	-1840	70	6.0 vs 0.0	68	8.5
	<151	68	-1486	70	8.0 vs 2.0	67	6.0
Reticulocytes† (10^{12}/L)	≥0.2	68	-1973	70	8.0 vs 0.0	67	6.0
	<0.2	69	-1296	70	6.0 vs 0.0	68	9.0
Steroid Usage	Yes	33	-1954	33	6.0 vs 2.0	32	8.0
	No	104	-1795	107	8.0 vs 0.0	103	7.0
Erythropoietin Usage	Yes	8	-1560§	8	7.5 vs 1.0§	8	5.0§
	No	129	-1835	132	7.0 vs 0.0	127	7.0
PNH Type III RBCs† (%)	≥31	69	-2161	70	5.0 vs 0.0	68	8.0
	<31	68	-1486	70	8.0 vs 2.0	67	6.0
Hemolysis† (LDH, U/L)	≥2042	69	-2534	70	7.5 vs 1.5	68	8.8
	<2042	68	-1104	70	6.0 vs 0.0	67	4.7
Hemoglobin† (g/dL)	≥9.8	70	-1699	70	8.0 vs 0.0	68	5.5
	<9.8	67	-1889	70	4.0 vs 1.5	67	8.0
Pre-treatment Transfusion Strata‡	<4	21	-1798	21	0.0 vs 0.0¶	21	9.0
	4-14	61	-1500	62	5.5 vs 0.0	60	9.0
	15-25	32	-1806	32	10.0 vs 2.0	32	4.0
	25+	23	-1954	25	16.0 vs 4.0	22	6.5
Transfusion Independence on Treatment	Yes	75	-1625	75	6.0 vs 0.0	74	6.0
	No	62	-1920	65	8.0 vs 6.0	62	7.0

*Positive change is an improvement in fatigue; >3 points is clinically meaningful
†Strata are above versus below median value at baseline
‡Units packed RBCs transfused per patient over 12-months pre-treatment
All comparisons are $P<0.001$, except for §$P\leq0.031$ and ¶P=not significant

Results. Statistically significant improvements in hemolysis, transfusion requirements and fatigue with eculizumab treatment were observed for

each of multiple PNH subpopulations (Table 1) including patients with: (1) a history of bone marrow dysfunction (AA or MDS); (2) thrombocytopenia; (3) lower levels of reticulocytes; (4) different concomitant medications including steroids or erythropoietin; (5) smaller proportions of PNH cells (PNH clone size); (6) lower levels of baseline hemolysis; (7) lower levels of anemia; (8) minimal pre-treatment transfusion requirements; (9) and without complete transfusion independence during eculizumab treatment. Significant improvements in hemolysis ($p<0.001$), transfusion requirements ($p<0.001$), and fatigue ($p<0.007$) were also demonstrated regardless of age, gender, race, weight, geography, disease duration, concomitant iron/folate or anticoagulant usage, and whether or not the patient had a history of thrombosis. Summary and conclusions. All patients treated with eculizumab respond with a marked reduction in hemolysis, the hallmark of PNH. Eculizumab significantly reduced hemolysis and transfusion requirements, and improved fatigue in patients regardless of whether they become entirely transfusion independent during treatment. Finally, the improvement in hemolysis with eculizumab directly leads to significant clinical benefit across all subgroups in a broad and diverse PNH population, including patients with poor bone marrow function, smaller PNH clone sizes, low levels of hemolysis, less anemia, minimal pre-treatment transfusion requirements and those receiving steroids or erythropoietin.

0380

THE TERMINAL COMPLEMENT INHIBITOR ECULIZUMAB REDUCES THROMBOSIS IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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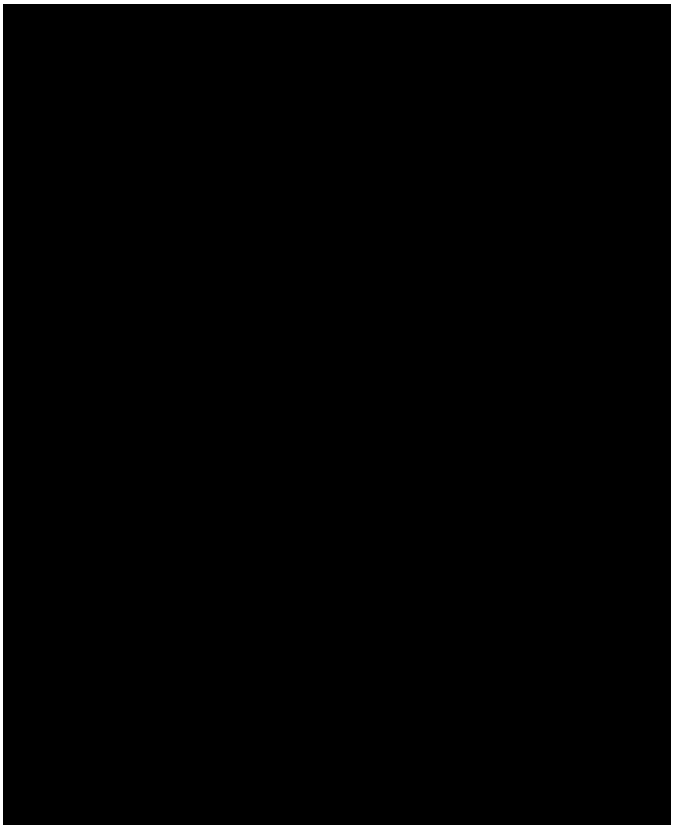
Background. Life-threatening thromboembolism (TE) is the most feared complication in paroxysmal nocturnal hemoglobinuria (PNH). TE accounts for approximately 45% of PNH patient deaths. Rapidly fatal TE without a previous thrombotic history occurs in PNH, highlighting the importance of effective preventative therapy in this population. Primary prophylactic anti-coagulation potentially reduces the thrombotic risk in PNH, although controlled studies have not been performed and there is a risk of life-threatening hemorrhage. Three studies of the terminal complement inhibitor eculizumab in PNH recently demonstrated dramatic reductions in intravascular hemolysis and units of red cells transfused with improvements in fatigue and other patient reported outcomes. *Aims.* To determine whether treatment with eculizumab reduced TE risk in patients with PNH. *Methods.* We systematically and prospectively examined the aggregate TE event rate in eculizumab-treated patients from the three parent studies and a subsequent common extension study compared to the TE rate in the same patients pre-eculizumab. *Results.* Before receiving eculizumab, 124 TE events in 195 patients were identified; 103 patients were on antithrombotics. Pre-treatment TE events in the study group occurred in both venous (85%) and arterial (15%) sites. The most common sites of venous thrombosis were lower extremity deep veins (18.5%), mesenteric/splenic veins (18.5%), hepatic/portal veins (16.9%), and other deep veins (14.5%), while the most common site of arterial thrombosis was cerebrovascular accident/transient ischemic attack (13.7%, median age of 38). Eculizumab reduced the TE rate in each of the three parent studies. The aggregate TE event rate during eculizumab treatment was significantly reduced by 85% when compared with the same patients before eculizumab treatment (see Table 1). With restriction of the pre-treatment observation period to the 12-months immediately preceding eculizumab treatment, the TE event rate during eculizumab treatment was significantly reduced by 94%. The TE event rate in patients with TE prior to the trials was also significantly reduced by 89%. Most TE events prior to eculizumab treatment occurred in patients receiving antithrombotics either therapeutically or prophylactically, indicating that this therapy may be insufficient to prevent thrombosis. Of 103 patients on antithrombotics, there were 54 TE events in 30 patients pre-eculizumab compared to one TE event during eculizumab treatment, demonstrating that eculizumab significantly reduces the risk of thrombosis in PNH patients despite treatment with antithrombotics. Pre-eculizumab, TE was frequent in patients with lower levels of hemolysis and with mild anemia; eculizumab significantly reduced TE across these and other subgroups. *Summary and conclusions.* These data show that in PNH, TE can occur in both the arterial as well as the venous systems, in the context of lower levels of hemolysis or with mild anemia, and patients may continue to experience thrombosis during antithrombotic therapy. Long-term eculizumab treatment results in a clinically and statistically significant reduction in thrombosis in PNH, including patients with mild anemia and lower levels of hemolysis. Considering that TE contributes to the majority of deaths in PNH, it is reasonable to expect that eculizumab treatment, by decreasing the risk of TE, will increase the life-expectancy in PNH.

Table 1. Effect of eculizumab on thrombosis in PNH Subpopulations.



Cytogenetics and molecular diagnostics

0381

KARYOTYPE IS AN INDEPENDENT PROGNOSTIC FACTOR IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Pre-treatment cytogenetics is a known predictor of outcome in haematological malignancies. However, its usefulness in adult acute lymphoblastic leukaemia is generally limited to the presence of the Philadelphia chromosome (Ph) because of the low incidence of other recurrent abnormalities and the rarity of the disease in this age group. We present centrally reviewed cytogenetic data from adult patients enrolled on the Medical Research Council (MRC) UKALLXII / Eastern Cooperative Oncology Group (ECOG) 2993 Trial MRC. Cytogenetic analysis was attempted in 1,366/1,522 (90%) patients. This rate did not vary by registration centre, year of diagnosis or patient age. Cytogenetic analysis was successful in 1,003 (73%) cases but was considered unacceptable in 363 (27%) cases. Among those patients with successful cytogenetics an abnormal clone was detected in 796 (79%). There was no trend towards a higher rate of unacceptable cytogenetics in the early part of the trial compared with the later years. Patients were classified according to the presence of each of the following chromosomal abnormalities: t(9;22)(q34;q11.2) (Ph) (n=267, 19%); t(4;11)(q21;q23) (n=54, 7%); other MLL/11q23 translocations (n=15, 2%); t(1;19)(q21;p13.3) (n=24, 3%); t(8;14)(q24.1;q32) plus variants (n=16, 2%); t(10;14)(q24;q11.2) (n=16, 2%); other T-cell receptor translocations (n=18, 2%); 14q32 translocations (n=45, 6%); 6q deletions (n=55, 7%); 7p deletions (n=23, 3%); -7 (n=19, 2%); + 8 (n=23, 3%); + X (n=34, 4%); 9p deletions (n=71, 9%); 11q abnormalities (n=29, 4%); 12p deletions (n=29, 4%); -13 / 13q deletions (n=40, 5%); 17p deletions (n=40, 5%); complex karyotype (five or more chromosomal abnormalities) (n=41, 5%); low hypodiploidy (30-39 chromosomes) / near-triploidy (60-78 chromosomes) (Ho-Tr) (n=31, 4%); high hyperdiploidy (51-65 chromosomes) (n=77, 10%); tetraploidy (80 or more chromosomes) (n=15, 2%); other abnormality (n=102, 13%); normal karyotype (n=195, 25%). Ph-positive patients had a significantly inferior 5 year overall survival (OS) compared with Ph-negative patients: 22% (95% CI 17%-27%) versus 41% (38%-44%) [p=0.0001 adjusting for age, gender and white cell count (WCC)]. Within the Ph-negative cohort, the following four chromosomal abnormalities were associated with a poorer outcome: t(4;11), OS 24% (13%-36%), p<0.001; t(8;14), OS 13% (2%-33%), p<0.001; complex karyotype, OS 28% (15%-43%), p=0.027; and Ho-Tr, OS 22% (9%-38%), p=0.001). In contrast, patients with high hyperdiploidy or 9p deletions had a significantly improved outcome compared to other Ph-negative patients: OS 53% (41%-64%), p=0.015 and OS 58% (46%-69%), p=0.032, respectively. We used a Cox proportional hazards model to assess the prognostic relevance of cytogenetic variables within the Ph-negative cohort in the context of other established survival indicators: gender, age, WCC and T-cell status. The adverse effect of t(8;14), Ho-Tr and complex karyotype was shown to be independent of these risk factors both within the overall cohort and a reduced cohort which excluded patients undergoing a bone marrow transplant. The observation that Ho-Tr and, for the first time, karyotype complexity confer an increased risk of treatment failure demonstrates that cytogenetic subgroups other than the Ph can and should be used to risk stratify adults with ALL in future trials.

0382

ETV6-NCOA2 FUSION DEFINES A NEW ENTITY OF ACUTE LEUKEMIA WITH COEXPRESSION OF T-LYMPHOID AND MYELOID MARKERS

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Background. In acute leukemia coexpression of T-lymphoid and myeloid markers is a rare finding and poses diagnostic and therapeutic challenges. Owing to the lack of specific genetic markers the molecular basis for a biphenotypic immunophenotype is unclear. Aims and Methods. To identify novel genetic lesions associated with biphenotypic leukemia displaying T-cell and myeloid markers determined by immunophenotyping, conventional cytogenetics, fluorescence *in situ* hybridization (FISH) and molecular analyses were employed. *Results.* We have identified a novel recurrent t(8;12)(q13;p13), which results in a fusion between the transcriptional repressor ETV6 (TEL) and the transcriptional coactivator NCOA2 (TIF2) in six cases of childhood leukemia. ETV6 encodes a transcription repressor and is frequently involved in chromosome rearrangements with a multitude of partners in acute leukemia. NCOA2 is a member of the p160 family of nuclear receptor transcriptional coactivators (NRCoAs), which have a common domain structure with a conserved N-terminal bHLH-PAS domain, a centrally located (nuclear) receptor interaction domain (RID/NID), and a C-terminal transcriptional activation domain (AD2). NCOA2 also contains a C-terminal CREB binding protein (CBP)-interaction domain (CID), and thus is a transcription factor that mediates transcriptional activation in a ligand-dependent fashion and involves chromatin remodeling. The ETV6-NCOA2 transcript encodes a chimeric protein that consists of the pointed protein interaction motif of ETV6 that is fused to the C-terminus of NCOA2 including the CBP interaction and the AD2 activation domain. The same C-terminal domains of NCOA2 are also involved in the previously identified MOZ-NCOA2 fusion, which is generated by a inv(8)(p11q13). The absence of the reciprocal NCOA2-ETV6 transcript in one of the cases, and the facts that MOZ-NCOA2 is transforming and that the reciprocal NCOA2-MOZ is not expressed, strongly suggest that the ETV6-NCOA2 chimeric protein and not the reciprocal NCOA2-ETV6 is responsible for leukemogenesis. In addition, heterozygous activating NOTCH1 mutations, which disrupt the heterodimerization (HD) or the PEST domains and are present in over 50% of childhood T-ALL, as well as in rare cases of AML, particularly in the context of lineage switch leukemia, were detected in 4 out of 5 samples analyzed. The blast cells of all six leukemias consistently expressed cytoplasmic CD3 and CD7, and additional T-cell specific markers such as CD2 and CD5 to a variable extent, and at least one myeloid marker, either MPO, CD33 or CD13 or a combination of these markers. Moreover, all blast cells of all samples were double negative for CD4 and CD8, and except for one case CD34 positive. As consequence of the myeloid antigen expression pattern and in accordance with the European Group for the Immunological Characterization of Leukemias (EGIL), the patients were assigned to different subtypes, namely three of the cases were classified as T-ALL (one as T-I My+) and three as biphenotypic leukemia (BAL). *Conclusions.* Together, these data suggest the ETV6-NCOA2 fusion defines a new entity of leukemia with T-lymphoid and myeloid features, and may either enforce a biphenotypic leukemia by reprogramming the target cell or may occur in a leukemic stem cell that precedes both T-lineage and myeloid commitment.

0383

TWO DISTINCT TYPES OF GENOMIC REARRANGEMENTS INVOLVE THE C-MYB LOCUS IN HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA, INCLUDING A TRANSLOCATION T(6;7) DEFINING A NEW LEUKEMIC SUBGROUP IN VERY YOUNG CHILDREN

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Background. Cytogenetics and expression studies have pointed out an increasing number of oncogenes in T-cell acute lymphoblastic leukemias (T-ALL), demonstrating the complexity of T-ALL oncogenesis and the requirement for a network of cooperative oncogenic events. Classifying T-ALL oncogenes include aberrantly activated transcription factors, namely bHLH (TAL1, TAL2, and LYL1), homeobox genes (HOXA, TLX1/HOX11, TLX3/HOX11L2), and CALM-AF10 and MLL fusion genes. These oncogenes are frequently activated by chromosomal translocation or intrachromosomal cryptic deletion. Combined analysis of the T-ALL genetic alterations and large scale gene expression has allowed the definition of several major T-ALL subtypes. *Aims.* We aimed to identify new oncogenic alterations in T-ALL and include them in the genetic and biological networks defining T-ALL. A series of 84 T-ALL including 80 primary samples from children and adults, and 4 cell lines, was investigated by conventional and molecular cytogenetics approaches. *Methods.* In order to identify new T-ALL oncogenes, TCR-related translocations were searched for using FISH flanking probes. Partner sequences were cloned using inverse circled PCR. In addition, a genome-wide copy-number analysis was performed using a customized 4K BAC/PAC array-CGH on the 84 T-ALL cases. *Results.* Two distinct recurrent genetic events were identified that both involved the C-MYB locus at 6q23. First, a reciprocal chromosomal translocation t(6;7)(q23;q34) was identified that juxtaposed the TCRB and the C-MYB loci (n=4 cases out of 84; two additional independant TCRB-MYB samples were also studied leading to a total of 6 cases). Second, the array-CGH analysis identified a cryptic duplication of a short genomic region including C-MYB (n=14 cases out of 84 T-ALL, 17%). The somatic origin of the duplication was demonstrated using paired leukemic and remission DNA, and a minimal duplicated region of approximately 230 Kb was mapped using 244K oligonucleotide array-CGH. RQ-PCR analysis showed an increased C-MYB expression in both the translocated and duplicated cases compared to other T-ALL cases and normal controls. Strikingly, the TCRB-MYB translocation occurred in very young children (median 2 years-old, p=10-4). Moreover, profiling of the T-ALL series by large scale expression analysis showed that the TCRB-MYB cases defined a unique T-ALL case cluster associated to a proliferation/mitosis signature. By contrast, the MYB-duplicated cases were found at various ages and in previously defined distinct T-ALL oncogenic subtypes (SIL-TAL1, TLX1, TLX3, CALM-AF10, MLL or Immature cases). *Conclusions.* The C-MYB gene has been a candidate oncogene in human hematologic malignancies for years, based on its homology with the viral oncogene v-Myb and frequent targeting by retroviral insertions in mouse leukemia. This work is the first clear demonstration of the recurrent involvement of the C-MYB locus in a human neoplasm, namely T-ALL, in two distinct genetic alterations. The C-MYB transcription factor is known to play a major role in early and definitive hematopoiesis, and it has been involved at several key steps throughout the normal thymic differentiation process. It is therefore likely that deregulated C-MYB expression due to genomic events is oncogenic in T-ALL. Importantly, the t(6;7) translocation defines a new subtype of T-ALL associated with a very young age and a proliferation/mitosis signature.

0384

SYSTEMATIC EVALUATION OF COPY NUMBER ALTERATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AND THEIR ASSOCIATION WITH TREATMENT RESPONSE

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Background. in vivo response to initial therapy, as assessed by determination of minimal residual disease on treatment day 33 and at week 12 of treatment, has evolved as the strongest prognostic factor in children with acute lymphoblastic leukaemia (ALL) treated according to the BFM regime (ALL-BFM-2000). However, it is not known so far, whether the individual treatment response might be influenced by copy number alterations, i.e. small deletions or amplifications, leading to altered gene expression. *Aims.* We therefore aimed to systematically evaluate copy number alterations (CNA) using high resolution array-comparative genomic hybridization (array-CGH) in different treatment-response groups. *Methods.* Leukemic DNA of 25 patients with a poor (MRD load > 10-3 at week 12, MRD-high risk, HR) and 25 patients with a good treatment response (no measurable MRD at weeks 5 and 12, MRD-standard risk, SR) were compared. For array-CGH, a DNA chip containing more than 8000 individual BAC/PAC clones leading to a genome-wide resolution of at least 1 Mb was used. Hybridization, image analyses and data processing was performed as described recently (Steinemann et al. 2006). Selected CNA were validated by fluorescence *in situ* hybridization. *Results.* Copy number alterations (CNA) were found in 46 patients (92%), in both patient groups. Microscopic alterations affecting the whole or nearly whole chromosome arm were frequently found, e.g. gain of 21 in 11/50, loss of 9p in 5/50, loss of 8p in 3/50, loss of 20q in 3/50 and loss of 7p in 2/50 or gain of 1q in 2/50. A gain of chromosome 1q23-qter was found in 10/25 SR-patients, whereas none of the HR-patients showed this gain with a breakpoint in 1q23 (p<0.002). This gain may result from an unbalanced translocation der(19)t(1;19)(q23;p13). Cytogenetically, two forms, a balanced t(1;19)(q23;p13) and an unbalanced der(19)t(1;19)(q23;p13) exist, both giving rise to the E2A/PBX1 fusion gene. To confirm our findings and to analyse, if additional cases may carry an unrecognized balanced t(1;19), we performed RT-PCR to detect the E2A/PBX1 fusion transcript. In consistence with array-CGH, all cases with gain of 1q23-qter -but no additional cases- showed the fusion transcript. A gain/amplification of 21q21-q22 was not only observed in the context of hyperdiploidy but also seen in six additional cases as an isolated gain. 8 patients of the SR-group in comparison to 3 patients of the HR-group showed a gain of 21q21-q22 (p<0.17). Moreover, amplifications of 21q21-q22 were observed only in the SR-group. The frequency of other CNA like loss of 9p did not differ between both patient groups. No recurrent genomic imbalances (>1-2 Mb) were found in the HR-group. *Conclusions.* This is the first study to evaluate the clinical significance of CNA as detected by array-CGH in childhood ALL. Array-CGH had a very high sensitivity to detect CNA. Gains of 1q23-1qter due to an unbalanced t(1;19)(q23;p13) and gains of 21q turned out as markers for good treatment response in the context of the ALL-BFM regime.

0385

CYTOPLASMIC MUTATED NUCLEOPHOSMIN DEFINES THE MOLECULAR STATUS OF A SIGNIFICANT FRACTION OF MYELOID SARCOMAS

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Detection of genetic lesions is critical for classification, prognostic stratification, and monitoring of minimal residual disease of acute myeloid leukaemia (AML). Information on genetic lesions associated with myeloid sarcoma (MS), a tumor mass consisting of myeloblasts or immature myeloid cells at an extra-medullary site, is still limited. This is mainly due to the fact that fresh cells are usually not available for cytogenetic and/or molecular studies, since the diagnosis of MS is frequently unexpected and/or the size of the sample is small, such as a skin punch biopsy. Since MS is usually treated in the same way as AML, the frequent lack of available cytogenetics in MS, represents a significant disadvantage, and the availability of techniques applicable to paraffin samples to detect specific genetic lesions would be of great help both for diagnostic and prognostic purposes. Aberrant cytoplasmic expression of nucleophosmin (NPM), a multifunctional shuttling protein usually located in the nucleus, is a key property of a large subgroup of AML exhibiting distinguishing biological and clinical features, that we termed NPMc$^+$ (cytoplasmic-positive) AML (Falini B. *et al.,* NEJM 352:254-266, 2005; Falini B. *et al.*, 109:874-885, 2007). In NPMc+ AML, leukemic cells harbour NPM1 gene mutations generating NPM leukemic mutants that are exported at high rate from the nucleus and accumulate in the cytoplasm (Falini B. *et al.*, Blood 107:4514-4523, 2006). Aberrant cytoplasmic NPM expression and/or NPM1 mutations have been investigated in AML. However, there is no information on the role of NPM in MS, a tumor that may develop de novo (preceding bone marrow involvement), present concurrently with AML, or represent manifestation of AML relapse or blastic transformation of a pre-existing chronic myeloproliferative disorder. Since NPM1 mutations cause aberrant cytoplasmic dislocation of NPM that is fully predictable by immunohistochemistry (Falini B. *et al.,* Blood 108:1999-2005, 2006), we used anti-NPM monoclonal antibodies recognizing both wild-type and mutated NPM to detect cytoplasmic NPM in paraffin-embedded samples from 181 MS retrieved from the archives of four large Institutions. Reactivity was evaluable in 173/181 cases: 146 (85.0%) with nucleus-restricted NPM (predictive of unmutated NPM1) and 26 (15.0%) with aberrant cytoplasmic NPM (predictive of NPM1 mutations). The presence or absence of NPM1 mutations was confirmed in paraffin sections by immunostaining with specific antibodies against the NPM mutants and, in a subset of cases, also by a polymerase chain reaction (PCR) assay of NPM1 sequence we developed for application on paraffin-embedded formalin-fixed samples. NPMc+ MS showed the same distinctive features as NPMc$^+$ AML, including frequent M4 or M5 morphology, CD34-negativity, association with normal karyotype, and no previous history of myelodysplastic or chronic myeloproliferative disorders. Thus, immunohistochemistry can serve as a surrogate of molecular studies for detecting NPM1 mutations in MS. This study identifies NPM1 mutations as the most frequent genetic lesion so far reported in MS, accounting for 15% of cases. Our genetic findings have clear implications for the upcoming WHO classification of myeloid neoplasms. Clinical prospective studies aimed to assess the prognostic value of NPM1 mutations (in combination with FLT3-ITD) in MS are also warranted.

Platelets, vascular biology and transfusion medicine

0386

A PLACEBO CONTROLLED, CROSS-OVER STUDY OF CITRATE EFFECTS ON BONE METABOLISM IN HEALTH VOLUNTEERS

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Background. Citrate is the anticoagulants of choice when performing apheresis donations. Less is known about the possible effects of citrate-induced hypocalcemia on bone turnover. *Aims.* Aim of the study was to investigate the possible effects of citrate on biochemical markers of bone metabolism. *Methods.* A placebo controlled, cross-over trial was performed in 10 male volunteers. Volunteers received two standardized infusion (80 min.) containing either citrate (1.5 mg/kg/min) or saline solution, separated by a wash-out period of 2 weeks. At each study time point blood and urinary samples were collected before, during, and after completion of the infusions. Samples were analysed for short- and long-term markers of bone turnover, PTH and electrolytes. *Results.* Application of citrate led to a profound decrease in serum level of iCa, potassium and phosphate. Decrease of iCa was inversely related to the increase in iPTH and the urinary excretion of Ca. Infusion of citrate resulted in a time-dependent steadily increased of serum levels of the bone formation marker osteocalcin (OC) and the bone resorption marker C-telopeptide of type 1 collagen (CTX). Peak levels of both bone markers were reached at the end of the citrate infusion (compared to base +34% for OC and +57% for CTX, respectively). Both bone parameters showed a positive correlation to the increase of PTH, as determined at the end of the citrate infusion. In addition, changes of OC but not CTX were related to the variations in serum iCa. Alterations in both bone markers were still detectable 90 min after completion of the citrate infusion. In contrast, no alterations were observed in the serum levels of the long-term bone markers bone specific AP, tartrate-resistant acid phosphatase 5b, osteoprotegerin or RANKL. *Conclusions.* Infusion of citrate equal to the dose used in voluntary platelet apheresis donation results in profound alterations of biochemical markers of bone turnover. The pattern of variations in the serum levels of the bone markers OC and CTX resembled to those of an increased bone resorption.

0387

A SMALL THROMBOPOIETIC MOLECULE NIP-004 IS EFFECTIVE FOR THE TREATMENT OF THROMBOCYTOPENIA INDUCED BY INTERFERON-ALPHA

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Background. Human interferon-α (IFN) is useful to treat chronic hepatitis C. However, doctors often reluctantly reduce the dose of IFN, or discontinue therapy, because of IFN-induced thrombocytopenia. Although IFN is believed to directly inhibit megakaryopoiesis, its mechanisms are not clearly understood. *Aims.* In this study, we evaluated the efficacy of a small non-peptidyl thrombopoietic molecule, NIP-004, for the treatment of IFN-induced thrombocytopenia and studied the inhibitory mechanisms of IFN for megakaryopoiesis. *Methods. In vivo* assay: After 2.4 Gy-irradiation, 1×10^5 of human umbilical cord blood-derived CD34$^+$ cells was intravenously injected into immunodeficient NOD/Shi-scid/IL-2Rγ-null (NOG) mice. Pegylated-recombinant human interferon-α2b (PEG-Intron®) and NIP-004 were subcutaneously administered into the mice. The number of platelets and human megakaryocyte ploidy were obtained by a flow cytometer. *in vitro* assay: The colony formation of human megakaryocytes (CFU-MK) assay was performed using human bone marrow (BM)-derived CD34-positive cells in collagen based semi-solid culture, and the proplatelet (PPT) formation assay was performed using human BM-derived CD34-positive cells in serum-free liquid culture, with the various combination of c-Mpl activator (thrombopoietin or NIP-004) and IFN. *Results.* When IFN at 30 microgram/kg was administered three times a week for 3 weeks into NOG mice transplanted with human CD34$^+$ cells, the number of human platelets was significantly reduced by 40% compared to the control mice. Seven weeks treatment with IFN resulted in 70% reduction of human platelets [$27.7\pm6.8\times10^6$/mL (control), $8.8\pm1.8\times10^6$/mL (IFN), n=5, $p<0.05$, Student t-test). When we administered NIP-004 to this IFN-induced thrombocy-

topenia model, the human platelet count was fully recovered. Interestingly, the numbers of $CD34^+CD41^+$ human megakaryoblasts and 4N to 128N human megakaryocytes in xenografted mice were not significantly changed compared to the control mice after treatment with IFN for 7 weeks. We also analyzed the inhibitory effects of IFN on megakaryocytes *in vitro*. Although IFN slightly reduced the number of CFU-MKs [146±13 colonies/5000 cells (control), 130±13 colonies/5000 cells (10 ng/mL of IFN)], the percentage of megakaryocytes bearing PPT was significantly reduced by IFN [20.4±3.0%(control), 12.5±3.6% (10 ng/mL of IFN)]. Currently, we are investigating the inhibitory mechanisms of IFN for PPT. *Conclusion*. These results indicated that NIP-004 is effective for preventing IFN-induced thrombocytopenia *in vivo*. IFN might inhibit the PPT formation rather than the proliferation of human megakaryocytopoiesis. We expect that the small thrombopoietin molecule, NIP-004, can be applied for the treatment of IFN-induced thrombocytopenia in the patients with chronic hepatitis C.

0388

IN VITRO AND *IN VIVO* MICROPARTICLE-ASSOCIATED ENDOTHELIAL PROTEIN C RECEPTOR CAN REVERSE PRO-INFLAMMATORY AND APOPTOTIC CELL SIGNALS AND EFFECTS

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Background. We have described a novel mechanism of endothelial protein C receptor (EPCR) release from cell surfaces *in vitro* and *in vivo*. Induced by Activated Protein C (APC), EPCR is released in microparticulate (MP) form. Unlike APC bound to truncated soluble EPCR, APC on MP-EPCR retains proteolytic anticoagulant activity. We now hypothesise that the MP EPCR-APC complex can also cleave endothelial protease activated receptor-1 (PAR1) to modulate inflammation and cytoprotection. *Aims.* 1. To compare the efficacy of MP EPCR-APC versus free-APC, using two different pools of endothelial cells, following exposure to a pro-inflammatory stimulus. 2. To determine if circulating MP EPCR-APC from patients can regulate gene expression in a PAR1-dependent manner. *Methods.* Using an *in vitro* system of human umbilical vein endothelial cells (HUVECs) or human coronary artery endothelial cells (HCAECs), TNFα, 10 ng/mL, was added to represent a cell model of sepsis. After 4 hours the cells were treated with an equal concentration of either free or MP-bound APC and left for a total of 24 hours. RNA was extracted from the cells and used to make probes for hybridization of GEArray Human Endothelial Cell Biology Gene Array (SuperArray). Using untreated cells as a baseline, the specificity of APC and the role of PAR1 were assessed by using the appropriate blocking antibodies and antagonist. Circulating MPs were isolated from plasma of APC treated patients by standard methods and platelet-derived MPs depleted using a CD41a antibody and magnetic beads. MP-associated APC was estimated from a standard curve and 17nM used for gene profiling. PAR1-mediated signalling was examined as above. *Results.* In TNFα stimulated cells, APC, selectively induced anti-inflammatory and suppressed pro-apoptotic gene regulation. In over 90% of these genes, MP-APC had a 2-7 fold greater effect in relation to free-APC. These observations were confirmed by Q-PCR for e-selectin, ICAM1, Bax, A20, caspase 10, GM-CSF and IL8. This translated into increased GM-CSF and interleukin 8 secretion, and cytoprotection against staurosporine-induced apoptosis. Protein C or PAR1 antagonism reversed these results to demonstrate that the induced effects by MPs were APC specific and PAR1-dependent. Further analysis using MP-associated EPCR from septic patients during APC treatment also demonstrated PAR1-mediated anti-inflammatory gene induction and anti-apoptotic function. *Conclusions.* APC on MP-associated EPCR can ameliorate TNF induced inflammatory changes and staurosporine induced apoptosis in both HUVECs and HCAECs. As these effects are reproducible with MPs isolated from patients' plasma, this may have clinical relevance in the septic patient treated with APC.

0389

ENDOTHELIAL COLONY FORMING CELLS FROM PATIENTS WITH CML, POLYCYTEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA, DERIVE FROM A STEM CELL LACKING THE DISEASE SPECIFIC MARKER

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Background. the persistence of a common progenitor to haematopoietic and vascular endothelial lineage in the adult haematopoietic tissue appeared to be validated when Bcr/Abl fusion gene was found in a variable proportion of endothelial like cells generated *in vitro* from the bone marrow and the peripheral blood of CML patients. However, the different colture 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: early EPC, i.e. colony forming unit-endothelial cells (CFU-EC), are haematopoietic derived progeny committed to the myeloid lineage while late EPC, namely ECFCs, are vessel-forming progenitors. *Aims.* we have reconsidered the issue of endothelial cell origin (status) in CML and other MPDs to verify whether circulating EPCs, isolated and expanded from the peripheral blood of patients, bear the disease specific genetic marker of the leukaemia clone or this is restricted to the haematopoietic lineage. *Methods.* Forty-three patients and 12 normal controls were included in this study. Twenty-four samples were Philadelphia and Bcr/Abl positive CML, 19 were Philadelphia and Bcr/Abl negative 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 the low frequency of late EPCs in the peripheral blood of patients, only 7/24 CML and 6/19 MPD samples gave rise *in vitro* to a progeny of EC. None of them carried the disease specific genetic marker. The expanded cells showed a complete outfit of endothelial associated antigens and were able to form capillary like structures *in vitro*. *Conclusions.* differently from previous reports and based on a clear distinction between early and late EPCs, this study shows for the first time that endothelial cells, derived in culture from Ph-positive, Bcr/Abl positive CML patients, lack the disease marker in the late EPC. In addition late EPCs 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, and multiple explanations could be hypothesized: the leukemogenetic event that starts the CML involves a stem cells below the common ancestor cells. Indeed, most T cells in CML do not belong to the leukemic clone. Alternatively, since in the majority of newly diagnosed patients with CML, the bone marrow contains a high number of normal Ph-negative early progenitor cells (LTC-IC), one could argue that Ph-negative stem cells still give rise to EPC therefore diluting those deriving from Ph-positive ones. The same apply to MPD where it is now evident that part of haematopoietic progenitors do not carry the JAK2-V617F mutation.

0390

ELTROMBOPAG RAISES PLATELET COUNT AND REDUCES BLEEDING COMPARED WITH PLACEBO DURING SHORT-TERM TREATMENT IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA: A PHASE III STUDY

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Background. Chronic idiopathic thrombocytopenic purpura (ITP) is a disease characterized by increased platelet destruction and impaired platelet production. In a previous, large phase II study, eltrombopag, a novel, non-peptide, oral platelet growth factor, increased platelet counts in adults with chronic ITP. *Aims.* In this global, randomized, double-blind, placebo-controlled, phase III trial, the safety and efficacy of eltrombopag was studied in adults with chronic ITP and platelet counts <30,000/μL. *Methods.* Patients received placebo or eltrombopag 50 mg (1:2 ratio) once daily for up to 6 weeks. Dose escalation to 75 mg (or matching placebo) was allowed after 3 weeks in subjects whose platelet count continued to be < 50,000/μL. The primary endpoint was the proportion of patients with a platelet count ≥50,000/μL after up to 42 days of dosing. Secondary endpoints included bleeding, safety and tolerability. Patients were stratified by splenectomy status, use of concomitant ITP therapy and platelet count ≥15,000/μL. *Results.* Patients (N=114) were randomly assigned to placebo (n=38) or eltrombopag (n=76); 61% were female and 74% were White. All patients had received prior ITP treatment and 52% had received ≥3 prior therapies. Stratification included 43% of subjects receiving concomitant ITP therapy (primarily corticosteroids); 39% refractory to splenectomy; and 48% with baseline platelet counts ≥15,000/μL. At the end of treatment, 16% of placebo patients and 59% of eltrombopag patients achieved the primary endpoint (platelet count ≥50,000/μL), with median counts of 18,000/μL (placebo) and 69,000/μL (eltrombopag). There was a significant increase in the odds of responding in the eltrombopag versus placebo arm (odds ratio [OR] = 9.61, p<0.001). Three percent of placebo and 25% of eltrombopag patients achieved a platelet count >200,000/μL. Response to treatment was observed regardless of strata. The odds of any bleeding, measured by the WHO bleeding scale, was significantly lower in the eltrombopag arm on day 43 (OR = 0.27, p=0.029) and over the entire course of treatment (OR = 0.48, p=0.018). Clinically significant bleeding (WHO Grade 2-4) between Days 15 and 50 was observed in fewer patients receiving eltrombopag (16%) compared with those receiving placebo (36%). The most common AEs were headache (11% and 8%), nausea (0 and 8%), nasopharyngitis (8% and 7%), diarrhoea (3% and 5%) and vomiting (0% and 5%) for placebo and eltrombopag, respectively. No apparent safety concerns were identified following thorough examination of clinical chemistries, haematology, electrocardiographic findings and preliminary results of ophthalmic examinations. *Conclusions.* Daily eltrombopag 50 mg was highly effective in raising platelet counts and reducing bleeding during the 6-week treatment period. No safety concerns were identified. Long-term and repetitive treatment studies are ongoing in patients with chronic ITP.

Hodgkin's lymphoma

0391

COMBINED ESCALATED BEACOPP-ABVD THERAPY GUIDED BY PET-CT FOR ADVANCED HODGKIN'S LYMPHOMA PATIENTS WITH HIGH IPS SCORE

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Background. The expected 5-year freedom from progression of advanced stage Hodgkin's lymphoma (HL) patients (pts) with IPS≥3, treated with COPP-ABVD, was reported as 55%. While the superiority of escalated (esc) BEACOPP regimen over COPP-ABVD was shown for all risk groups, it was more pronounced in pts with a poor IPS. However, pts receiving escBEACOPP had more acute and long-term toxicities including a higher incidence of AML/MDS. In an attempt to reduce this toxicity, while preserving improved initial tumor control in this high risk group of pts, we conducted a phase II study, which tested the feasibility, toxicity and efficacy of a regimen which utilized the combination of escBEACOPP and ABVD. *Methods.* Newly diagnosed HL pts, with unfavorable stage IIB or stages III-IV with IPS≥3 were initially received two cycles of escBEACOPP followed by reevaluation with PET-FDG/CT scans. When complete or partial response (CR, PR) was achieved, pts then continued to receive four cycles of ABVD, while pts who failed to obtain this response received salvage therapy. *Results.* Since starting in late 2001, 40 eligible pts received this regimen. Median age at diagnosis was 27 years (range 18-56) and 29 (73%) were males. Histology included nodular sclerosis (n=30), mixed cellularity (n=6) and unclassified (n=4). Stage IV, III and IIB were evident in 29 (73%), 8 (20%) and 3 (7%) pts, respectively and extranodal involvement was noted in 28 (70%). Following the first two cycles of escBEACOPP the overall response rate (CR+PR) was 100% and at the end of all therapy 36 (90%) pts were in CR, 2 (5%) in PR and 2 (5%) pts had progressive disease. After a median follow-up of 30 months (range 7-61), 38 pts are alive while two pts died from progressive HL. The estimated 5-year event free survival (EFS) and overall survival rates were 78% (95% CI, 64-92%) and 91% (95% CI, 78-100%), respectively. The 5-year cumulative incidence of relapse is 13% (95% CI, 5-33%). These survival rates are higher than those expected for ABVD containing regimens and comparable with the reported estimated long term survival rates achieved with the poor prognostic subgroup of pts, receiving eight cycles of escBEACOPP in the GHLSG HD9 trial. Furthermore, the estimated 5-year EFS rate for early PET negative pts (n=27) and for early PET positive pts (n=11) was 82% (95% CI, 66-98%) and 64% (95%, CI 35-92%), respectively (p=0.14) (in 2 pts early PET results were not conclusive). As expected, the incidence of acute hematologic toxicities was more common in the escBEACOPP than in the ABVD phase. Non hematologic adverse effects included grade III-IV infection (n=1), avascular necrosis of the hip (n=1) and cognitive impairment (n=1). *Conclusions.* Combined escBEACOPP-ABVD therapy is well tolerated and may improve the outcome in pts with advanced HL who have high IPS scores. Larger scale randomized studies, comparing this combination regimen with previously reported dose-intensified chemotherapy regimens, are required in order to verify its true merit in this high risk subgroup of pts.

0392

EARLY INTERIM FDG-PET DURING INTENSIFIED BEACOPP THERAPY FOR ADVANCED-STAGE HODGKIN DISEASE SHOWS A LOWER PREDICTIVE VALUE THAN DURING ABVD

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Background. FDG-PET scan performed early during standard ABVD chemotherapy for Hodgkin's disease (HD) is a powerful prognostic tool (Hutchings: Blood 2006, Gallamini: Haematologica 2006). So far, no data have been published on the role of early FDG- PET in HD patients treated with BEACOPP. *Aims.* Starting from November 2002, 29 new,

advanced-stage HD patients consecutively admitted to seven Italian hematological institutions were enrolled in a prospective multicenter clinical trial to study the predictive role on treatment outcome of early interim FDG 'PET scan in HD patients treated with BEACOPP (4 escalated + 4 baseline cycles). Patients The mean age was 35,3 years (18-60); advanced disease (stages IIB-IVB) was present in 26, and stage IIA with adverse prognostic factor (> 3 nodal sites involved, sub-diaphragmatic presentation, bulky disease and ESR > 40) in 3. Bulky disease and extra nodal sites were recorded in 16 and 14 patients, respectively. All pts were staged at baseline, after 2 courses of chemotherapy at the end of treatment by FDG-PET scan (PET-0, PET-2, PET-8, respectively). All the PET-2 positive studies were reviewed. The mean interval between the end of the second BEACOPP course and PET-2 was 11.6 (5-20). At the end of chemotherapy in 15/29 pts. with bulky disease consolidation radiotherapy was given. All patients were given the therapy programmed at baseline. No treatment change depending on PET-2 result was allowed, except in case of overt progression. *Results.* the mean follow-up was 637 days (259-1428). Twenty-six pts attained CR while 3 were chemoresistant and showed disease progression during therapy; three patients relapsed + 55, + 224 and +266 days after CR entry. In univariate analysis, besides PET-2 (p<0.05), the clinical factors that were significantly associated with a higher probability of treatment failure were age older than 45 (p<0.05) and hemoglobin lower than 10.5 g/dL (p< 0.05). In multivariate analysis only age and hemoglobin retained their significance(p<0.05). In terms of treatment failure, the Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 60% and 88%, respectively. The sensitivity, specificity and overall accuracy of PET-2 were 50%, 91% and 83%, respectively. The 2-y Failure-Free Survival (FFS) probability for PET-2 negative and for PET-2 positive patients were 88% and 0, respectively (FFS Log Rank test = 5.0, p<0.05). *Conclusions.* With the caution due to the relatively small number of patients these results seems to indicate that early interim PET during intensified chemotherapy with BEACOPP regimen in advanced stage HD has an equivalent prognostic meaning than during standard ABVD therapy. However, PET-2 during BEACOPP showed a lower sensitivity and PPV (50% versus 86% and 60% vs. 92%, respectively, p< 0.05). This could depend on the relatively high number of false negative PET-2 results, probably due to an higher effectiveness of this intensified regimen.

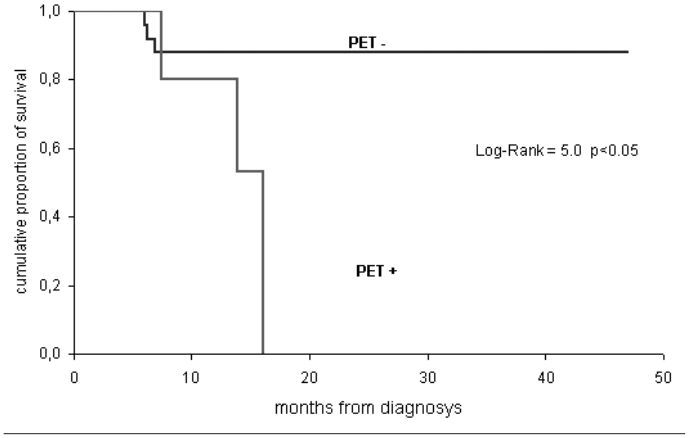

Figure 1. Failure free survival.

0393

HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN 58 PATIENTS WITH HODGKINS LYMPHOMA. OUTCOME AND PROGNOSTIC FACTORS

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Backgound. HL is a potentially curable disease in at least 75% of the patients treated with ABVD chemotherapy (CT)±radiotherapy (RT). For patients either refractory to or relapsing after first line treatment, HDT/ASCT is the most widely accepted option with curative intent. *Aims.* To analyze the outcome and prognostic factors for relapsed or refractory HL patients treated with HDT/ASCT. *Methods.* We retrospectively analyzed 58 patients with refractory or relapsed HL treated with HDT/ASCT in a single Hematology Unit. Event free survival (EFS) was calculated from time of ASCT to relapse/progression, death of any cause or last follow-up. Overall survival (OS) was calculated from time of ASCT to death of any cause or last follow-up. *Results.* Median age at ASCT was 29 years (19-57) and 71% were males. At diagnosis 14%, 52%, 19% and 16% of the patients had clinical stage I, II, III and IV respectively, 43% had B symptoms, and 36% bulky disease. 97% had received an anthracycline-based regimen and 3% MOPP, while 44% had received RT. At relapse 21%, 42%, 5% and 32% of the patients had clinical stage I, II, III and IV respectively, 18% B symptoms and 4% bulky disease. 47% of the patients were transplanted in first relapse, 22% after multiple relapses and 31% were primary refractory. All patients received salvage chemotherapy before ASCT, mostly ESHAP or GIN. The median number of CT regimens prior to ASCT was 2 (2-5). Following the last salvage regimen, 36% of the patients were in complete remission (CR), 43% in partial remission (PR) and 21% were chemoresistant. The conditioning regimen was BEAM in all patients. The median time to neutrophil and platelet recovery was 10 (8-19) and 14.5 days (10-102) respectively. One patient died during the procedure for a transplant related mortality (TRM) rate of 1.7%. Two additional patients required mechanical ventilation due to respiratory and cardiac failure. 27 patients experienced an event at a median of 5.5 months (0.7-45.5) after ASCT. At a median follow-up of 25 months (0.5-104) for surviving patients the 3- and 6- year EFS were 49±7% and 39±9% respectively. The corresponding 3- and 6- year OS rates were 73±8% and 66±10% respectively. Survival analysis did not identify statistically significant prognostic factors for the outcome with the exception of bulky disease at relapse (both patients with bulky relapses progressed and died soon after ASCT, p<0.001). However, there was a non-significant trend for inferior EFS and OS in primary refractory patients. Furthermore, patients achieving CR prior to ASCT had a superior OS (p=0.02) compared to PR and unresponsive patients, although EFS was not significantly different. *Conclusions.* HDT/ASCT can salvage 40% of relapsed/refractory HL patients with a low TRM.

0394

EFFECT ON CLINICAL OUTCOME OF GENETIC POLYMORPHISMS IN HODGKIN LYMPHOMA PATIENTS

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Background. Most drugs demonstrate an inter-patient variability in their effect These differences can be due in part to the presence of polymorphisms in genes encoding enzymes related to drug metabolism that may lead to either reduce or increase their activity. *Aims.* We investigated whether distinct patterns of functional polymorphisms in genes involved in drug metabolic pathways (SXR, GSTT1, GSTP1, SULT1C2), multidrug resistance (MDR1), DNA repair (XPD, XPA, ERCC5, XRCC5, TOP2A), apoptosis (FAS, FASL) and inflammatory cytokines (IL6, IL10) predict clinical outcome in patients with Hodgkin lymphoma (HL). *Patients and Methods.* One hundred and seventy five adult patients (median age 33, range 13-89 years; males 49%) diagnosed with HL at a single institution between January 1996 and January 2006 have been studied. Distribution according to histological subtypes was: nodular sclerosis (60%), mixed cellularity (17%), lymphocytic predominance (6%), and lymphoid depletion (3%). First-line treatment consisted of MOPPABV (38%) or ABVD (52%). Allelic discrimination of single nucleotide polymorphisms (SNPs) (ABI Prism 7500; TaqMan) of DNA obtained from formalin fixed paraffin embedded lymph nodes was performed. The characteristics considered were: age (<45 vs >45 years), gender, WHO histological classification, EBV status (LMP1+ vs LMP1-), B symptoms, bulky mass, high LDH, high β-2-microglobulin, ESR (EORTC criteria), Ann Arbor stage (I-II vs III-IV), Hasenclever prognostic index (<3 vs >3), and polymorphisms of the above-mentioned genes. Clinical outcomes analyzed were probability to achieve complete remission (CR), toxicity due to the treatment, relapse rate, disease-free survival (DFS) and overall survival (OS). *Results.* Out of 175 patients, 144 (82%) achieved CR, 10 (5.7%) a partial response, 10 (5.7%) were chemoresistant, 6 (3.4%) died during the initial treatment and 5 (2.9%) were not evaluable. After a median follow-up of 50.8 (range, 1.2-144.1) months, OS was 82% and DFS 75%. In the multivariate analysis, the only prognostic

factor for the achievement of CR was SXR -1567T>C polymorphism (RR=0.5; p=0.06). A lower probability of DFS was associated with IL10 -116A>G polymorphism (RR=0.2; p=0.02), and a lower probability of OS to higher Hasenclever prognostic index (RR=6.4; p<0.001) and MDR1 Ile1145Met polymorphism (RR=3.3; p=0.03). A close association between Asp5Glu genotype of the phase II drug-metabolizing enzyme SULT1C2 and pulmonary toxicity was found. Thus, all 9 patients with pulmonary toxicity due to bleomicine had the wild type SULT1C2 genotype (p=0.004). Thereby, 9 (12%) of the 76 wild type patients presented pulmonary toxicity (p=0.006). *Conclusions.* Germline polymorphisms in IL10, MDR1 and SULT1C2, which can easily be analyzed in paraffin embedded samples, have prognostic value in patients with HL.

0395

EARLY RESPONSE TO CHEMOTHERAPY MAY FACILITATE OMMITTING RADIOTHERAPY IN CHILDREN WITH HODGKINS LYMPHOMA

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Background. ABVD (Doxorubicin, Bleomycin, Vinblastine Dacarbazine) chemotherapy is commonly used to treat Hodgkin's lymphoma (HL) in adults. It used less frequently in children because of the potential anthracycline related cardiotoxicity. Chemotherapy and radiation are used in most childhood HL protocols. Avoiding radiation therapy or reducing its dose may decrease its potential long-term toxicities. *Aims.* To evaluate the efficacy of 4 courses of ABVD in children with low risk HL, and 4 courses of ABVD and 2 courses of COPP (cyclophosphamide, vincristine, prednisone, procarbazine) in children with high risk HL. In addition to evaluate the efficacy of chemotherapy alone in patients who are rapid responders to chemotherapy. *Methods.* 42 children were diagnosed and treated for HL at King Hussein Cancer Center in Jordan between January 2004 and December 2006. 8 patients were excluded from analysis because they were treated on different protocols. The outcome of 34 eligible patients was retrospectively analyzed. Median age at diagnosis was 11 years (range 2-18 years). Patients were stratified into 2 risk groups: Low risk (LR) and high risk (HR). The LR group included stage IA or IIA without bulky disease or extranodal involvement, while the HR included all other patients. Patients in LR group were treated with 4 courses of ABVD, while HR patients received 4 courses of ABVD followed by 2 courses of COPP. In both groups, radiation was omitted in patients who achieved complete remission (CR) after 2 cycles of ABVD (rapid responders to chemotherapy). Patients who were slow responders to chemotherapy (i.e. achieved CR after 4 cycles of ABVD) received radiation at a dose of 25.5 Gy. CR was defined as 75% reduction in the size of each of the nodal masses, or return to normal size along with a negative gallium or PET scan. *Results.* 26/34 (75%) patients were rapid responders to chemotherapy and did not require radiation. All patients achieved CR after 4 cycles of ABVD. 6/34 (25%) patients received radiation at a dose of 25.5 Gy since they were slow responders to chemotherapy. 2 patients relapsed (one was a slow responder to chemotherapy and received radiation and one was a rapid responder and did not receive radiation during his initial therapy). At a median follow up time of 19 months (range 6-38 months), event free survival (EFS) and overall survival (OS) were 94% and 100%, respectively. No major treatment related toxicities were observed. *Conclusions.* 4 courses of ABVD chemotherapy without radiation are probably adequate in children with low risk HL who achieve CR after 2 cycles. 4 courses of ABVD followed by 2 courses of COPP chemotherapy without radiation are probably adequate in children with HR HL who achieve CR after 2 cycles of ABVD. Radiation therapy can be reserved for patients who are slow responders to chemotherapy. This can be an important issue for countries with limited resources in radiotherapy. Limiting ABVD courses to 4 by utilizing COPP may decrease the potential cardiotoxicity. These results have to be confirmed by longer follow up and larger number of patients.

Acute myeloid leukemia - Clinical

0396

PROGNOSTIC IMPACT OF NPM1 MUTATIONS IN CYTOGENETICALLY NORMAL (CN) ACUTE MYELOID LEUKEMIA (AML) IS ADVERSELY AFFECTED BY HIGH EXPRESSION OF THE ERG GENE: A CANCER AND LEUKEMIA GROUP B (CALGB) STUDY

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Background. Mutations in the NPM1 gene (NPM1+) are the most frequent molecular lesions in patients (pts) with CN-AML and, in the absence of FLT3 internal tandem duplication (FLT3-ITD), define a subset of pts with favorable prognosis. We have recently shown that overexpression of the ETS-related gene, ERG, is predictive of poor outcome in CN-AML. However, the prognostic impact of NPM1+ has not yet been evaluated in the context of ERG expression. *Aims.* To evaluate the prognostic impact of NPM1+ in CN-AML pts when FLT3-ITD and ERG expression status are also determined. *Methods.* Diagnostic samples from 216 CN-AML pts aged <60 years and treated on one of two similar frontline CALGB trials (9621 and 19808) were analyzed by PCR amplification and subsequent sequencing for the presence of mutations in exon 12, the major mutational cluster in NPM1. The samples were also assessed for the presence (+) or absence (-) of the FLT3-ITD by GeneScan fragment analysis of PCR products encompassing FLT3 exons 14 and 15. ERG expression was measured by quantitative Real-Time RT-PCR. *Results.* NPM1 mutations were found in 65% of pts. NPM1+ pts had higher white blood cell counts (p<0.001) and higher% bone marrow blasts (p=0.01) than NPM1- pts. A trend towards worse overall survival (OS) was observed for the NPM1- pts (p=0.11). No significant differences in complete remission (CR) rates or event-free-survival (EFS) were found between the two groups. In NPM1+ pts, high ERG and FLT3-ITD were detected in 40% and 36% of the cases, respectively. Among the NPM1+ pts, high ERG expressers had worse EFS than low ERG expressers (p=.0002; estimated 3-yr rate 55% vs 15%), and high ERG independently predicted a worse EFS (p=0.003) once adjusting for FLT3-ITD status (p<.001). The subset of NPM1+/FLT3-ITD- pts (36%) tended to have a higher CR rate (p=.06; 90% vs 80%) and had significantly better EFS (p<.0001; 3-yr rate 58% vs 29%) and OS (p<.0001; 3-yr rate 70% vs 41%) when compared with the remaining pts. Importantly, when all three molecular markers were considered together, NPM1+/FLT3-ITD-/low ERG pts had better EFS than NPM1+/FLT3-ITD-/high ERG pts (p<0.001; estimated 3-yr rate 72% vs 14%). In a multivariable analysis, ERG remained the only variable independently associated with EFS (p=0.002); high ERG expressers had almost four times the risk (hazard ratio: 3.8) to experience an event compared with low ERG expressers. *Conclusions.* NPM1+ as a single molecular marker is not a strong predictor for outcome in CN-AML pts, but its prognostic value can be enhanced by further molecular characterization. Within the favorable NPM1+/FLT3-ITD- group, a subset of pts with an extremely dismal outcome can be identified based on high levels of ERG expression.

0397

PRONOSTIC VALUE OF MOLECULAR FINDINGS IN AML: RESULTS OF THE FRENCH ALFA-9802 TRIAL

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Background and aim. Beside cytogenetics, mutations of several genes of interest have emerged as major prognostic factors in patients with acute myeloid leukemia (AML). In this study we investigated 305 of 450 patients aged 15-50 years, enrolled in ALFA-9802 protocol (median fol-

low-up: 3-y). Mutations of FLT3 (ITD and D835), NPM, and CEBPa genes were analyzed and their prognostic value evaluated. *Results.* Mutation frequencies were: FLT3-ITD 14% (#44), FLT3-D835 5% (#16), NPM 24% (#74), and CEBPa 6.8% (#21). NPM mutations were associated with female sex (p=0.004), high WBC (p=0.01), M4/M5 FAB subtypes (p=0.04), intermediate cytogenetics (p<.0001), and normal karyotype (p<0.0001) and were strongly associated with FLT3-ITD (p=0.006). FLT3-ITD was associated with high WBC (p=0.0001), intermediate cytogenetics (p=0.009), and normal karyotype (p=0.003). CEBPa mutations were associated with FAB (M1/M2) and normal karyotype (p=0.03) and were mutually exclusive with FLT3-ITD+ and NPM+ (p=0.03). Overall CR rate was 90%, with a significant correlation with FLT3-ITD (77% in FLT3-ITD+ versus 90% in FLT3-ITD-neg, p=0.008). FLT3-ITD+ patients had a worse EFS (p=0.001) and NPM+ patients a better EFS (p=0.007). FLT3-D835 or CEBPa as isolated mutation did not interfere with EFS. Only FLT3-ITD+ patients presented a shorter OS (median, 15.7 versus 60 months, p=0.0009). In the intermediate cytogenetic group (n=173), FLT3-ITD and NPM mutations showed negative (p=0.0006) and positive (p=0.0017) correlation with EFS, FLT3-ITD remaining a bad prognostic factor for OS (p=0.0006). When analyzing the normal karyotype subgroup (n=117), EFS correlations with FLT3-ITD and NPM status were still present (p=0.011 and p=0.0043, respectively). Worse OS was observed in FLT3-ITD patients (p=0.00016) and a trend for longer OS in NPM+ and CEBPa+ patients (both p=0.09). Finally we divided the intermediate cytogenetic group in 4 subgroups: CEBPa+, NPM+, FLT3-ITD-neg (G1); CEBPa+, NPM+, FLT3-ITD+ (G2); CEBPa-neg, NPM-neg, FLT3-ITD+ (G3), and the remaining patients (G4). Significant differences were found between those 4 subgroups in terms of EFS and OS (p<0.0001). Median EFS was 9.9 (G2), 4.8 (G3), and 18.8 months (G4), while not reached in the G1 group. Median OS was 16.6 (G2), 22 (G3), and 43.4 months (G4), while not reached in the G1 group. *Conclusions.* Here we confirm the good prognosis of NPM mutations and the bad prognosis of FLT3-ITD and show a negative effect of FLT3-ITD for CR achievement. The bad prognostic of FLT3-ITD is predominant in NPM+/FLT3-ITD+ patients (our G2). According to clinical data, we can observe that patients with NPM+/FLT3-ITD-neg or CEBPa+ (G1) are similar to CBF patients, while FLT3-ITD+ patients (G3) are near to those with complex karyotypes.

0398

THE IMPACT OF THERAPY-RELATED AND SECONDARY AML IN RELATION TO KARYOTYPE AND MOLECULAR MARKERS: A STUDY OF THE AMLSG

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Background. Patients with therapy-related acute myeloid leukemia (t-AML) or secondary AML (s-AML) after a myelodysplastic syndrome are considered to have an inferior outcome compared to *de novo* AML. Whether this is due to an adverse cytogenetic risk profile or by the fact of s/t-AML per se has not been determined yet. *Aims.* To evaluate the frequency and prognostic impact of cytogenetic abnormalities and molecular markers in t-AML and s-AML in comparison to *de novo* AML in a large cohort of adult AML patients. *Methods.* Patients were entered on several AMLSG treatment trials (AML HD93, AML HD98A, AML HD98B, AMLSG 07-04, AMLSG 06-04). The key inclusion criteria for this analysis were availability of cytogenetics and molecular markers in cytogenetically normal (CN) AML. Genotypic groups were defined as follows: favorable [t(8;21), t(15;17), inv(16)/t(16;16)], NPM1pos/FLT3-ITDneg-CN-AML, other-CN-AML, and unfavorable [all other cytogenetic aberrations]. *Results.* Between 1993 and 2006, 2530 adults (age range 16-85 years) were registered and 2287 were included in this. Of the 2287 patients, 170 (7.5%) had t-AML, 360 (16%) s-AML and 1757 (76.5%) *de novo* AML. The median age was significantly lower in *de novo* AML compared to t-AML and s-AML. Median WBC and LDH levels were significantly higher (p<.0001) in *de novo* AML compared to t-AML and s-AML. The distribution of genotypic groups was different with a lower incidence of CN-AML subsuming both CN-AML subgroups in t-AML and a lower incidence of favorable genotype in s-AML (p<0.0001). The incidence of NPM1pos/FLT3-ITDneg-CN-AML patients was nearly half in t-AML and s-AML compared to *de novo* AML (p=0.03). Response to induction therapy was significantly higher in *de novo* AML (CR 72%) compared to t-AML (59%) and s-AML (52%). Cox regression models on event-free (EFS) and overall survival (OS) revealed the genotypic groups other-CN-AML (HR 2.2 and 2.3) and unfavorable (HR 3.4 and 3.2), t-AML (HR 1.3 and 1.5) and s-AML (HR 1.4 and 1.2), age (10 years difference HR 1.4 and 1.2) and log(LDH) (HR 1.1 and 1.1) as significant unfavorable prognostic variables. To better define the role of t-AML and s-AML subgroup analyses were performed according to genotypic groups; favorable group subsuming favorable and NPM1pos/FLT3-ITDneg-CN-AML, other-CN-AML and unfavorable. These analyses revealed no impact of t-AML and s-AML within the favorable group (*de novo* n=510, t-AML n=33, s-AML n=34) for EFS (p=0.73) and OS (p=0.8), a marginal impact within the other-CN-AML group (*de novo* n=381, t-AML n=19, s-AML n=69) for EFS (p=0.02) and no impact for OS (p=0.1), and finally a marked significant impact within the unfavorable group (*de novo* n=649, t-AML n=102, s-AML n=196) for EFS (p<0.0001) and OS (p<0.0001). In t-AML and s-AML OS of patients with either favorable or NPM1pos/FLT3-ITDneg-CN-AML genotypes was 58% and 68% and was significantly superior compared to that of all other patients with 11% and 19% after 3 years, respectively. *Conclusions.* The prognostic impact of t-AML and s-AML is restricted to the group of patients defined by unfavorable cytogenetics.

0399

INCIDENCE OF RETINOIC ACID SYNDROME DURING INDUCTION THERAPY FOR ACUTE PROMYELOCYTIC LEUKEMIA (PETHEMA PROTOCOLS LPA 96 &9 99) REVEALS TWO PEAKS WITH DIFFERENT INDUCTION MORTALITY

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Migration-maduration of tumoral promyelocytes following treatment with All-Trans Retinoic Acid (ATRA) in Acute Promyelocytic Leukemia (APL) is the cornerstone of the pathophisiology of Retinoic Acid syndrome (RAS). RAS is a potential life-threatening complication whose incidence ranges from 2 to 30% of patients with APL treated with ATRA. We have analyzed the incidence of RAS in 733 patients with acute promyelocitic leukaemia (APL) treated *de novo* with PETHEMA protocols LPA 96 and 99 (175 and 558 patients respectively). Induction therapy consisted in all-trans retinoic acid (ATRA) plus Idarrubicin, followed with three-consolidation courses of anthracycline monochemotherapy. Treatment was risk adapted depending on the platelets and leukocytes number (three groups: low, medium and high). In LPA99 patients, ATRA was added in each cycle of consolidation, except in low risk patients ($<10\times10^9$/L leucocytes and platelets $>40\times10^9$) and oral prednisone (0.5 mg/kg) was added in induction as prophylaxis of RAS. Treatment of suspected RAS consisted in ATRA discontinuation and intravenous Dexametasone administration. Definite RAS was defined when four of the seven following criteria was found: unexplained fever (non infectious related), respiratory distress, radiological pulmonary infiltrates, pericardial/pleural effusion, hypotension, renal failure, and weight gain over 5kg. 87 out 733 patients developed RAS (11.46%). ATRA was discontinued in 63% of patients. The incidence of RAS was not statistically different between the LPA96 and LPA99 trials (15% vs 11%, p=0.16).

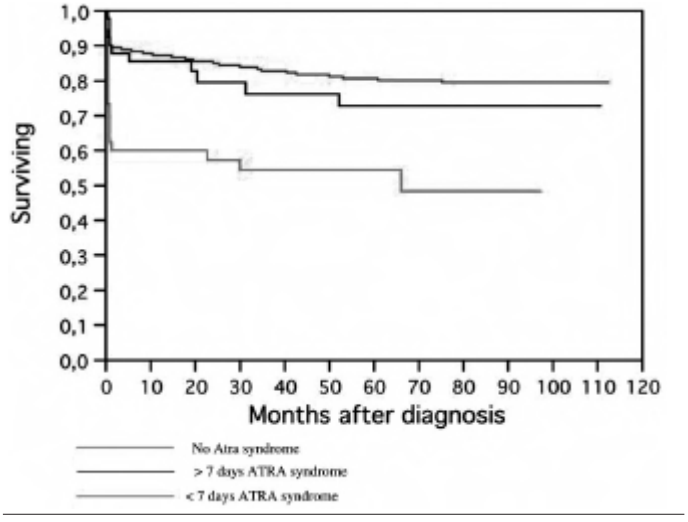

Figure 1.

The cases of RAS showed two peaks of incidence, the first one at <7 days (47 patients, 54%, median 3 days) and a second peak from 8 to 30 days (36 patients, 46%, median 22 days). Univariate analysis showed that the occurrence of the first peak was associated with high ECOG score, more leukocytes (p<0.001), more patients in high risk (p<0.001), higher LDH (p=0.025) and expression of CD2 (p=0.003). No differences in number of patients in both peaks were appreciated between LPA protocols 96 and 99. The first peak was associated with high induction mortality (36% vs. 9.11% in the second peak; 7% in no RAS patients) and decrease overall survival (p<0.001). In LPA 96 both peaks were associated with increased risk of LPA relapsed and perhaps related to ATRA discontinuation. Noteworthy, this increased risk of relapse was not observed in LPA 99 trial, in which ATRA was added in each consolidation course. In conclusion we observed a bimodal distribution incidence of RAS, in which the first peak is associated with high mortality. Patients who develop a second peak have mortality similar to patients with no RAS. The patophisiology of the second peak of RAS is intriguing since the use of Idarrubicine in induction therapy is associated with leukocyte nadir or perhaps normal leukocyte recovery.

0400

PCR-BASED MRD DETECTION IN NPM1 MUTATED AML: A PROSPECTIVE FOLLOW-UP STUDY IN 97 PATIENTS

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Background. Around 50% of all normal karyotype AML have been shown to carry mutations in the NPM1 gene that mostly are comprised of 4 bp insertions. This suggests that NPM1 may be used as an MRD marker applicable to a large subset of AML with normal karyotype. *Aims.* To evaluate the applicability of NPM1 mutations as follow up marker and to evaluate whether there is any prognostic relevance we studied 97 NPM1 mutated AML during and after therapy. *Methods.* NPM1 mutations are heterogeneous with 80% being belonging to type A, 10% to types B and D, and further 10% to more than 40 different yet described rare types. We have established NPM1 specific real time PCR assays for 10 different NPM1 variants. All assays are RNA based rendering high sensitivities between 1:100,000 to1:1,000,000. Most of these assays are specific, while in some of them a minimal detection of the wildtype allele cannot be avoided. In total 367 samples (spl) of 97 patients (pts) were analysed by quantitative real time PCR. Out of these 72 pts had type A, 7 type B, 8 type D, and 10 had 7 different rare types. 2-12 follow-up samples were analysed per patient (mean 3.7, median: 3). Mean follow-up time was 249 days (median: 175 days). *Results.* Three cases were resistant to chemotherapy as identified by non-decreasing NPM1 levels. Relapses were detected in 27 cases. All relapses had high NPM1 levels comparable to those at diagnosis. Of these 23 relapses 10 were predictable by increasing NPM1 levels 3-6 months before clinical relapse. Further four cases did not reach more than a three log reduction after consolidation and relapsed within the first year after start of therapy. In 9 cases early detection of relapse was not possible due to lack of samples within the last 6 months before relapse was diagnosed.To analyse the impact of NPM1 mutation levels on prognosis four different follow-up intervals were defined: interval 1: day 21-60 after start of therapy; interval 2: days 61-120; interval 3: day 121-365, 4: later. Using Cox regression analysis a significant impact of MRD levels on EFS was detected for interval 1 (p=0.014), interval 2 (p=0.004), and interval 3 (p=0.002). So far no impact of the initial expression ratio of the mutation on survival could be shown. In addition, the log change from diagnosis to the defined follow-up intervals was analysed. A rapid decline of median transcript ratios was observed: interval1: 2 log, interval 2: 4 log, interval 3 and 4: 6 log. *Conclusions.* 1) NPM1 mutations are highly effective and sensitive markers for PCR-based MRD detection in normal karyotype AML, 2) mutation levels highly correlate with the clinical course of the disease, 3) mutation levels during follow up have significant impact on prognosis, 4) early detection of relapse is possible by increasing MRD levels, 5) the NPM1 MRD pattern reflects a biological subgroup of AML with early response and high log reduction during follow-up.

Myeloma / MGUS / WM - Clinical

0401

ORAL MELPHALAN, PREDNISONE, THALIDOMIDE VERSUS ORAL MELPHALAN, PREDNISONE IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS

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Background. Since 1960, oral melphalan and prednisone (MP) has been considered the standard treatment for elderly patients. In this multicentre randomized trial we compared oral MP plus thalidomide (MPT) with MP alone in 60 to 85 years old patients. *Aims*. The primary objectives were response rates and event-free survival (EFS). Secondary end points included overall survival (OS), prognostic factors and incidence of any grade 3-4 adverse event. *Methods.* Inclusion criteria were previously untreated myeloma patients, age > 65 years of age or younger but excluded from transplant procedure, Durie and Salmon stage II or III and measurable disease. Patients agreed to use contraception, and women of childbearing age had a pregnancy test before enrolment. The criteria for exclusion were another cancer, psychiatric disease and any grade 2 peripheral neuropathy. MPT consisted of oral MP (melphalan 4 mg/m^2 and prednisone 40 mg/m^2 for 7 days) for six four-week cycles plus thalidomide 100 mg per day continuously until any sign of relapse or progressive disease (Pharmion LTD, Windsor, UK). No anticoagulation prophylaxis was administered until December 2003 when the protocol was amended and enoxaparin at 40 mg per day was delivered subcutaneously during the first four cycles of therapy. Two-hundred and fifty-five patients were randomly assigned to receive oral MPT (N=129) or MP (N=126). *Results*. Patients treated with MPT experienced higher response rates and a longer event-free survival than patients who were not. In intention-to-treat analysis, the complete and partial response rates were 76.0% for MPT and 47.6% for MP alone (absolute difference +28.3%, 95% CI 16.5 to 39.1), and the near complete and complete response rates were 27.9% and 7.2%, respectively. The 2-year EFS rate was 54% in patients receiving MPT and 27% in patients receiving MP (p=0.0006). The 3-year OS rate was 80% in patients taking MPT and 64% in patients taking MP (p=0.19). In MPT arm, no significant differences in term of OS were observed between patients with high or low B2-microglobulin (cut-off: 3.5 mg/L): at 18 months OS rate was 80% and 84%, respectively (HR 1.21, 95% CI 0.50-2.93, p=0.67). By contrast, in MP group, B2-microglobulin remained a prognostic factor: at 18 months OS rate was 71% for patients with high B2-microglobulin and 86% for patients with low B2-microglobulin (HR 2.8, 95% CI 1.26-6.18, p=0.01). Grade 3-4 adverse events were 48% in MPT patients and 25% in MP patients (p=0.0002). In the MPT group, the most frequent grade 3-4 toxicities were haematological, thromboembolism, infections and peripheral neuropathy. The introduction of enoxaparin prophylaxis significantly reduced the incidence of thromboembolism from 20% to 3% (p=0.005). *Conclusions*. Oral MPT is superior to MP as first-line treatment for elderly patients with multiple myeloma. Longer follow-up is needed to assess the effect on overall survival. Oral MPT seems to cancel the adverse prognostic effect of high β2-microglobulin.

0402

ORAL MELPHALAN, PREDNISONE AND LENALIDOMIDE FOR ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS

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Background. Lenalidomide plus dexamethasone is an effective treatment in advanced and newly diagnosed multiple myeloma (MM). In newly diagnosed patients the addition of thalidomide to the standard oral melphalan and prednisone (MP) significantly increase response rate and event free-survival (EFS) compared with MP. No data are available on the potential additive and synergistic effect of the combination of MP plus lenalidomide. *Aims.* This trial was a phase 1/2 multicenter, dose-escalating, open-label study designed to evaluate the dosing, safety and efficacy of the association of MP plus lenalidomide (MPR) in newly diagnosed symptomatic elderly MM patients. Primary end points were definition of the maximum tolerated dose (MTD) and response rate; secondary objectives were EFS and overall survival (OS). *Methods.* Fifty-four patients (median age 71 years, range 57-77) were enrolled in the study and received 9 cycles of lenalidomide (5-10 mg/day for 21 days) plus melphalan (0.18-0.25 mg/kg for 4 days) and prednisone (2 mg/kg for 4 days) every 4-6 weeks, followed by maintenance therapy with lenalidomide alone (10 mg/day for 21 days every month). Four different dose-levels were tested. All patients received aspirin (100 mg/day), as antithrombotic prophylaxis. *Results*. The MTD was lenalidomide 10 mg/day for 21 days and melphalan 0.18 mg/kg for 4 days every 4-6 weeks. At this dose-level partial response (PR) was observed in 81% of patients, including 47.6% with at least a very good partial remission (VGPR) and 23.8% who showed immunofixation negative complete response (CR). The 1-year EFS and OS rates were 92% and 100%, respectively. The presence of deletion of chromosome 13 or chromosomal translocation (4;14) did not affect response rate and survival, while high values of β2-microglobulin predicted a shorter EFS. Seventy percent of patients received maintenance treatment with Lenalidomide alone (median follow-up on maintenance 3,8 months). Major grade 3'4 adverse events consisted of hematological toxicities (69.8%); major grade 3'4 non-hematological toxicities included febrile neutropenia (9.4%), cutaneous rash (7.5%), and thromboembolism (5.7%); two of three thromboembolic events occurred after aspirin discontinuation. *Conclusions*. Oral MPR is a promising first-line treatment for elderly patients with multiple myeloma. Hematological adverse were manageable and non-hematological adverse events showed a low incidence. Aspirin appears to be an effective antithrombotic prophylaxis. Un update of these data will be presented at the meeting.

0403

TANDEM AUTOGRAFTING-NONMYELOABLATIVE ALLOGRAFTING FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: THE GITMO EXPERIENCE

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Background. Allografting induces long-term molecular remissions and possibly cure in myeloma patients. The development of nonmyeloablative conditionings has reduced the transplant-related mortality (TRM) 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. *Aims.* We report the results of a GITMO (Gruppo italiano trapianto midollo osseo) multicenter trial which evaluated efficacy and safety of a hematopoietic stem-cell autograft followed by a nonmyeloablative allograft from an HLA-identical sibling in patients with newly diagnosed myeloma. *Methods.* One hundred and six patients from 15 Italian centers were enrolled. Four patients did not complete the program because of consent withdrawal and were then excluded from the analysis. The median age was 54 years (range 30-65), 70 patients out of 102 had stage III myeloma and β2microglobulin was greater than 3,5 mg/dL in 33. All patients were initially treated with vincristine, doxorubicin, and dexamethasone, after which peripheral-blood stem cells (PBSC) mobilized by cyclophosphamide and granulocyte colony-stimulating factor (G-CSF) were collected. Upon recovery, patients were treated with 200 mg/m^2 melphalan followed by autologous stem-cell rescue. About 2 months later, they received 2 Gy total-body irradiation and infusion of G-CSF mobilized PBSC from an HLA-identical sibling. Graft-vs-host disease (GVHD) prophylaxis included cyclosporin and mycophenolate mofetil. *Results.* After autologous transplantation overall response rate was 77% (79/102), with 7/102 complete remission (CR). Following allografting, all patients promptly achieved donor engraftment. The overall response rate was 91% (93/102), with 56 patients achieving CR. Among patients progressed after allografting, 16 were treated with donor lymphocyte infusion, 8 of them received prior cytoreductive therapy, and 4/16 obtained a transient disease response. Incidence of grade 2-4 acute GVHD was 40% (41/102), including 4 patients with grade 4. Overall, chronic GVHD was observed in 74% (73/99) of patients alive at least 3 months after allografting, GVHD was graded as extensive in 50 of them. After a median follow-up of 48 months (range, 14-103) from diagnosis, median overall survival was not reached with follow-up extending to 7 years. Causes of deaths were TRM in 14 patients, disease progression in 6 and second tumor in 3. Median event-free-survival (EFS) was 38 months (range 10-103). *Summary and conclusions.* This reports shows that the combination of autologous transplant followed by allografting from an HLA-identical sibling is feasible in patients with newly diagnosis myeloma up to 65 years of age and demonstrates efficacy in terms of disease control and reduced toxicity by separating the cytoreductive effect of high dose chemotherapy from the graft-versus-myeloma effect of allografting.

0404

BORTEZOMIB PLUS MELPHALAN AND PREDNISONE (VMP) IN ELDERLY UNTREATED PATIENTS WITH MULTIPLE MYELOMA: PROGNOSTIC FACTORS INFLUENCING TIME TO PROGRESSION

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Between January 2004 and April 2005, the Spanish PETHEMA group conducted a phase I/II trial in which 60 newly diagnosed MM patients older than 65 years were included and received four 6-weeks cycles of bortezomib 1.0 or 1.3 mg/m^2 on days 1, 4, 8, 11, 22, 25, 29 and 32 followed by a 10-day rest period, in combination with oral melphalan 9 mg/m^2 and oral prednisone 60 mg/m^2, both on days 1 to 4. This was followed by five cycles of weekly bortezomib in combination with melphalan and prednsone. No Dose Limiting Toxicity occurred during the phase I; therefore, the 1.3 mg/m^2 dose was expanded to the phase 2. With a median follow-up of 26 months (range: 15-38) we confirm the response rate of 88% previously reported including 32% of IF-negative CRs, 11% IF-positive CRs and 45% of PR. The median time to progression (TTP) time was 27,2 months (95% CI: 22-32) and the median overall survival (OS) time has not been reached and the estimated 3-year OS

is 85%. Seven out of the 25 relapsed patients are considered to have a biological but asymptomatic relapse and remains untreated with a median follow-up from the biological progression of 2,5 months (range: 1-8). We analyzed the influence of variables with known prognostic factors in MM in TTP and we observed that hypoalbuminemia (<3 g/dL), poor Karnofsky performance status (<70%), and high S-phase (>2,5%) of bone marrow plasma cells were associated with a significantly shorter TTP (p value < 0,05). In addition, patients with high LDH showed also a trend to have short TTP (p:0,1). By contrast neither age (similar survival for patients younger and older than 75 years) nor cytogenetics abnormalities influenced outcome in terms of TTP (p value >0,05). Toxicity was manageable and predictable; principal toxicities were haematologic, gastrointestinal, and peripheral neuropathy and were more evident during early cycles and in patients older than 75 years old. In conclusion, prognostic factors such as hypoalbuminemia, poor Karnofsky performance status and high S-phase were associated with a shorter TTP; by contrast, VMP overcomes the adverse prognosis conferred by age and cytogenetics abnormalities.

0405

POOR PROGNOSIS ASSOCIATED WITH GAIN OF CHROMOSOME 1Q21 IN MULTIPLE MYELOMA MAY BE OVERCOME BY TREATMENT WITH A BORTEZOMIB COMBINATION

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Background. Bortezomib is an active agent for treatment of multiple myeloma (MM) and may even be effective in patients (pts) with adverse prognostic factors including unfavorable cytogenetic abnormalities. However, it is unknown whether or not bortezomib may overcome the negative prognostic impact of a chromosome 1q21 (CKS1B) gain, which has recently been reported as a negative prognostic factor even in the setting of a total therapy approach. *Aims.* We therefore evaluated chromosome 1q21 among other abnormalities in 46 pts with relapsed/refractory MM who were treated with single-agent bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11 every 3 weeks) and in 28 pts treated with a bortezomib combination (bortezomib/dexamethasone in 43%, bortezomib/ chemotherapy in 46%, bortezomib/ thalidomide/dexamethasone in 11%). *Patients and Methods.* Median age of pts was 63 years (range, 40 - 82) and median time to bortezomib therapy was 40 months (median number of prior therapies: 3; 96% of pts had high-dose pulsed dexamethasone, 61% thalidomide, 85% alkylating agents, and 41% high-dose melphalan). Chromosome 1q21 was evaluated by interphase FISH with a CKS1B-specific probe. Results were correlated with clinical outcome. *Results.* Among patients treated with single-agent bortezomib, gain of 1q21 was observed in 20 of the 46 pts (43.5%). Treatment outcome after bortezomib was negatively affected by presence of a 1q21 gain: The overall response rate was 30% (versus 58% in pts with normal 1q21; p=0.06) and the CR/near-CR rate was 10% (versus 23%). Moreover, gain of 1q21 was associated with shortened time to treatment failure (TTF) (median, 2.4 versus 6.6 months; p=0.043) and overall survival (OS) (median, 4.4 versus 19.8 months; P = .003) compared to pts with normal 1q21. β-2-microglobulin and 14q32 translocations were unrelated to treatment outcome after single-agent bortezomib, but median OS was short in the presence of low serum albumin (4.8 versus 17.8 months; p=0.036). In the group of pts treated with a bortezomib-combination, 11 of the 28 pts had a 1q21 gain (39%). There were no significant differences between pts with 1q21 gain and normal 1q21 regarding overall response (54.5% versus 64.7%), CR/near-CR rate (27% versus 29%), median TTF (8.2 versus 6.9 months; p =0.57) and median OS (not reached versus 17.8 months; p=0.49). *Conclusions.* FISH-defined gain of 1q21 is associated with poor response, short TTF and short OS after single-agent bortezomib; however, these differences disappeared in the context of a bortezomib-combination therapy. These results provide further evidence for the efficacy of bortezomib-combinations in MM patients with high-risk features.

Non-Hodgkin's lymphoma - Clinical I

0406

PROSPECTIVE, MULTICENTER RANDOMIZED GITMO/IIL TRIAL COMPARING INTENSIVE (R-HDS) VERSUS CONVENTIONAL CHEMOIMMUNOTHERAPY (CHOP-R) IN HIGH-RISK FOLLICULAR LYMPHOMA AT DIAGNOSIS: THE SUPERIOR MOLECULAR REMISSION (MR) RATE OF R-HDS EXPLAINS ITS BETTER CLINICAL PERFORMANCE

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Background. The superiority of intensified chemotherapy with autologous stem cell transplantation (ASCT) as the first-line treatment in follicular lymphoma (FL) is uncertain, particularly since the introduction of rituximab. 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. Objectives. This is a multicenter randomized open-label phase III trial. The analysis was intention to treat with event-free survival (EFS) as the primary endpoint. Crossover from CHOP-R to R-HDS was allowed. Centralized PCR-based molecular analysis was performed. The whole patient (pt) population is now evaluable for analysis with a median follow-up of 39 months. Methods. Eligibility required a FL with aaIPI>1 or IIL>2 score and an age of 18-60. 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. Cross-over was allowed after CHOP-R failure. Centralized PCR-based molecular analysis was planned on BM cells.

Figure 1.

Results. Age, sex, histological grade, stage, LDH, bulky disease, B-symptoms, ECOG PS, frequency of bone marrow, spleen and other extranodal disease, high aaIPI and IIL scores were similar in the two arms. CRs were 62% with CHOP-R and 85% with R-HDS (p<0.001). At a median follow-up of 39 months EFS and PFS are 33% and 36% for CHOP-R and 62% and 70% for R-HDS (Figure 1A). OS is 81% in each arm. 56% of relapsed R-CHOP pts underwent R-HDS with a CR/VGPR rate of 66%. PCR analysis showed a MR rate of 44% after CHOP-R and 80% after R-HDS (p<0,001). MR was associated to a better PFS (p<0,001) (Figure 1B). Of note, 3yrs 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: 21% for CHOP-R and 30% for R-HDS). MR was the strongest independent prognostic factor for PFS, EFS and DFS by multivariate analysis. *Conclusions.* 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.

0407

LONG-TERM RESULTS OF THE GELA STUDY COMPARING R-CHOP AND CHOP CHEMOTHERAPY IN OLDER PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA SHOW A LONG TERM BENEFIT FOR THE ADJUNCTION OF RITUXIMAB TO CHOP

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We present an update with 7 years median follow-up of the prospective randomized study LNH-98.5 that was first reported in the N Engl J Med and J Clin Oncol with a median follow-up of 2 and 5 years. The 399 patients included in the study had untreated diffuse large B-cell lymphoma and were 60 to 80 years old with a median age at diagnosis of 69 years. 60% had a poor risk lymphoma as defined by the aaIPI risk score of 2 or 3. 197 patients were randomized in CHOP arm and 202 in R-CHOP arm. Treatment consisted of 8 cycles of CHOP every 3 weeks with rituximab, 375 mg/m^2, the same day in R-CHOP. With a median follow-up of 7.1 years, 76% of the patients had an event in CHOP compared to 58% in R-CHOP, p=0.0002 (Table 1). 65% of patients died in CHOP arm compared to 47% in R-CHOP arm: 80% and 71% of them from lymphoma or treatment toxicity, 5% and 5% from another cancer, and 15% and 22% in CR from other causes, respectively. Survival curves show the same difference as reported before with a large difference in favour of R-CHOP (Table 1). Patients not expressing bcl-2 protein treated with R-CHOP have a statistically longer PFS but only a trend for OS because they responded better to salvage treatment. No statistically significant difference was observed for patients <70, 70-74, or >75 years old. Patients treated with R-CHOP have good survival even with poor risk parameters: 43% are alive for age >75 years, 38% for PS=2, 54% for B symptoms, 47% for stage IV, 45% for high LDH level, 54% for Hb <10 g/dL, and 42% for high aaIPI score. Death in CR was associated with high risk aaIPI score and presence of other diseases before lymphoma diagnosis. This analysis confirms the long term benefit associated with the combination of rituximab and CHOP and shows that older patients must be treated as younger patients even in presence of high risk characteristics or concomitant diseases.

Table 1. Survivals of patients treated with R-CHOP.

0408

ADDITION OF RITUXIMAB TO DOSE-DENSE AND HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS TRANSPLANTATION IN UNTREATED POOR-PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS OF PHASE II TRIAL

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Background. The efficacy of Rituximab (R) with high-dose chemotherapy in DLBCL is under investigation. *Aims.* We evaluated efficacy and safety of adding Rituximab to dose-dense and HDC as first line treatment in untreated patients with aa-IPI at Intermediate-High (IH) or High (H) risk with DLBCL. *Methods.* 94 previously untreated patients<61 years with DLBCL, stage III-IV at aaIPI IH or H risk were enrolled into R-HDC trial (study group; January 2001-December 2004). Treatment was: an induction lasting two months with 4 courses of R-MegaCEOP chemotherapy (R 375 mg/m^2 day1, CTX 1200 mg/m^2 + EPI 110 mg/m^2 + VCR 1.4 mg/m^2 day3 and PDN 40 mg/m^2 days 3-7) every 14 days with G-CSF support; 2 courses of intensified chemoimmunotherapy R-MAD (Mitoxantrone 8 mg/m^2 + ARAC 2000 mg/m^2/12h + Dexamethasone 4 mg/m^2/12h for 3 days and R 375 mg/m^2 day4 and before PBSC harvest) followed by ASCT conditioned by BEAM regimen. *Results.* median age was 47 yrs (19-60); 47% were at H risk; 28% had bone marrow involvement, 79% had LDH level >normal and 35% extranodal sites>1. Complete Response at the end of the treatment was achieved in 77 patients (82%), PR in 1 (1%) and 11 patients (12%) did not response; 5 patients (5%) died of toxicity. Few severe early toxicities (WHO grade 3-4) were reported and late toxicity was minor, with no MDS or ANLL or solid tumour. With a median follow-up of 41 months, 4-yr FFS and 4-yr OS rates were: 73% and 80%. These results were compared to those ones achieved into 41 patients, with the same clinical characteristics, enrolled in a previous phase II clinical trial with up-front HDC and ASCT but without R. Treatment in HDC control group was: an induction treatment lasting two months with MACOPB chemotherapy for 8 weekly infusions followed by the same intensified and HDC regimens (MAD x 2 courses + BEAM and ASCT). Four-yrs 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 competing risk factors (age, IPI, BM involvement, number of extranodal sites). In this multivariate analysis the risk of failure and death was confirmed as significantly reduced in R-HDC group: adjusted hazard ratio (R-HDC vs HDC) was 0.46 (95% CI=0.25-0.85, p=0.01) for FFS and 0.46 (95% CI=0.23-0.93, p=0.03) for OS. PBSC harvest and time to engraftment were similar into two groups, with no statistically significant differences: all patients in both groups collected more than 2×10^6 CD34$^+$/kg; median time to neutrophils engraftment (neutrophils >500/mm^3) was 9 days in R-HDC group and 9.5 days in HDC group and median time to platelets engraftment (platelets >50000) was 13 vs 11 respectively. *Conclusions.* these results suggest that Rituximab as adjuvant to dose-dense and HDC may improve the outcome of DLBCL at poor prognosis. This promising new treatment strategy need to be compared to Rituximab dose-dense chemotherapy without HDC as R-CHOP14. Such a randomized trial is currently undergoing conducted by Intergruppo Italiano Linfomi.

0409

FRACTIONATED RADIOIMMUNOTHERAPY IN NHL WITH DOTA-CONJUGATED, HUMANIZED ANTI-CD22 EPRATUZUMAB AT HIGH CUMULATIVE 90Y DOSES

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Background. The advantages of fractionated delivery of external radiation therapy may also apply to radioimmunotherapy. *Aims.* A phase I/II, multi-center, dose-escalation trial was undertaken to determine the maximum tolerated dose (MTD) for 2 or 3 weekly infusions of 90Y-epratuzumab in non-Hodgkin's lymphoma (NHL). *Methods.* Patients (pts) with B-cell NHL who failed >1 regimen of standard chemotherapy were eligible if they had <25% bone marrow involvement, platelets >100,000 cells/mm^3, and measurable disease by CT with no single mass >10 cm. The 90Y dose was escalated separately in increments of 2.5 mCi/m^2 (92.5 MBq/m^2) in cohorts of 3-6 pts with or without prior bone marrow transplant (BMT) until 2 occurrences of dose limiting toxicity (DLT). *Results.* Fifty-five pts (31 male, 24 female; median age 63) with a median of 3 prior therapies have now completed treatment. Therapy was well tolerated, and other than hematological DLT, no serious adverse events were considered treatment-related. For 17 pts with prior BMT, escalation stopped with 2 DLTs (platelet recovery delayed >12 wks) at 10 mCi/m2 total dose (5.0 mCi/m^2×2 wk). For 38 pts without prior BMT, no DLTs have yet occurred at 45 mCi/m^2 total dose (15 mCi/m^2×3 wk), the highest level currently administered. Of 44 pts with evaluated treatment responses, 23 (52%) had an objective response (OR) by International Working Group (IWG) criteria, across all histologies [follicular NHL, 11/20 (55%); DLBCL, 3/9 (33%); mantle cell, 6/12 (50%), marginal zone, 3/3 (100%)], and including pts after prior BMT (7/17, 41%), or pts not responding to the last rituximab-containing regimen (6/9, 67%). Most ORs were complete responses (CR/CRu, 17/23, 74%), and with long-term follow-up now available in 11 CR/CRu responders, 8 pts had responses >6 mo, including 3 pts with continuing response >1 yr. OR rates increased at higher cumulative dose for the 44 patients currently evaluated [5-10 mCi/m^2, 7/17 (41%), 15-20 mCi/m^2, 7/12 (58%), 22.5-37.5 mCi/m2, 9/15 (60%)] and remain to be determined for other pts currently being evaluated, including those who completed treatment at the highest cumulative doses, 40 and 45 mCi/m^2. *Conclusions.* Fractionated radioimmunotherapy with epratuzumab appears feasible and safe, achieving high response rates at cumulative 90Y doses several-fold higher than the 32-mCi (1200-MBq) limit approved for a single dose of Zevalin.

0410

HIGH CURE RATE OF ADULT BURKITT'S AND OTHER HIGH GRADE NHL BY THE COMBINATION OF SHORT INTENSIVE CHEMOTHERAPY CYCLES WITH RITUXIMAB

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Survival rates for adults with Burkitt'S NHL and B-ALL were improved by mostly childhood derived regimens with HDMTX, HD alkylators and HDAC to CR rates of 80% and overall survival (OS) of 50-70%. Further intensification of chemotherapy in other and our study group failed to improve these results (B-NHL90 trial, Blood 100 (11), #595). Therefore the GMALL study group invented in 2002 a new B-NHL protocol including 6x Rituximab 375 mg/m^2 before each chemo cycle and of two Rituximab maintenance cycles. In addition two cycles HDAC 2 g/m^2 were included. HDMTX was reduced from 3 to 1,5 g/m^2 in the protocol for younger pts (<55 yrs) since no improvement but increased toxicity had been observed in the previous study B-NHL90. A dose reduced regimen without HDAC and with 500 mg/m^2 HDMTX was given in older pts (>55 yrs) (Figure 1).

Figure 1. Multicentre study to optimize therapy of B-ALL, Burkitt's NHL and other high-grade non-Hodgkin's lymphoma in adults (GMALL-B-ALL/NHL 2002).

In this study 376 pts with high-grade B-NHL were enrolled between 09/02 and 12/06. 272 were evaluable for response after the first two cycles. The median age was 38 (16-78) yrs; 23% (N=63) were older than 55 yrs. The distribution of subtypes was as follows: 115 Burkitt's NHL (stage III-IV 52%, extranodal inv. 78%, aaIPI >1 47%), 70 mature B-ALL, 62 DLBCL (42 mediastinal, stage III-IV 63%, extranodal inv. 77%, aaIPI >1 63%), 14 B-LBL and 11 LACL. The CR rate after two cycles was 90% in Burkitt's NHL, 81% in B-ALL and 74% in DLBCL; death under therapy occurred in 3%, 11% and 2% respectively. The OS at 3 yrs was 91% for Burkitt's NHL, 79% for B-ALL and 91% for DLBCL in pts 15-55 yrs and 84%, 39% and 67% (N=9) in pts >55 yrs. Since in elderly pts with mature B-ALL CNS relapses were observed, HDAC will be included in the future. In younger pts the overall CNS relapse rate was 0. There was no difference in OS between pts with Burkitt (92%) vs Burkitt-like NHL (86%). Since no prognostic factors could be identified in younger pts, there was no need for SCT in CR1. Major grade III/IV toxicity was hematological (28-37%) and mucositis (36%, 37%, 28% in cycles A1, B1, C1 respectively). Compared to the trial B-NHL90 with 270 pts the OS at 3 yrs improved significantly from 54% to 80% (p<0.0001) overall, 56% to 85% (p<0.0001) in younger and 39% to 65% (p=0.01) in older pts. In this largest prospective study of adult Burkitt's NHL and other high grade NHL the combination of Rituximab and 6 short intensive chemo cycles was feasible and lead to an OS of 91% in NHL and 79% in mature B-ALL in the younger patient cohort. Even in older pts with Burkitt's NHL survival was 84%. The next generation studies will mainly focus on reduction of toxicity.

Myeloproliferative disorders - Biology

0411

ACQUIRED ISODISOMY IS COMMON IN ATYPICAL MYELOPROLIFERATIVE DISORDERS

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Recent evidence has indicated that acquired isodisomy is a novel mechanism by which mutations in cancer may be reduced to homozygosity. Typically, acquired isodisomy is associated with oncogenic changes rather than tumour suppressor genes, eg. the activating JAK2 V617F mutation in patients with myeloproliferative disorders (MPD) is often associated with acquired isodisomy for chromosome 9p. We have undertaken a screen for regions of acquired isodisomy as a means to identify genomic regions that may harbour novel oncogenes in patients with atypical MPD (n=30), blast crisis of chronic myeloid leukaemia (CML-BC; n=20) and chronic lymphocytic leukaemia (CLL; n=20). Genomewide SNP analysis was performed using Affymetrix 50K XbaI arrays and analysed for copy number changes and length of homozygous tracts. Chromosome deletions and gains identified by cytogenetic analysis were also detected by SNP analysis. Large tracts of homozygosity (defined as >20Mb running to a telomere), strongly suggesting acquired isodisomy, were seen 12 (40%) aMPD patients, a single case (5%) of CML-BC but were not seen in CLL. The homozygous tracts encompassed diverse genomic regions in aMPD, but two common regions (3 cases for each region) were identified at 7q and 11q. Because of the recurrent involvement of tyrosine kinases in MPDs, we focused our initial screen on this class of gene. The entire coding regions of EPHB6 and EPHA1 were sequenced in patients with 7q isodisomy but no sequence changes were identified. Similarly, no changes were found in selected regions of BRAF and PIK3CG. Ongoing sequence analysis is focused on other candidate genes within the two regions.

0412

JAK2V617F-NEGATIVE ET PATIENTS DO NOT DISPLAY CONSTITUTIVELY ACTIVE JAK/STAT SIGNALLING

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Background. Presence of the Jak2V617F mutation in only 40-60% of patients with Essential Thrombocythemia (ET) underscores the heterogeneity of this myeloproliferative disorder (MPD). Several distinct mutations, either in Jak2 (exon 12) or in c-Mpl (W515L) have been described in subsets of other Jak2V617F-negative MPDs, Polycythemia vera (PV) and Idiopathic Myelofibrosis (IMF). Analogous to Jak2V617F, these mutations cause constitutive Jak2 and STAT activation. It has therefore been proposed that constitutive activation of the Jak/STAT pathway underlies the molecular etiology of all MPDs. *Aims.* We investigated the alternative hypothesis that distinct alterations, separate from the Jak/STAT signal transduction pathway, underlie a subset of Jak2V617F-negative ET. We therefore compared gene expression profiles of ET patients with and without the Jak2V617F mutation. *Methods.* 32 patients with ET, diagnosed according to the PVSG criteria, were included in the study after giving informed consent. Jak2V617F allele expression was quantified in purified granulocytes by real time qRT-PCR. cDNA microarrays were processed according to the protocol of Eisen and Brown using the Lowess (Locally weighted scatter plot smoother) subgrid normalization method for comparison across slides. Statistical significance of differences was determined after correction for the false discovery rate by the method of Benjamini and Hochberg. Differences in gene expression were verified with qRT-PCR and protein phosphorylation detected by Western Blotting. *Results.* Unsupervised clustering of gene expression patterns in ET patients revealed two distinct subclasses of patients. These subclasses differed in presence or absence of the Jak2V617F mutation. Patients lacking the Jak2V617F mutation displayed significantly lower expression of the STAT target genes Pim-1 and SOCS2. In addition, Jak2V617F-negative patients showed lower levels of STAT phosphorylation. *Conclusions.* These data demonstrate that a large proportion of Jak2V617F-negative ET patients do not display constitutive Jak/STAT signaling. Hence, we propose that alterations in different signal transduction pathways can lead to the clinical phenotype of ET. Elucidation of novel ET-inducing changes will facilitate both a molecular classification of ET and the development of rationally designed therapies.

0413

MPL MUTATIONS IN PRIMITIVE MYELOFIBROSIS ARE NOT RESTRICTED TO MPL 515 MUTATIONS BUT ONLY MPL 515 MUTATIONS ARE ONCOGENIC EVENTS PRESENT AT THE STEM CELL LEVEL

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Background. The MPL 515 mutations have recently been described in 10% of primitive myelofibrosis (PMF) as an essential oncogenic event. Here we report other MPL mutations found in PMF patients. These new mutations raise the question whether these molecular events occur in a true stem cell (ie: lymphoid/myeloid progenitor cell), as it has already been shown for JAK2 V617F occurrence in PMF and PV. *Aims.* The aim of this work was to study the presence of MPL exon 10 mutations in a cohort of 100 PMF, determine the functions of the different mutations, and if these mutations occurred in both myeloid and lymphoid lineages in JAK2 V617F negative PMF. *Methods and Results.* We therefore sequenced MPL exon 10 in 100 PMF patients. Six different mutations were found. In order to determine whether these mutations were only genetic polymorphism or real gain of function mutations, we introduced all the mutations in Baf3 cells. We found that only the recently described MPL 515 mutations and a 506+519 mutant induced factor independence growth, activation of JAK/STAT, RAS/MAPK, and PI3K transduction pathways as well as P21 overexpression, a spontaneous cell cycling with an increase in S and G2M phases, and tumorigenesis in NUDE mice. Then we looked for the MPL 515 mutations in stem cells compartments: First in mature myeloid and lymphoid cells and second in lymphoid/myeloid progenitors progeny after $CD34^+$ and $CD34^+CD38^-$ cell isolation from peripheral blood. Three PMF patients harboring MPL515 mutations were studied. Peripheral blood granulocytes and platelets were purified by standard methods and B, T, NK cells and monocytes were isolated by combined immunomagnetic and flow cytometric procedures. The same techniques were used to sort CD34+ and $CD34^+CD38^-$ subpopulations from peripheral blood. Clonal B/NK/Myeloid differentiation from $CD34^+CD38^-$ cells and T cell differentiation from $CD34^+$ cells were performed respectively onto a MS5 layer in the presence of cytokines and in Fetal Thymic Organ Cultures (FTOC). Genotyping of mature cell populations, B/NK/Myeloid clones and $CD34^+$ derived T cells were performed by direct sequencing. Moreover, $CD34^+$ cells were cultured in a one cell per well experiment to determine the sensitivilty of these patient's cells to low dose TPO. The MPL515 mutations were present in granulocytes and platelets from all patients, and in monocytes in one. We detected the mutation in NK cells in two cases. The MPL 515L and MPL 515K mutations could be subsequently detected in $CD34^+$ cells and in B/NK/Myeloid and/or NK/myeloid $CD34^+CD38^-$ derived clones from all IMF patients. Interestingly, MPL 515L homozygous clones were detected in B/NK/Myeloid and/or NK/Myeloid clones from 1 patient. However, using the FTOC assay, the mutations were not detected in T cell fractions derived from $CD34^+$ cells. At least we found that MPL mutations induce a spontaneous megakaryocytic growth in cell culture but also a hypersensitivity to low dose TPO. *Summary.* These results demonstrate that the MPL 515 mutations occur in a lymphoid/myeloid progenitor cell and give rise to a hypersensitivity to TPO. Thus, these MPD take their origin in a true lymphoid/myeloid progenitor cell. In accordance with mouse model, this represent a good argument for a causative role of MPL mutations in PMF.

0414

COMPARATIVE ANALYSIS OF THE CONSTITUTIVE ACTIVE JAK2V617F, JAK2T875N AND MPLW515L ALLELES IN A MURINE BONE MARROW TRANSPLANT MODEL OF MYELOPROLIFERATIVE DISEASE

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Present in the 95% of polycythemia vera (PV) patients and in 50-60% of patients with essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF), the JAK2V617F mutation, resulting in constitutive activation of the JAK/STAT pathway, is the most frequent genetic event in myeloproliferative disease (MPD). Little is know about additional mutations which contribute to JAK2V617F negative MPD. Very recently, two additional mutations were identified in JAK2V617F$^-$ patients: JAK2T857N, detected in the human AMKL cell line CHRF-288-11 and the somatic mutation in the thrombopoietin receptor, MPLW515L, detected in ~10% of patients with JAK2V617F$^-$ MF. JAK2V617F, JAK2T875N and MPLW515L were analysed in murine models of bone marrow transplantation and sufficiency for the development of MPD has been shown for each of them. Comparison of these three models provides insight into the JAK2-positive and JAK2$^-$ MPDs that may inform therapeutic strategies. Furthermore, developing murine models of disease for each of these alleles may provide novel insights into phenotypic variance among the MPDs. The JAK2V617F mouse model is characterized by a striking, longterm erythrocytosis, a strain dependent degree of leukocytosis and the development of bone marrow and splenic reticulin fibrosis. Despite the presence of megakaryocytic hyperplasia, there is impaired megakaryocytic polyploidization, and platelet counts are not augmented. JAK2T875N constitutively activates downstream effectors including STAT5 in hematopoietic cells *in vitro*. In a murine bone marrow transplantation model JAK2T875N, although the T875N substitution is localized in a different domain of the JAK2 kinase, results in a phenocopy of JAK2V617F disease, causing a MPD with features of AMKL including megakaryocytic hyperplasia in spleen, a polyploidization defect and increased reticulin fibrosis in bone marrow and spleen, with normal platelet and leukocyte counts. Similarly, the MPLW515L mutation also transformed hematopoietic cell lines to cytokine independent growth through activation of the JAK/STAT pathway. *in vivo* expression of MPLW515L, caused a rapidly fatal, fully penetrant MPD that was characterized by a marked leukocytosis, splenomegaly, bone marrow reticulin fibrosis with a strain specific disease latency. However, - in contrast to both JAK2 mutations - there was megakaryocyte hyperplasia with normal megakaryoctye ploidy and marked thrombocytosis in the 3-4 million/uL range that was accompanied by thrombotic complications. Our data demonstrate important phenotypic differences between JAK2V617F, JAK2T875N and MPLW515L induced MPDs. Most striking was the effect on the megakaryocyte lineage. JAK2V617F and JAK2T875N induced megakaryocytic hyperplasia with a block in megakaryocyte maturation and lack of thrombocytosis, whereas MPLW515L enhanced megakarypoiesis resulting in severe thrombocytosis. Furthermore, marked leukocytosis developed in MPLW515L but not in JAK2V617F and JAK2T875N expressing C57Bl/6 mice. These findings indicate that the three mutations have different impacts on proliferation and/or survival of various hematopoietic progenitors. Since all three mutations signal through the JAK/STAT pathway, these data suggest that the MPLW515L allele activates transcriptional programs not activated by JAK2 mutations that confer the ability for megakaryocytic development and thrombocytosis.

0415

EXPRESSION AND EFFECTS OF NUCLEAR FACTOR-ERYTHROID DERIVED 2 (NF-E2) IN MURINE AND HUMAN HEMATOPOIESIS

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The basic region-leucine zipper transcription factor NF-E2 plays an important role in terminal erythroid and megakaryocytic maturation and regulates the differentiation of erythroblasts and megakaryocytes. NF-E2 knock out mice die at birth due to lack of platelets, but only display mild defects in the erythroid lineage. NF-E2 expression has been reported in murine cKit+/Sca-1+/Lin- (KSL) cells. However, its specific role in stem cells and early progenitors remains unclear. NF-E2 is overexpressed in patients with Polycythemia vera (PV). Several studies have demonstrated that the degree of NF-E2 overexpression in individual PV patients correlates with the proportion of Jak2V617F'positive granulocytes and the ability to form endogenous erythropoietin-independent colonies *in vitro*. Interestingly, the Jak2V617F activating mutation is present in uncommitted progenitor cells (Lin-/CD34$^+$/CD38$^-$/CD90$^+$) and results in enhanced erythroid differentiation capacity. However, the role of NF-E2 in stem and progenitor cells and its potential cooperation with Jak2V617F in PV is unclear. In an approach to clarify the function of NF-E2 in normal hematopoiesis and PV we investigated nf-e2 mRNA expression in human and murine hematopoietic cells at different developmental stages as well as the effects of NF-E2 overexpression in murine models. Nf-e2 expression was evaluated in FACS sorted human and murine stem and progenitor populations by qRT-PCR. A lentiviral construct (NF-E2-IRES-GFP) was used to transduce KSL and cKit+/Sca-1-/Lin- cells, which were subsequently assayed for colony formation in methylcellulose. A tetracycline-inducible mouse model (TET-OFF) was generated by introducing an nf-e2 transgene under control of the tetracycline responsive element and subsequent crossing to a CD34-promoter-tTA transgenic strain. Nf-e2 displayed a similar differentiation-specific expression pattern in murine and human hematopoiesis: nf-e2 mRNA was detected in LT-HSCs and ST-HSCs. During lineage commitment nf-e2 was downregulated in both CMPs and GMPs but was again upregulated in MEPs. In murine cells, highest nf-e2 expression levels were found in proerythroblasts followed by a consecutive decrease during maturation to orthochromatic erythroblasts. Enforced expression of NF-E2 following lentiviral transduction of murine KSL and cKit+/Sca-1-/Lin- progenitor cells caused markedly decreased BFU-E and CFU-E colony formation in the presence of erythropoietin. In the tetracycline-inducible mouse model NF-E2 overexpression in KSL and cKit+/Sca-1-/Lin- cells similarly led to impaired erythroid colony formation *in vitro*. Lentiviral NF-E2 overexpression did not appear to affect maturation of committed erythroid progenitor cells in *in vitro* differentiation assays of murine Ter119-spleen and fetal liver (E14.5) cells. In these assays, Jak2V617F likewise showed no effect. Our study shows that NF-E2 expression is regulated in a biphasic manner in both murine and human erythropoiesis. Forces expression of NF-E2 in various human and murine stem- and progenitor cells decreases erythroid colony cloning efficiency.

Gene therapy and immunotherapy

0416

DUAL SYSTEM OF RNA TRANS-SPLICING ELEMENT AND SLEEPING BEAUTY TRANSPOSON CORRECTS DNA PROTEIN KINASE MUTATION IN SEVERE COMBINED IMMUNE DEFICIENCY

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Background. Spliceosome-mediated RNA trans-splicing (SMaRT) can repair defective mRNA molecules by exon replacement between two individual RNA molecules: endogenous mutated mRNA and exogenous wild type mRNA. SMaRT technology, though, can only be effective long-term if the correcting sequence persists in target cells. While virus-mediated SMaRT delivery can be effective in achieving long-term correction, there are technical issues for production. In contrast, non-viral vectors are typically much more easily produced. Therefore, we tested whether the non-viral Sleeping Beauty (SB) transposon system could mediate stable delivery of trans-splicing molecules designed to correct the genetic defect responsible for severe combined immune-deficiency (SCID). This immunological disorder is caused by a point mutation within the 12.4 kb gene encoding the DNA protein kinase catalytic subunit (DNA-PKcs) and is associated with aberrant DNA repair, defective T- and B-cell production, and hypersensitivity to radiation-induced injury. Importantly, the currently available SB transposon plasmid vectors are not practical for delivering large genes, such as the DNA-PKcs gene mutated in severe combined immune deficiency (SCID). *Aims.* To use the SB transposon/SMaRT dual system to achieve integration and repair of the mutated mRNA endogenous transcripts in a T-cell line and in adult stem cells derived from SCID mice. *Methods.* To select the preferred trans-splicer for correction of the DNA-PKcs gene we designed six binding domains targeting a distinct region adjacent to DNA-PKcs mutation. The vector resulting in the highest radiation resistance was chosen for further studies. SB/SMaRT dual vector and transposase were nucleofected to a T-cell thymoma cell line (scid.adh) and SCID adult stem cells (ASC). ASC were derived from the bone marrow of SCID mice and induced to differentiate *in vitro* into neurons, hepatocytes and endothelium. Pyrosequencing (a quantitative sequencing method) and automated sequencing were used to determine the correction to wild type. *Results.* Gene transfer of the SB-based trans-splicing vector and radiation selection in scid.adh cells resulted in a 4.3-fold increase in number of surviving cells over irradiated untreated scid.adh cells (p=0.029). The degree of mutation correction in treated scid.adh cells was 79.1% as determined by pyrosequencing. For adult stem cells, in the presence of the SB/SMaRT dual vector and selection by radiation, 64.3% correction to wild type was achieved. Corrected DNA-PKcs protein was detected by Western blotting in radiation resistant scid.adh and ASC treated with SB/SMaRT dual vector and transposase. Multiple genomic integration sites of the SB/SMaRT dual vector were determined with splinkerette PCR in genomes of corrected SCID cells. *Summary.* Using this novel SB-based trans-splicing vector, we demonstrate stable mRNA correction, proper DNA-PKcs protein production, and conference of a radiation-resistant phenotype in a T-cell thymoma cell line (scid.adh) and SCID adult stem cells. These results suggest that SB-based trans-splicing vectors offer a potential alternative to viral gene therapy for treatment of genetic diseases, especially those involving large genes such as the DNA-PKcs mutation in SCID.

BRB and JT have contributed equally to this work.

0417

RHAMM/CD168-R3 PEPTIDE VACCINATION OF PATIENTS WITH ACUTE MYELOID LEUKEMIA, MYELODYSPLASTIC SYNDROME, MULTIPLE MYELOMA AND CHRONIC LYMPHATIC LEUKEMIA ELICITS IMMUNOLOGICAL AND CLINICAL RESPONSES

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We initiated a phase I/II R3 peptide vaccination to induce immunological and hematological responses for patients with AML, MDS, MM or CLL overexpressing the receptor for hyaluronic acid mediated motility (RHAMM/CD168). RHAMM/CD168 is expressed on tumor cells of most patients with AML, MDS, MM and CLL. We characterized RHAMM/CD168 as a leukemia-associated antigen (LAA) eliciting both humoral and cellular immune responses in patients with different hematological malignancies. CD8 positive T cells primed with the RHAMM/CD168-derived peptide R3 (ILSLELMKL) were able to lyse autologous tumor cells expressing this LAA. In this study, patients were included with positive RHAMM/CD168 expression but with a limited tumor load. At a biweekly interval, RHAMM R3 peptide (300 mcg for the first 12 patients and 1000 mcg for patients 13-24) emulsified with the incomplete Freund's adjuvant (day 3) and GM-CSF (100 mcg, days 1-5) was administrated four times subcutaneously. The primary aim of the study is safety and feasibility of this peptide vaccination, secondary aims the evaluation of a specific T cell immune response to RHAMM/CD168 R3 peptide and the assessment of the influence of the R3 peptide vaccination on the remission status. Since January 2005, 19 patients (3 AML, 8 MDS, 6 MM, 2 CLL) have been enrolled in the study. The first ten patients (2 AML, 4 MDS, 4 MM) have completed the course of four vaccinations and have been completely evaluated. Drug-related adverse events observed under R3-peptide vaccination were erythema and induration of the skin at the site of injection (CTC I°). In 5/10 patients, we detected an increase of CD8+ R3 tetramer+ CD45RA+CCR7-CD27-CD28- effector T cells in flow cytometry in accordance with R3-specific CD8+ T cells in ELISPOT analysis. In chromium release assays specific lysis of RHAMM-positive leukemic blasts were shown for AML patients responded to peptide vaccination. 3/6 patients with myeloid disorders (1/3 AML, 2/3 MDS) achieved clinical responses: one partial and one complete remission (1 PR, 1 CR), and one hematological improvement (1 HI). One patient with MDS did not need any longer erythrocyte transfusion after four vaccinations. Two MM patients responded as assessed by free light chains, two progressed (PD). Taken together, RHAMM/CD168 induced both immunological and clinical results and therefore constitutes a promising target antigen for immunotherapies in patients with hematological malignancies.

0418

FEASIBILITY STUDY OF RITUXIMAB FOLLOWED BY ANTI-IDIOTYPE IMMUNOTHERAPY WITH TUMOR-SPECIFIC IDIOTYPE-KLH (FAVID) AND GM-CSF IN INDOLENT B-CELL LYMPHOMAS

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Background. Anti-idiotype immunotherapy appears a promising strategy for lymphoma treatment. *Aims.* A pilot study was started at the Oncology Institute of Southern Switzerland (IOSI) with the primary objective of testing the feasibility of a idiotype-KLH vaccine (FavId®) therapeutic program in Switzerland with a patient-specific vaccine produced in the USA. *Methods.* Patients with B-cell lymphoma of the following subtypes were eligible: small lymphocytic lymphoma (CLL/SLL), lymphoplasmacytic lymphoma (LPL), follicular lymphoma (FL), marginal zone lymphoma (MZL) either nodal, splenic or extranodal (MALT type), and mantle cell lymphoma (MCL). A recent tumor biopsy from lymph nodes (LN), bone marrow (BM) or other tissues suitable for preparation of FavId® was required. Eleven patients have thus far been enrolled, 10 with pretreated disease,1 (with FL) treatment naïve. All the enrolled patients received 4 weekly Rituximab infusions (375 mg/m^2) followed approximately 8 weeks later by 6 monthly subcutaneous treatments with FavId® (1 mg on day 1) and GM-CSF (250 mcg on days 1-4). Patients with lack of disease progression following 6 doses were allowed to continue to receive additional cycles of FavId® and GM-CSF every 2 months for 6 doses and then every 3 months until disease progression. A tumor cell suspension, prepared within 2 hours after the biopsy in the IOSI laboratory in Bellinzona, was frozen and shipped by courier to the Favrille laboratories in San Diego according to CDC guidelines for shipment of biohazardous body fluids and tissues. *Results.* In all the cases viable tumor cells suitable for proper vaccine manufacturing arrived in San Diego and the patient-specific FavId® was successfully prepared and sent back to Switzerland under controlled temperature in suitable condition to be administered to the patients. Vaccine therapy has been thus far given to 9 patients. The main side effect was a transient mild erythema (grade 1-2), sometimes with itching, in the site of FavId® injection, which was observed in nearly all cycles and usually disappeared within 3-4 days. In two patients only the erythema was more severe and was

successfully managed with GM-CSF discontinuation. The table summarises the preliminary results (a final sample size of 15 patients is planned). *Conclusions.* Our experience suggests that a large scale trial of anti-idiotype vaccine (Id-KLH active immunotherapy) may be conducted in Europe with the vaccine manufacturing taking place in the USA. Clinical efficacy was not the main endpoint of the study, however, our preliminary findings indicate a possible activity in disseminated MZL, which might merit further evaluation

Table 1. Summary of preliminary results.

#	NHL type	Biopsy Site	Successful Favid® production	Response after Rituximab	Vaccine Therapy	Outcome (from start of Favid®)
02	FL	LN	yes	complete response (CR)	completed (6 cycles)	PD at 9 months
03	MZL	BM	yes	CR	ongoing (12 cycles)	CR at 18 months
04	FL	LN	yes	partial response (PR)	ongoing (6 cycles)	PR at 6 months
05	CLL	LN	yes	no change (NC)	discontinued (progression after 4 cycles)	dead (septic shock)
06	MCL	LN	yes	NC	ongoing (7 cycles)	stable disease at 8 months
07	MZL	BM	yes	CR	ongoing (6 cycles)	CR at 6 months
08	FL	LN	yes	PR	discontinued (progression after 4 cycles)	alive, with disease at 7 months
09	FL	LN	yes	CR	ongoing (6 cycles)	CR at 6 months
10	MZL	soft tissue	yes	PR	ongoing (1 cycle)	not assessed
11	FL	LN	ongoing	PR	planned	not assessed
12	MZL	BM	yes	PR	planned	not assessed

0419

ACUTE MYELOID LEUKEMIA (AML)-REACTIVE CYTOTOXIC T LYMPHOCYTE CLONES RAPIDLY EXPANDED FROM CD8+ CD62L(HIGH)+ T CELLS OF HEALTHY DONORS PREVENT AML ENGRAFTMENT IN NOD/SCID IL2R GAMMA NULL MICE

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Donor-derived $CD8^+$ cytotoxic T lymphocytes (CTLs) eliminating host leukemic cells mediate curative graft-versus-leukemia (GVL) reactions after allogeneic hematopoietic stem cell transplantation (HSCT). The leukemia-reactive CTLs recognize hematopoiesis-restricted minor histocompatibility and leukemia-associated peptide antigens that are presented by HLA class I molecules on recipient cells. Current *in vitro* techniques for generating AML-reactive CTLs from unprimed healthy donors are of relatively low efficiency and yield responder populations with unknown biological significance. We established a new allogeneic mixed lymphocyte-leukemia culture (MLLC) approach by stimulating donor $CD8^+$ T cells at comparably low numbers (i.e. 10^4/well) with HLA class I-matched primary AML blasts in 96-well microtiter plates. Using this so-called mini-MLLC strategy, we aimed at creating multiple different responder-stimulator compositions in order to provide for the growth of leukemia-reactive CTL optimized culture conditions by chance. Before culture, $CD8^+$ T cells were immunomagnetically separated into $CD62L^{high}$+ and $CD62L^{low}$+/neg subsets enriched for naïve/central memory and effector memory cells, respectively. The culture medium was supplemented with interleukin (IL)-7, IL-12, and IL-15. On day 14, IL-12 was replaced by IL-2. In 8 different related and unrelated donor/AML pairs with complete HLA class I match, numerous CTL populations were isolated that specifically lysed myeloid leukemias in association with various HLA-A, -B, or -C alleles. These CTLs recognized neither lymphoblastoid B cell lines of donor and patient origin nor primary B cell leukemias expressing the corresponding HLA restriction element. CTLs expressed T cell receptors of single V-β chain families, indicating their clonality. The vast majority of CTL clones were obtained from mini-MLLCs initiated with $CD62L^{high}$+ cells. Using antigen-specific stimulation, multiple CTL populations were amplified to 10^8-10^{10} cells within 6-8 weeks. We also investigated the capability of mini-MLLC derived AML-reactive CTL clones to inhibit the engraftment of human primary AML blasts in immunodeficient nonobese diabetic/severe combined immune deficient IL-2R common γ-chain deficient (NOD/SCID IL2R gamma null) mice. The leukemic engraftment was specifically prevented if inoculated AML blasts had been preincubated *in vitro* with AML-reactive CTLs, but not with anti-melanoma control CTLs. Taken together, our results provide the first evidence that CD8+ CTL clones raised from healthy donors against AML blasts using primary *in vitro* stimulation might carry biologically significant anti-leukemic activity. The efficient *in vitro* generation and expansion of AML-reactive CTL clones from $CD8+CD62L^{high}$+ precursors of healthy donors by the mini-MLLC approach allows several potential applications. First, CTLs can be used within T cell-driven antigen identification strategies to extend the panel of molecularly defined AML antigens that are recognizable by T cells of healthy donors. Second, because these CTLs can be readily isolated from the stem cell donor by mini-MLLC prior to transplantation, they could be infused into AML patients as a part of the stem-cell allograft, or early after transplantation when the leukemia burden is low. Our current work focuses on the identification of CTL-defined AML peptide epitopes using cDNA expression cloning, and on the translation of the mini-MLLC approach into a protocol that is compatible with good manufacturing practice guidelines.

0420

A COMPARATIVE ANALYSIS OF THE LEUKAEMIC POTENTIAL OF MATURE T CELLS VERSUS HEMATOPOIETIC STEM CELLS

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Background. After the report of two cases of leukaemia caused by insertional mutagenesis of a retroviral vector in children with SCID, it became clear that safety issues of therapeutic gene transfer must be addressed more thoroughly. *Aims.* We wanted to analyse whether gene transfer into mature T cells and haematopoietic stem cells bear the same risk of generating T cell leukaemia through activation of specific T cell oncogenes, such as LMO2, TCL1 and TrkA. *Methods.* To address this issue, we used the Rag-1 mouse model, which allows long term analysis of transplanted T cells and haematopoietic stem cells. We transduced mature T cells and haematopoietic stem cells of C57BL/6 (Ly5.1) donor mice with oncoretroviral vectors expressing LMO2, TCL1 and TrkA. *Results.* Transduction efficacies of up to 70% were achieved for mature T cells and approximately 90% for haematopoietic stem cells. After transplantation into Rag-1-deficient recipients, stem cell transplanted animals developed T cell lymphomas/leukemia for all investigated oncogenes after characteristic incubation times, mostly of a $CD8^+CD4^+$ double positive phenotype. T cell lymphomas were characterised by gross thymic mass, splenomegaly and heavily enlarged lymph nodes, although none of the control- vector- transduced mice developed lymphoma/leukaemia. LM PCR analysis revealed mono- or oligoclonality of the tumours. T cell transplanted animals showed no signs of leukaemia development so far. *Summary and conclusions.* Our results so far indicate that mature T cells are less susceptible to transformation by known T cell proto- oncogenes, but the studies are still ongoing.

Stem cell transplantation II

0421

GRAFT-VERSUS-LEUKEMIA INDUCED BY GRAFT-VERSUS-HOST DISEASE EARLY AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION IN CHILDREN WITH DETECTABLE LEVELS OF RESIDUAL ACUTE LYMPHOBLASTIC LEUKEMIA PRIOR TO TRANSPLANTATION

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Children with high risk acute lymphoblastic leukemia who receive an allogeneic stem cell transplantation (SCT) nevertheless have a high chance of relapse (40%). It has been reported that the presence of minimal residual disease (MRD) detected by sensitive molecular techniques immediately prior to SCT predicts a very high probability of relapse (80%). This criterion was used to select patients for an intervention study. In patients with MRD levels > 1×10^{-4} in their bone marrow pretransplantation, we have attempted to induce a graft-versus-leukemia effect by early tapering of cyclosporin A (CsA, 4-5 weeks post-SCT) followed, if needed, by infusion of incremental doses of donor lymphocytes (DLI). Dosage varied between 1×10^{5} and 4×10^{6} $CD3^{+}$ cells/kg depending on transplantation characteristics. When GVHD grade II occurred, therapy was initiated to suppress GVHD. Patients with low (<1×10^{-4}) or undetectable levels of MRD were not eligible for intervention. As of October 2006, 42 patients were transplanted and included in the protocol with a median follow-up of 11 (1-65) months. In 13/42 patients MRD levels were > 1×10^{-4}. In 11 of these patients CsA was tapered 4-5 weeks after SCT. Four patients developed GVHD, grade II after tapering of CsA, which was controlled by treatment. Two relapsed, the other two are still in remission. The remaining 7 MRD-positive patients received DLI, and did not develop GVHD. Of those 7 patients, 5 had a relapse and 2 are in remission as of to date. Of the 7 relapses 4 were extramedullary. Of the remaining 29 patients in whom MRD was either below the cut-off level of 1×10^{-4}, positive but not quantifiable, or undetectable, 9 relapsed (31%) with GVHD greater than grade I, in 2 patients. As no transplant related mortality was seen, due to induction of GVHD, these data indicate that early reduction of immunosuppression and low dosis DLI can safely be performed following SCT. The timing of (mostly) extramedullary relapses in the MRD high group suggests an antileukemic effect of the intervention.

0422

A PROSPECTIVE RANDOMIZED TRIAL OF TWO DOSE-INTENSIVE MELPHALAN REGIMENS (100 VS 200 MG/MQ) IN NEWLY DIAGNOSED MYELOMA PATIENTS

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Background. Several trials have shown the superiority of high-dose melphalan (usually 200 mg/m^2, MEL200) versus standard therapy in myeloma patients. Intermediate-dose melphalan (100 mg/m^2, MEL100) is also superior to the standard dose, but has not been clinically compared with MEL200 in a randomized study. In this prospective, randomized, phase III trial, we compared MEL200 with MEL100. *Aims*. The primary end points were complete remission (CR) rate, event-free survival (EFS) and incidence of gastrointestinal toxicity, infections and early deaths. *Methods*. Between January 2002 and July 2006, 298 patients were enrolled. Inclusion criteria were previously untreated myeloma, age < 65 and Durie and Salmon stage II or III. Exclusion criteria were prior treatment for myeloma, abnormal cardiac function (systolic ejection fraction <50%), respiratory disease (vital capacity or carbon monoxide diffusion <50% of normal), abnormal liver function (serum aminotransferase value >2.5 of normal), abnormal renal function (serum creatinine > 3 mg/dL), HBV, HCV, or HIV positivity, concomitant cancer or psychiatric disease. The institutional review board approved the protocol and written informed consent was obtained from all patients. All patients received: 2 DAV debulking courses (dexamethasone-doxorubicin-vincristine), 2 cycles of cyclophosphamide (4 mg/m^2) plus G-CSF followed by stem cell harvest. The MEL200 group was conditioned with 2 cycles of melphalan 200 mg/m^2. The MEL100 group was conditioned with 2 courses of melphalan 100 mg/m^2. All MEL courses were followed by stem cell infusion. *Results.* Two-hundred and forty-six patients (median age 57) were evaluable: 124 in the MEL200 arm and 122 in the MEL100. Patient characteristics were similar in both groups. In intention-to-treat analysis, the very good partial response rate was higher in MEL200 arm (38% versus 22%, p=0.011), but CR was 17% in the MEL200 group and 10% in the MEL100 group (p=0.2). The median follow-up for censored patients was 26.5 months. The 3-years EFS was 48% in the MEL200 and 31% in the MEL100 arm (p=0.31). The 3-years overall survival was 86% in the MEL200 and 71% in the MEL100 group (p=0.51). Forty-six patients did not complete tandem MEL200; 36 did not complete tandem MEL100. Severe hematologic toxicity was comparable in two arms, but 84% of MEL200 patients received more than 4×10^{6} $CD34^{+}$/Kg compared with 52% of MEL100 (p=0.0001). Grade 3-4 non-hematologic adverse events were more frequent in the 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 was 51% after MEL200 and 21% after MEL100 (p<0.001). The incidence of grade 3-4 infections was similar in both group (54% in the MEL200 versus 45% in the MEL100 patients, p=0.25). Early deaths were 6% in the MEL200 and 4% in the MEL100 arm (p=0.9). *Conclusions.* MEL200 resulted in a significantly higher very good partial response rate but this did not translate in a superior EFS and overall survival.

0423

A RAPID CLEARANCE OF MINIMAL RESIDUAL DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION PREDICTS THE CLINICAL OUTCOME OF HIGH RISK ADULT ALL PATIENTS

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Background. Allogeneic stem cell transplantation represents a curative option for patients with acute lymphoblastic leukemia (ALL) with high-risk features at diagnosis. However, the percentage of relapse after transplant is still high (30-40%). The molecular analysis of Minimal Residual Disease (MRD) may provide informative results as to the risk of recurrence in patients with acute lymphoblastic leukemia. *Aims*. Objective of this study is to correlate the kinetics of MRD clearance after allogeneic transplantation with the clinical outcome. *Methods.* Patients were diagnosed and treated in the context of multicenter Northern Italian Leukemia Group (NILG) protocol for adult ALL 09-00 (ClinicalTrials.gov Identifier: NCT00358072). MRD was evaluated by Real Time Quantitative Polymerase Chain Reaction (RQ-PCR) using probes derived from fusion chimeric genes (BCR/ABL and MLL/AF4) or rearrangements of the T-cell receptor or the Immunoglobulin genes. 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.* Diagnostic samples from 43 ALL patients were analyzed to identify a molecular probe suitable for MRD evaluation. Twenty patients with B precursor ALL were positive for the BCR/ABL chimeric transcripts and two for MLL/AF4 transcript. The remaining patients (6 T-ALL and 15 B precursor ALL) were studied for the presence of clonal Ig or TCR gene rearrangements. The sensitivity of the probes by which patients were actually monitored during the follow-up was ranging from 10-3 (7% of cases) to 10-5 (70% of cases). At day +30 after transplant, a median 3-log reduction of leukemic cells was documented so that 71% of patients converted to a molecularly negative status. At day +100 evidence of leukemia persistence/progression was documented in 44% of patients. As expected, a ten times higher median MRD value at conditioning was documented in the BM of most patients who failed to achieve the molecular remission by day +30 or +100. With a median follow-up of 23 months (range 4-138), the OS at 36 months of these patients was 48% (95% CI 31-63). The OS of patient who underwent transplant in hematological remission was 80% for those who proved PCR negative before transplant as compared to

49% for PCR positive patients (95% CI, 20-67), (p=0.17). For the same patients, 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. *Conclusions*. These observations may help in identifying patients at high risk of leukemia relapse after allogeneic stem cell transplantation. More importantly, our results strongly suggest that patients not achieving an early molecular remission after transplantation require timely and appropriate preemptive treatments such as immunosuppression modulation, infusions of donor lymphocytes or new experimental drugs.

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DIRECT INTRA BONE TRANSPLANT OF UNRELATED CORD BLOOD CELLS IS ASSOCIATED WITH FAST AND COMPLETE HEMATOLOGIC RECOVERY

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Background. Cord blood transplants (CBT) are associated with delayed or failed engraftment in a significant proportion of patients. Two previous our observations suggested (i) that, in the animal model, direct intrabone (i.b.) injection of hematopoietic cells improves seeding efficiency and (ii) that the delayed engraftment was not related to an insufficient number of hematopoietic stem cells but rather to a reluctance to differentiation and maturation. *Methods.* Unrelated CB (4/6 or 5/6 HLA antigen matched) were selected for 19 consecutive patients. Median cell dose was 2.6×10^7/kg (range 1.6-3.5). CB cells were concentrated in a total volume of 20 mL and infused in the supero-posterior iliac crest (SPIC) under rapid general anesthesia (10 minutes with propofol). Patients median age was 40 years (18-60), 15 had acute leukemia, 2 chronic myeloid leukemia, 2 Hodgkin disease. Fifteen patients had refractory or advanced disease, whereas 4 had high risk first remission leukemia. Most patients (n=15) were prepared with conventional CY-TBI. *Results.* The infusion of cells i.b. in supero-posterior iliac rest (SPIC), in the operating room, under short general anaesthesia, was uneventful. All patients engrafted. Cumulative incidence of neutrophil and platelet engraftment was 100% at 50 days. Median for neutrophil engraftment ($>0.5\times10^9$/L) was day 23 (14-40), whereas for platelets ($>20\times10^9$/L) it was day 38 (range 22-47). Three patients are not evaluable because died within day 10 from transplant. Two patients relapsed and two died of infection and multiorgan failure. Twelve patients are alive and well in hematologic remission at a median follow up of 6 months (range 2-11). 100% donor chimerism was documented since 30 days onward after transplant, including those in which CB cells were injected monolaterally in one SPIC. From day +60, CFC and LTC-IC had already reached the lower values of the range of normal individuals in bilateral sites. This is particularly relevant considering that hemopoietic progenitor reconstitution remains deficient years after allo-transplant. Thus, direct injection of CB cells in the bone marrow spaces, produces *in situ* proliferation and maturation, rapid recovery of peripheral blood counts, and early recirculation of stem cells to other un-injected bone marrow sites. Acute GvHD grade II was seen in 1/20 patient (5%). Since lymphocyte trafficking is known to be an essential part of immune response, two combined factors might contribute: (i) injected T cells come immediately in direct contact with mesenchymal stem cells (MSC) and osteoblasts which are known to be potent immunomodulators; (ii) the T cells present in the graft do not reach primarily in the lymphatic organs, where they would be immediately confronted with host antigen presenting cells, as it probably occurs after intra-venous injection. *Conclusions.* This study shows that CB cells injected directly into the iliac crests are associated with fast and complete hematologic recovery and almost absent acute GvHD. The direct intra-bone transplant of CB cells could now be offered to many more adult patients. This may change our policy of hemopoietic cell transplants.

0425

THE ROLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN THE TREATMENT OF WALDENSTRMS MACROGLOBULINAEMIA PATIENTS. ANALYSIS OF 201 CASES FROM THE EUROPEAN BONE MARROW TRANSPLANT REGISTRY (EBMT)

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Background. Waldenström's Macroglobulinaemia (WM) is a relatively rare lymphoma, which primarily affects elderly patients. Standard doses of alkylating agents, purine analogues and anti-CD20 monoclonal antibody produce response rates of up to 60%. Nevertheless, complete responses are infrequent and there is no cure. Due to the indolent nature of the disease and the fact that patients are old and present with comorbidities, the evaluation of the role of high-dose therapy with autologous transplantation (ASCT) has been infrequent in the past. *Aims.* Herein we report a retrospective multicenter analysis of 201 WM patients (132 male, 69 female), who an ASCT between 1992 and 2005 were reported to the database of the Lymphoma WP of the EBMT. *Patient and Methods.* Median age at transplantation was 53 years (22-73), the median time from diagnosis to transplant was of 18 months (3-239) and the patients had received a median number of 2 (1-10) lines of therapy before ASCT. Forty patients (20%) were in CR1, 24 (12%) in CR2, 83 (41%) in PR1, 27 (13%) in PR2, 21(10%) with primary refractory at the time of transplantation. Conditioning regimens used were BEAM in 44% of the cases, the combination of cyclophosphamide or melphalan/TBI in 28% of the cases, high dose melphalan in 4% and other protocols in the remaining 14% of the cases. Peripheral blood was used as the source of stem cells in 188 patients, bone marrow in 10 patients and the combination of both in 3 patients. *Results.* All patients but 3 had a successful engraftment. With a median follow-up of 26 months (5-163), 112 (56%) patients are alive and free of disease and 73 (36%) patients have relapsed after a median of 14 months (1-110) post ASCT. Fifty-two patients have died, 36 (18%) from disease progression and 16 (8%) from regimen toxicity. Non- relapse mortality was 6% at 1 year. The actuarial overall survival was 86% at 1 year, 75% at 3 years and 61% at 5 years. The probability of relapse was 20% at 1 year, 38% at 3 years and 55% at 5 years and the estimated progression free survival of 74%, 54% and 33% at 1, 3 and 5 years, respectively. *Conclusions.* In conclusion, this study shows that ASCT is a safe procedure and is able to rescue a significant proportion of heavily pre-treated patients with WM.

Myelodysplastic syndromes

0426

CYTOGENETIC HYBRIDIZATION BY ARRAY COMPARATIVE GENOMIC HYBRIDIZATION AND FLUORESCENCE *IN SITU* HIBRIDIZATION OF MESENCHYMAL STEM CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDS) are a group of clonal disorders of hematopoietic stem cell (HSC). The hematopoietic microenvironment plays a role in the physiology of the hematopoietic system, and mesenchymal stem cells (MSC) are a key component of this microenviroment. Recently, some data has been published showing that these MSC could be involved in the MDS pathophysiology. Moreover, the presence of cytogenetic aberrations on these cells is controversial. The studies performed, so far, have used conventional cytogenetics and fluorescent *in situ* hybridization (FISH). *Aims*. To characterize bone marrow derived MSC from patients with MDS, by two approaches: array comparative genomic hybridization (array-CGH), and FISH, in order to know genomic changes in these cells and whether they could show clonal cytogenetic alterations. *Methods.* 13 patients with untreated MDS were included in the study. The median age was 72 years (range: 54-89) The male/female ratio was 7/6. Diagnosis of MDS was established according to the WHO classification as follows: 5q- syndrome (n=7), refractory anemia (RA) (n=2), refractory anemia with ringed sideroblasts (RARS) (n=1) and refractory anemia with excess blasts type II (RAEB-II) (n=3). Standard cytogenetic and FISH studies on hematopoietic cells were performed at diagnosis according to standard methods with the following *Results.* 5q- (n=7), 8 trisomy (n=2) and normal cytogenetics (n=4). MSC were obtained by plating mononuclear cells from BM and cultured in a DMEM + 10% fetal calf serum medium, and expanded following standard procedures, until the third passage, when adherent MSC were harvested to perform cytogenetic and phenotypical studies. Array-CGH was performed in 11 cases and FISH studies in 5 (3 cases had both techniques). To perform Array-CGH a total of 3500 genomic targets were compounded from RP-11 libraries. The PCR products after purification were arrayed onto glass slides using a BioRobot. DNA was labelled, denaturalised and hybridizated. Images were analysed by using GenePix Pro 4.0 software. *Results.* MSC showed characteristic phenotypical data and their purity was 91%. In all cases analysed MSC showed DNA genomic changes. The most frequent aberrations were 1q24q32 region gains, in 72% of the cases, and 1p13 and 11q12 losses in 55% of patients. When the cells from patients carrying the 5q- alteration were analysed we observed that in half of them 5q losses were present in MSC. FISH analysis was carried out in MSC from 3 patients with 5q- and 2 patients diagnosed of RARS and RAEB-II, who had trisomy 8, and the same changes were present in 11% of MSC. *Conclusions.* We conclude that 100% of MSC from MDS show chromosomal aberrations when CGH arrays are used for this analysis. Even clonal specific MDS alterations are also shown when both, FISH and array-CGH analysis are performed. This is the first study that has showed the gains and losses in DNA of MSC from MDS by Array-CGH. MSC aberrations were also confirmed by FISH.

0427

HAS TREATMENT WITH EPO+/- G-CSF AN IMPACT ON PROGRESSION TO AML AND SURVIVAL IN LOW/INT-1-RISK MDS? A COMPARISON BETWEEN FRENCH-EPO PATIENTS AND THE IMRAW DATABASE

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Backround. We recently reported the results of treatment with EPO ±G-CSF in 419 patients (Park, ASH 2006, n°522) treated between 1998 and 2005 in France. *Aims.* Here we compared the outcome (progression to AML and survival) of these French patients (French-EPO) to MDS patients who received only supportive care (IPSS/IMRAW database). *Methods.* We restricted the analysis to patients with low and int-1 IPSS and also excluded CMML, RAEB-t, unclassifiable MDS, poor- risk karyotype, and 3q26 deletion, that are classically not the best candidates for EPO treatment. Progression to death and progression to AML since MDS diagnosis were compared using Kaplan-Meier estimates and Log-rank test. Multivariate cox analyses were adjusted for the main known prognostic factors (age at diagnosis, sex,% of marrow blasts, FAB diagnosis, IPSS score and karyotype). EPO introduction was modeled as a time-dependent covariate (dependent on the delay between MDS diagnosis and EPO introduction). *Results.* In the French-EPO (F), 284 pts, and in the IMRAW database (I), 447 pts were finally compared. Median follow-up was 26 months (IQR 13-53) and 33 months (IQR 15-61) in French-EPO and IMRAW, respectively. Both cohorts were different for: age (median 71 vs 68 y, p=0.0013),% of blasts (median, 3% [IQR 2-5] vs 2% [IQR 1-4], p=0.0006), FAB diagnosis [RA= 39% vs 56%, RAEB= 22% vs 17%, and RARS=38% vs 26%, (p<0.0001)], and karyotype [favorable=90% vs 84%, intermediate= 10% vs 16%, (p=0.02)] for F and I, respectively. At 5 years, 8% pts in the F cohort vs 16% in the I cohort progressed to AML (p=0.0002). In multivariate regression analysis, EPO treatment (HR=0.2, CI 95% [0.1-0.3]) was independently associated with a lower progression to AML. Other factors significantly associated with a lower progression to AML were: feminine sex (HR=0.6, CI 95% [0.5-0.8]), RARS vs RAEB (HR=0.6, CI 95% [0.5-0.9]), and favorable karyotype (HR=0.6, [CI 95% 0.4-0.8]). Overall survival (OS) from diagnosis of MDS was also higher in the French-EPO with a 5-year OS of 82% vs 47% for F and I, respectively (p<0.0001). In multivariate analysis, the same factors were found independently associated with survival as for AML progression. EPO treatment (HR=0.26, [CI 95% 0.18-0.38)]) was also independently associated with a better survival. *Conclusions.* These results suggest that EPO treatment was associated with reduction of the risk of progression to AML and improvement of survival in MDS patients. Reasons underlying this improvement are being examined. Confirmation of these results by other studies and with prospective patient series and clinical trials are required.

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TREATMENT OF HIGH RISK MDS AND AML POST-MDS WITH AZACYTIDINE (AZA): CURRENT RESULTS OF THE FRENCH ATU PROGRAM

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Background. Following FDA approval of AZA for MDS, a compassionate program of AZA (ATU nominative) was started in France for higher risk MDS unfit for intensive chemotherapy and with contra indication to a phase III trial randomizing AZA to conventional treatment. Patients (pts): IPSS int-2 and high-risk MDS (and some int-1) received AZA 75 mg/mÇ/d (d 1-7) (SC) every 4 weeks. *Results.* From Sept 2004 to October 2006, 145 pts from 34 centres were included. We currently had data for 105 of them who had completed at least one course of AZA: M/F: 69/36, median age 71 y [17-102]. WHO at inclusion: 9 RAEB1, 40 RAEB2, 42 AML post-MDS, 4 CMML, 1 RAS, 2 RCMD, 7 unclassified MDS. 36 pts had previously received intensive Anth-AraC, 7 low dose AraC, 22 EPO and 9 non cytotoxic treatments. 31 (29%), 24 (23%) and 50 (48%) pts had fav, int and unfav karyotype, resp. 48 pts (46%) were IPSS high, 48 pts (46%) int-2 and 9 pts (8%) int-1. Pts received a median of 5 cycles [range 1-18+]. Response was generally assessed after 4 cycles, unless pts progressed before. Thus, 20 pts were not yet evaluable for response. Of the remaining 85 pts, 10 (12%) achieved CR (2006 IWG criteria), 29 (34%) achieved PR and 15 (18%) achieved HI (Overall response rate OR=64%). 31 pts (36%) were considered failure: 6 of them died before evaluation and 10 of them were considered failure because early evaluation after only 2 cycles showed stable disease only, and AZA was then stopped. OR was 88%, 65%, 75% and 63% for RAEB1, RAEB2, CMML and AML post-MDS resp (p<0.1); 76%, 58% and 59% for fav, int and unfav karyotype, resp (p=NS). 4/7 pts with monosomy 7 responded (2 CR+2 PR), 2/3 pts with

+8 (1 PR+1 HI) and 14/24 pts with complex karyotype (3 CR+9 PR+2 HI). OR was 59%, 65% and 75% for IPSS high (p=NS), int-2 (p<0.1) and int-1 pts (p=NS) resp and 63%, 67% in pts previously treated or not by cytoreductive agents resp (p=NS). Median survival from inclusion was 8 months [range 1-24+]. AZA induced myelosuppression lead to dose reduction in 16% pts and hospitalization in 23% pts but was not responsible for any death. Other side effects included frequent local reactions (reversible with local NSAID) (54%), grade I-II gastro-intestinal disorders (54%), unexpected cardiac arythmias (2%). *Conclusions.* AZA, in this population with overall unfavorable features, gave response rates at least similar to those of CALGB studies, that may have been higher if treatment had not been stopped in some of the pts stable after 2 courses. Pts with unfavorable karyotype (-7, complex) or +8 had about 59% response rates. Accrual in this program is continuing. Data on response duration will be presented.

0429

PROGNOSTIC VALUE OF BONE MARROW HISTOLOGY IN MYELODYSPLASTIC SYNDROMES CLASSIFIED ACCORDING TO WHO CRITERIA

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Background. In 2002, the WHO proposed a new classification of myelodysplastic syndromes (MDS) that has been shown to have a relevant prognostic value [J Clin Oncol 2005;23:7594, Haematologica. 2006;91:1596]. Previous studies had also shown that bone marrow (BM) histopathology is associated with outcome of MDS, but its prognostic value in patients classified according to WHO criteria has not been defined. *Aims.* To determine the prognostic value of BM histology in MDS classified according to WHO criteria. *Design and Methods.* We retrospectively evaluated 452 patients with a diagnosis of MDS formulated at the Department of Hematology, Fondazione IRCCS Policlinico San Matteo, University of Pavia Medical School, Pavia, Italy, between 1992 and 2004. Patients receiving a diagnosis before 2002 were reclassified according to the WHO criteria. BM biopsies were analyzed by independent pathologists for CD34 immunoreactivity, cellularity, and bone marrow fibrosis according to the European consensus guidelines [Haematologica 2005;90:1128]. Cox proportional hazards regression was used to identify the most significant prognostic factors, and hierarchical clustering analysis was performed to recognize subsets of patients with homogeneous clinico-pathological features. *Results.* Moderate or severe BM fibrosis (grade ≥2) was detected in 57 out of 408 evaluable cases (14%), and was significantly associated with multilineage dysplasia (p<0.001), low hemoglobin and platelet count (p<0.001), higher red cell transfusion need (p<0.001) and poor cytogenetics (p=0.001). In multivariate analysis, BM fibrosis and CD34 clusters showed a significant prognostic value on both OS (p<0.001 and p=0.03, respectively), and LFS (p<0.001 and p=0.006, respectively). The negative effect of BM fibrosis was more significant in MDS patients without excess blasts (OS p<0.001 and LFS p<0.001). A hierarchical clustering analysis allowed us to identify 3 distinct groups of patients. Two of the subsets (n=74 and n=67) included patients with no or mild BM fibrosis, clustered according to WHO classification, while a third cluster (n=67) consisted of patients with increased BM fibrosis (including the 57 patients with BM fibrosis of grade ≥2), high BM cellularity, multilineage dysplasia, and high transfusion need. Patients classified in the latter cluster had significant lower OS and LFS compared to clusters 1 and 2 (p=0.001 and p<0.001). Finally, we applied Cox regression in order to identify the most significant prognostic factors in the cluster with increased BM fibrosis: marrow blasts was the only variable with a significant effect on both OS (p=0.02) and LFS (p=0.01). *Conclusions.* BM histology provides useful clinical information in patients with MDS classified according to WHO criteria. Bone marrow fibrosis identifies a distinct subset of MDS patients with multilineage dysplasia, high transfusion requirement and poor prognosis. This parameter should be taken into account for implementing risk-adapted therapeutic strategies.

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A ROLE FOR THE ENDOPLASMIC RETICULUM IN THE APOPTOSIS OF ERYTHROID PRECURSORS IN LOW RISK MYELODYSPLASTIC SYNDROMES

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Background. The anemia that characterizes low grade myelodysplastic syndromes (MDS) has been explained by an excessive death by apoptosis of bone marrow erythroid precursors. The pathways leading to the death of these precursors have been partially depicted, e.g. we have identified the Fas-dependent activation of caspase-8 as a key initiating event. The endoplasmic reticulum (ER) was shown to play a role in some apoptotic pathways. AIMS. The present study was designed to determine whether the ER was involved in the death of MDS erythroid precursors. METHODS. These erythroid precursors were derived in liquid culture from bone marrow $CD34^+$ progenitors. Intracellular or ER Ca^{2+} stores were measured by spectrofluorimetry. Cells were loaded with Fura-2/AM and stimulated with ionomycin or thapsigargin. These experiments were performed in the presence of the extracellular Ca2+ chelator EGTA. *Results.* We first observed that the basal ER Ca2+ stores were increased in MDS compared to normal erythroid precursors. We also specifically detected a spontaneous cleavage of the ER-associated caspase-4 in MDS erythroid precursors. To further explore the role of the ER in erythroid precursor cell death, we ectopically expressed an ER-targeted Bcl-2 protein in $CD34^+$ cells using a lentiviral vector before inducing their erythroid differentiation. We observed that ER-targeted Bcl-2 protein prevented the spontaneous death of MDS erythroid precursors by acting upstream of the mitochondrial events such as cytochrome c release in the cytosol and decrease in mitochondrial membrane potential. This observation suggested a role for the ER in the pathway to death of MDS erythroid cell precursors, upstream of the mitochondria. Thapsigargin is an inhibitor of sarcoplasmic Ca^{2+} (SERCA) pumps that can induce apoptosis through ER stress. MDS erythroid precursors did not demonstrate a higher sensitivity to thapsigargin-induced apoptosis than normal erythroid precursors. Thapsigargin enhanced mitochondrial Ca^{2+}, which was inhibitable by ruthenium red, suggesting that Ca^{2+} import into the mitochondria was operational in both cell types. ER-targeted Bcl-2 protected MDS erythroid precursors from thapsigargin-induced apoptosis. This protective effect of Bcl-2 in MDS erythroid precursors could not be related to a depletion of ER Ca^{2+} stores, as observed in other cell types including normal erythroid precursors. Rather, Bcl-2 targeted to ER appeared to prevent caspase-4 activation in MDS erythroid progenitors undergoing spontaneous and thapsigargin-induced apoptosis. *Conclusions.* Altogether, our data indicate that ER plays a central role in apoptosis of MDS erythroid precursors, upstream of the mitochondria, through a Ca^{2+}-independent and Bcl-2-sensitive pathway that appears to involve caspase-4.

Stem cell biology and microenvironment

0431

INVERSE REGULATION OF HEMATOPOIETIC PROGENITOR CELL EGRESS BY MT1-MMP AND RECK

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Background. hematopoietic stem and progenitor cells continuously exit from the bone marrow (BM) reservoir to the blood circulation as part of homeostasis and host defense. Clinical recruitment of progenitors to the peripheral blood (PB) is achieved by chemotherapy and repeated G-CSF stimulations, which mimic physiological egress of stem and progenitor cells during injury and inflammation. Yet, mechanisms governing progenitor cell trafficking during steady state homeostasis and clinical mobilization are not fully understood. Membrane type-1 metalloproteinase (MT1-MMP) and its endogenous inhibitor, reversion-inducing-cysteine-rich protein with Kazal motifs (RECK), are established key regulators of tumor and endothelial cell motility. *Methods and Results.* In our study we found that egress of human hematopoietic CD34$^+$ progenitors requires cell autonomous changes in their motile properties inversely mediated by MT1-MMP and its endogenous inhibitor, RECK. By flow cytometry analysis we detected higher MT1-MMP and lower RECK expression on circulating human CD34$^+$ cells and maturing leukocytes as compared to immature BM cells. MT1-MMP expression was even more prominent on CD34$^+$ cells obtained from PB of G-CSF-treated healthy donors whereas RECK labeling was barely detected. In addition, five daily injections of G-CSF to NOD/SCID mice, previously engrafted with human cells, increased MT1-MMP and decreased RECK expression on human CD45$^+$ leukocytes, human immature CD34$^+$ and primitive CD34$^+$/CD38$^-$/low cells, in a PI3K/Akt1-dependent manner, resulting in elevated MT1-MMP activity. Inverse regulation of MT1-MMP and its inhibitor RECK by G-CSF induced mobilization was confirmed by *in situ* immuno-labeling of BM sections, as well as by human MT1-MMP and RECK mRNA expression analysis of leukocytes repopulating the BM of chimeric mice. Blocking MT1-MMP function halted mobilization, while RECK neutralization promoted egress of human CD34+ progenitors in the functional model of NOD/SCID chimeric mice. Treatment with MT1-MMP neutralizing Ab or with tissue inhibitor of metalloproteinase-2 (TIMP-2) reduced the *in vitro* chemotactic response to SDF-1 of human G-CSF-mobilized CD34$^+$ cells via matrigel coated filters. In contrast, blocking RECK function by neutralizing Ab, thus abrogating RECK-mediated inhibition of MT1-MMP, facilitated SDF-1-induced migration of steady state human BM CD34$^+$ cells. Following G-CSF-induced mobilization, we also observed a reduction in CD44 expression on human leukocytes and, specifically, on immature CD34$^+$ progenitor cells in the BM of chimeric mice. This was accompanied by accumulation of CD44 cleaved products of molecular weights, expected for MT1-MMP activity, in the BM supernatants. In chimeric mice co-injected with MT1-MMP-neutralizing Ab, less cleavage of CD44 was detected upon G-CSF mobilization, whereas in the absence of a mobilizing signal, increasing MT1-MMP activity by anti RECK Ab injection facilitated CD44 proteolysis on the BM cells. Finally, MT1-MMP expression correlated with the number of CD34$^+$ cells, collected on the first apheresis day in 29 consecutive patients with lymphoid malignancies and in 21 healthy donors treated with G-CSF. *Conclusions.* our results indicate that MT1-MMP and RECK oppositely control G-CSF induced-hematopoietic progenitor cell mobilization, by regulating CD44 surface expression and ultimately, cell adhesion and motility. These molecules might serve as targets for new approaches to improve clinical stem cell mobilization and repopulation.

0432

SNX5, A NOVEL GENE LINKED TO FANCONI ANAEMIA CAUSES HAEMATOPOIETIC FAILURE WHEN KNOCKED DOWN IN ZEBRAFISH

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Background. Sorting nexin 5 (SNX 5) is a member of the sorting nexin family, a diverse group of cellular trafficking proteins unified by the presence of a phospholipid binding domain (PHOX) which is involved in protein-protein interactions. While the function of SNX5 is unknown, there is evidence from yeast two hybrid studies that SNX5 binds to FANCA, and SNX5 is one of the 20 genes located on a region on mouse chromosome 2 associated with accelerated stem cell aging. More recently SNX5 has been shown to be a partner of Mindbomb- an E3 ubiqitin ligase essential for notch signal activation. *Aims.* We have shown that SNX5 is significantly higher expressed in CD34$^+$CD33$^-$CD38$^-$Rho$^+$ (Rho-hi) cells from umbilical cord blood (UCB) and bone marrow (BM), depleted of SCID repopulating cells (SRCs) than in CD34$^+$CD33$^-$CD38$^-$cKIT$^+$ Rho- cells (Rholo), containing all SRCs. We are investigating the role of SNX5 in Fanconi anaemia and developmental haematopoiesis. *Results.* A large scale high throughput functional analysis was carried out in zebrafish and genes differentially expressed between Rholo and Rhohi cells were knocked down using morpholino (MO) antisense oligonucleotides. Morpholinos are useful tools which can specifically inhibit the translation of target mRNA. 16 out of 70 genes knocked down, including snx5, demonstrated decreased circulating blood in the zebrafish injected. Further analysis has shown that MO knock down of snx5 in zebrafish (n=152) results in haematopoietic failure with normal vasculature, with normal expression of scl and gata-1 by *in situ* hybridization, indicating a defect at or beyond the haematopoietic stem cell (HSC) stage. Quantitative RT-PCR was used to further confirm the phenotype. Levels of haemoglobin, l-plastin and myeloperoxidase mRNA in snx5 morphants compared to uninjected controls, were significantly lower, consistent with a multi-lineage haematopoietic differentiation defect. The decrease in circulating blood could be partially rescued by overexpressing human SNX5 cDNA, confirming specificity of the morphant phenotype. To determine whether SNX5 might be linked to Fanconi anaemia (FA), we measured levels of SNX5 mRNA and protein in EBV transformed cell lines from patients with Fanconi-complementation group A (FANCA), -complementation group C (FANCC) or unknown complementation group (FANC-NX). We have found no significant difference between SNX5 mRNA and protein expression in 11 Fanconi cell lines tested compared to Raji controls. The association of SNX5 and Fanconi anaemia in mammalian cell lines has been inconclusive. Studies examining SNX5 over expression and knockdown in human UCB using lentivirus, the impact on haematopoietic engraftment and differentiation in a NOD SCID murine model are in progress and this data will be presented. *Conclusions.* Knockdown of snx5 in zebrafish results in decreased blood, with an effect at or beyond the definitive HSC. There is no quantitative defect of SNX5 in Fanconi anaemia cell lines and mammalian studies are ongoing to determine its association with the HSC.

0433

IMPACT OF STOCHASTIC DYNAMICS ON THE ACTIVE HEMATOPOIETIC STEM CELL POOL

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Background. Hematopoiesis is maintained by a small group of hematopoietic stem cells (HSC) that may contribute to blood formation for many years, if not for the lifetime of the individual. HSC replicate approximately 1/year and this slow replication rate is one mechanism to minimize the risk of acquiring mutations in this important pool of cells. However, HSC do acquire mutations that can lead to either neoplastic proliferation (e.g. chronic myeloid leukemia, CML) or marrow failure (e.g. paroxysmal nocturnal hemoglobinuria, PNH). *Aims.* Given that the size of the HSC is small, we aim to determine the potential role of stochastic effects on the evolution of mutant clones that originate in this pool of cells by mathematical modeling. *Methods.* We develop a stochastic model of HSC dynamics based on the Moran process where the cell population is maintained strictly constant. Cells are chosen for reproduction based on their fitness and each time a cell is chosen for reproduction, it has a probability to mutate into a cancer stem cell (CSC). In this model we consider that one mutation is enough to lead to cancer

(e.g. bcr-abl leading to CML). CSC have a fitness advantage r compared to normal cells with fitness 1. Cells are selected for reproduction proportional to their fitness. After each reproductive event, one cell chosen at random from the pool is eliminated. We follow this birth-mutation-export process for a HSC pool of ~400 cells and perform stochastic simulations to follow the evolution of the clone and determine time probability density functions for either extinction or complete invasion by the mutant stem cells. *Results.* Stochastic dynamics leads to three possible scenarios: clonal extinction, latency/stability or complete takeover of the pool of the HSC pool by CSC. Although stochastic extinction is rare, this is possible even for mutants with a significant fitness advantage. The mutant clone can remain latent for many years. The time required for the mutant clone to reach a threshold compatible with a diagnosis of cancer varies significantly and has a very broad distribution, especially if the fitness advantage conferred by the mutation is small. As the fitness advantage increases, the time to diagnosis decreases and while extinction is still possible this becomes a rare event. *Summary and conclusions.* Stochastic considerations with respect to the active HSC pool are important and can possibly explain observations such as stability of JAK2 mutant clones in patients with essential thrombocythemia, disappearance of bcr-abl clones in healthy adults or transient leukemia in children with Down syndrome. Single mutations have to give a significant fitness advantage (r>1.7) to the cell for the disease to appear within the observed timeframes.

0434

A PUTATIVE ROLE FOR VEGFR-1 (FLT-1) IN B CELL COMMITMENT AND DIFFERENTIATION

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VEGF and its receptors are expressed in the hematopoietic system. A role for FLT-1 in particular was described in monocyte-macrophage migration and differentiation, megakaryocytes maturation and dendritic cell differentiation. Given that the expression of this receptor in the lymphoid lineage is not known, we studied FLT-1 expression and a its functions in normal lymphoid progenitors (B cells). To address this question we induced *in vitro* $CD34^+$ cord blood cells differentiation into the B cell lineage using a well established assay (on S17 stromal cells). With this approach, we observed that FLT-1 is expressed throughout B cell differentiation increasing along the differentiation process, and reaching its highest at the *small pre-B cell* stage. We also neutralized FLT-1 during B cell differentiation *in vitro*. Surprisingly, in the presence of the FLT-1 neutralizing antibody (6.12 monoclonal Ab, from ImClone systems), at the end of the assays (4 different experiments) a significantly higher number of $CD19^+$ cells were detected. We observed that the expression of PU.1, Pax5 and E47 was also up-regulated by FLT-1 neutralization. To understand if VEGF/PlGF signalling through FLT-1 promoted myeloid differentiation, suppressed B cell differentiation or simply regulated the quiescent state of hematopoietic stem cells, we studied the *in vitro* differentiation of $CD34^+/FLT\text{-}1^-$ and $CD34^+/FLT\text{-}1^+$ cells (10% of $CD34^+$ population) using the assay described above. Interestingly, $CD34^+/FLT$-1- differentiation *in vitro* largely promoted B cell differentiation, while $CD34^+/FLT$-1+ cells originated mostly myeloid cell differentiation. These results pointed out for a role of FLT-1 in B cell commitment. In order to confirm it, we used FLT-1 positive and negative BM precursors to reconstitute the BM of immunocompremised (sub lethally 350 rad irradiated) mice. Surprisingly, $FLT\text{-}1^+$ precursors lacked the capacity to engraft and/or reconstitute the BM of irradiated recipients and at the end of 5 days all mice in this group were dead. On the other hand, $FLT\text{-}1^-$ precursors not only reconstituted the recipient BM but also originated B cells, which were detected/quantified in both BM and in the spleen. Next, given that FLT-1 function was mainly associated with cell migration, and since it is expressed at the BM latest B cell stages, we reasoned that FLT-1 might have a role in B cells exit from the BM. For this purpose, we used LPS to induced B cells exit from BM and pre-treated some of the mice with a FLT-1 neutralizing Ab. FLT-1 neutralization not only prevented LPS-induced B cells exit from BM but also decreased the number of activated B cells in the peritoneal cavity. Taken together, our results reveal a role for FLT-1/VEGF in regulating B cell commitment from precursor cells and later during B cell differentiation as a chemoattractive signal.

0435

RXR α DIFFERENTIALLY REGULATES MYELOID SUBLINEAGE DIFFERENTIATION

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Cells of the myeloid lineage including neutrophil granulocytes (G), monocytes (M) and dendritic cells (DC) are continously regenerated by bone marrow resident hematopoietic stem cells. Nuclear receptor (NR) family members are ligand-activated transcription factors that play key roles in cellular proliferation and differentiation processes including myelopoiesis. Retinoid X receptor alpha (RXRα) represents the predominant NR type I and II homo- and heterodimerization partner in myeloid cells. RXRα partner availability regulated by intracellular RXRα abundance is thought to determine NR signaling. However its regulation in primary human neutrophil versus M versus DC differentiation remained uncharacterized. Here we show that human myeloid progenitors express RXRα protein at sustained high levels during M-CSF-induced monopoiesis. In sharp contrast, RXRα is downregulated during G-CSF-dependent late-stage neutrophil differentiation from myeloid progenitors. Downregulation of RXRα is critically required for neutrophil development since ectopic RXRα inhibited granulopoiesis by impairing proliferation and differentiation. Moreover ectopic RXRα was sufficient to redirect G-CSF-dependent granulocyte differentiation to the monocyte lineage and to promote M-CSF-induced monopoiesis. Functional genetic interference with RXRα signaling in hematopoietic progenitor/stem cells using a dominant-negative RXRα promoted the generation of late-stage granulocytes in human cultures *in vitro* and in reconstituted mice *in vivo*. Therefore, our data suggest that high levels of RXRα?are not compatible with neutrophil development but redirect common progenitors towards the M lineage. Furthermore we show that a third sublineage of myeloid origin - Langerhans-type DC (LC) - are also regulated by RXRα. RXRα activation inhibited TGFβ1-dependent LC proliferation and differentiation and arrested LC development at a CD14loCD11blo/- common LC/intDC/M progenitor stage. Ectopic expression of the myeloid transcription factor PU.1 - but not RelB - fully restored LC development from arrested progenitors in human primary cultures, suggesting that RXRα functionally interferes with PU.1 signaling Taken together we conclude that RXRα abundance and activation determine the fate of myeloid progenitors towards G, M and DC in a differentiation stage and cytokine-dependent manner.

Bone marrow failure

0436

IMPACT OF SOMATIC CELL MOSAICISM IN FANCONI ANEMIA ON DEVELOPMENT OF BONE MARROW FAILURE

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Background. Determination of the degree of somatic mosaicism providing functional correction of Fanconi anemia (FA) hematopoiesis has direct implications for gene therapy for FA: it may help assess the percentage of FA hematopoietic cells corrected by gene therapy approaches that are needed to achieve clinically meaningful effects. *Aims.* Hypersensitivity to DNA inter strand cross-linking agents, such as diepoxybutane (DEB) and mitomycin C (MMC), is a cellular marker for diagnosis of FA. However, in some FA patients a population of DEB-resistant PHA-stimulated lymphoblasts (PHA-L) was observed, and this population sometimes varied over time. Our aim was to assess the significance of this finding on hematopoietic function. *Methods.* To that end, we evaluated the MMC sensitivity of bone marrow mononuclear cells (BMMC) and DEB sensitivity of PHA-L and cultured lymphoblastoid cell lines (LCL) in 42 consecutive FA patients referred to the University of Minnesota. In cases where LCL were DEB-resistant, cultured fibroblasts were also studied. BMMC were cultured in the presence of increasing concentrations of MMC. PHA-L and LCL were cultured in DEB at 0.1 μ/ml. *Results.* Wild type BM progenitors (N=17 subjects) proliferated regardless of increasing MMC concentrations (albeit at decreased efficiency at the highest concentrations) as follows: 0 MMC (normalized to 100%), 5 nM MMC (99% [standard deviation, SD, 16%]), 10 nM MMC (90% [SD 22%]), 25 nM MMC (77% [SD21%]), and 50 nM MMC (44% [SD 30%]). Of the 42 FA patients, BMMC failed to proliferate at 0 nM MMC in 10 patients and at 5 nM MMC in 20 patients. Twelve FA patients had MMC resistant BMMC: cells cultured in 5, 10, 25 and 50 nM MMC grew 44% (SD 28%), 35% (SD 24%), 24% (SD 30%) and 17% (SD 32%) of colony numbers in MMC free culture, respectively. Six of these 12 subjects were PHA-L mosaics as determined by DEB sensitivity testing. Four patients with no growth of BMMC at 0 or 5 nM MMC were also somatic mosaics in their PHA-L and LCL. Thus there was no clear correlation between somatic mosaicism as demonstrated by DEB testing in peripheral blood and sensitivity of BMMC to growth in MMC. Clinically, two patients with hematopoietic somatic mosaicism (out of six subjects with simultaneous sensitivity of BMMC to MMC and PHA-L to DEB) developed severe marrow aplasia, one of which received hematopoietic stem cell transplantation. The remaining four hematopoietic mosaic patients had normal or near normal peripheral blood counts. While patients with hematopoietic somatic mosaicism had mixed populations of DEB sensitive cells in their peripheral blood, all their fibroblast cultures were DEB sensitive. *Summary.* These data show that the presence of somatic mosaicism per se does not necessarily prevent bone marrow failure. Moreover, the data suggest that patients with stigmata of FA may have chromosomal breakage studies showing few cells (or no cells) with the characteristic changes of FA; in these cases, skin fibroblasts should be tested as well.

0437

INCREASED PRODUCTION OF TGF-Β1 BY BONE MARROW STROMAL CELLS IS ASSOCIATED WITH DOWNREGULATION OF IL-10 AND OVEREXPRESSION OF SOLUBLE FLT-3 LIGAND IN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

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Background. Chronic idiopathic neutropenia (CIN) is a bone marrow (BM) failure syndrome characterized by low number of circulating neutrophils for prolonged period of time. The disease has been attributed to low number of BM myeloid progenitor cells due to an inflammatory BM microenvironment. Among multiple cytokines normally involved in regulation of myelopoiesis, transforming growth factor-β1 (TGF-β1), interleukin-10 (IL-10), and soluble flt-3 ligand (sFL) play a significant role. The involvement of these cytokines in the pathogenesis of CIN has not been extensively studied. *Aims.* The aim of the study was to evaluate the production of TGF-β1, IL-10 and sFL in the BM microenvironment of CIN patients and investigate the possible involvement of these cytokines in the pathogenesis of neutropenia in the affected subjects. *Materials and Methods.* BM was obtained from 70 patients with CIN and 35 healthy controls. All patients fulfilled the diagnostic criteria for CIN as we have previously published (Blood 2003; 101: 2591-2600). Aliquots of BM mononuclear cells (BMMCs) were evaluated by flow cytometry for $CD34^+/CD33^+$ expression. Long-term BM cultures (LTBMCs) were initiated from BMMCs according to a standard technique and TGF-β1, IL-10 and sFL were measured in the supernatants by means of ELISA. LTBMC supernatant IL-10 and sFL were also measured in patient cultures after the addition of anti-human TGF-β1 mouse monoclonal neutralizing antibody. *Results.* The levels of TGF-β1 in LTBMC supernatants were significantly increased in CIN patients compared to controls ($p<0.0001$). Individual values of the cytokine inversely correlated with the number of circulating neutrophils (r=-0.3776, $p<0.0001$). In contrast, LTBMC supernatant IL-10 levels were significantly decreased in the patients compared to controls ($p<0.0001$), and individual values of the cytokine inversely correlated with the values of TGF-β1 (r=-0.6117, $p<0.0001$) and positively with the number of circulating neutrophils (r=0.4957, $p<0.0001$. The levels of culture supernatant sFL were significantly increased in the patients, compared to controls ($p<0.0001$). sFL values inversely correlated with the values of supernatant IL-10 (r=-0.4401, $p<0.0001$) and the number of circulating neutrophils (r=-0.4370, $p<0.0001$) and positively with the values of supernatant TGF-b1 (r=0.4807, $p<0.0001$). Moreover, culture supernatant TGF-β1 values inversely correlated with the proportions of $CD34^+$ (r=-3599, $p=0.0016$) and $CD34^+/CD33^+$ (r=-0.2613, $p=0.0243$) cells, while the levels of supernatant IL-10 correlated positively with the percentages of $CD34^+$ cells. An inverse correlation was also found between the values of LTBMC supernatant sFL and the proportions of $CD34^+$ (r=-0.4119, $p=0.0007$) and $CD34^+/CD33^+$ (r=-0.3801, $p=0.0019$) cells. Interestingly, the addition of anti-TGF-b1 neutralizing antibody in patient LTBMCs significantly increased IL-10 but not sFL levels in culture supernatants. *Summary and Conclusions.* BM stromal cells produce excessive amounts of TGF-b1 in CIN patients which affect the number of $CD34^+$ and $CD34^+/CD33^+$ cells. TGF-β1 exerts a direct inhibitory effect on the production of the anti-inflammatory cytokine IL-10. The effect of TGF-β1 on sFL production however, seems to be indirect as was shown by the neutralizing antibody experiments. We postulate that the increased sFL levels in patient LTBMC supernatants represent a compensatory effect of the BM microenvironment to the low number of granulocytic progenitor cells in CIN.

0438

TERC MUTATIONS ANALYSIS IN PATIENTS WITH APLASTIC ANEMIA: RESULTS OF A DHPLC SCREENING AND CLINICAL IMPLICATIONS

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Background. Telomerase RNA Component (TERC) is an essential subunit of the telomerase ribonucleoprotein complex. TERC mutation results in a reduction of telomerase activity leading to premature telomere shortening. Heterozygous mutations of TERC gene are responsible for the autosomal dominant form of Dyskeratosis Congenita (DC). Recently TERC mutations were also described in individuals with aplastic anemia (AA) acquired apparently late in life. Denaturing High Performance Liquid Chromatography (DHPLC) is a relatively novel technique of mutation detection based on the separation of heteroduplex PCR products from their corresponding homoduplex by reverse phase liquid chromatography. *Aims.* To investigate the presence and the frequency of TERC mutations in a population of Italian AA patients including pediatric and adults subject. *Methods.* DNA from 111 AA patients (75 pediatrics and 36 adults) and 156 normal controls (94 pediatrics and 62 adults was obtained from blood or bone marrow samples previously collected and frozen. The TERC coding region (GenBank NR_001566) was amplified in 2 PCR fragments and then analyzed by DHPLC. For each abnormal elution profile PCR products were directly sequenced using ABI prism 3100 genetic analyzer. *Results.*Two new mutations, c53T>A and c210C>G, of TERC gene were identified. The c53T>A mutation results in a nucleotidic change in the template sequence inside the highly conserved region CR1, but it does not alter TERC-RNA secondary structure. This mutation was found in a 14 year old boy who, throughout his 10 year clinical follow-up, on CyA and Steroid to which he was not thoroughly compliant, maintained hypocellular marrow and a mild cytopenia (WBC 2.8-7.0×10^9/L, PMN 1.0-4.5×10^9/L, Hb 14-16 g/dL, Plt 60-80 ×10^9/L). The c210C>G mutation was found in a 47 year old woman and localized in the P1 region. This mutation gives rise to a radical conformational change of the TERC-RNA secondary structure. This patient was diagnosed 10 years ago with severe AA, she did not respond to initial treatment with ATG plus CyA. Afterwards, androgens and steroids were added to CyA, she improved her counts (Platelets 50×10^9/L, Hb 9.4 g/dL, PMN 3.0×10^9/L), after 3 years of this therapy. In the last year, without treatment, her Hb is 12 g/dL, Platelets 180×10^9/L and PMN 3.0 ×10^9/L). Marrow committed progenitors are far lower than normal controls. *Conclusions.* The frequency of TERC mutations in our cohort of AA patients is low (1.75%) but consistent with previously published data. We developed a fast, effective and low cost DHPLC-screening protocol which allows TERC gene mutation analysis in all new cases of AA. Identification of TERC mutations in AA patients has some important clinical implications: i) the genetic test is useful for a correct diagnosis and an adequate therapeutic protocol since TERC mutated patients often have no other signs of DC and invariably fail to respond to immunosuppressive therapy. ii) TERC mutation carriers, as they have a hereditary disease with an elevated cancer-susceptibility, require an adequate cancer surveillance program and genetic counseling.

0439

ADIPONECTIN IS PRODUCED BY LYMPHOCYTES AND INHIBITS GRANULOPOIESIS

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Background. Previous studies by our group have shown that normal unstimulated lymphocytes produce a protein which inhibits colony formation of granulopoietic progenitors, but has no effect on erythroid progenitors. Therefore, this inhibitor was initially designated GIA (granulopoietic inhibitory activity). GIA was identified as a glycoprotein of approximately 30 kDa, with a pI of 7.9-8.4. Furthermore, we demonstrated that this inhibitor may have physiological significance in that its production is altered in patients with neutropenia. GIA has proved difficult to characterise to date since it is produced in relatively low amounts although it has a high specific biological activity. *Aims.* Adiponectin is an adipokine reported to share many of the inhibitory characteristics of GIA and has been demonstrated to act as a negative regulator of haemopoiesis and immune response. This study aimed to determine whether GIA is adiponectin or if it represents an adiponectin-like molecule. *Methods.* Lymphocyte conditioned medium (LCM) from lymphocytes cultured at 1×10^6 cells/ml in HL-1 minimal medium was used as a source of GIA. Inhibition of granulopoiesis was tested by co-culturing LCM with normal bone marrow cells in a myeloid colony assay. Western blot analysis and ELISA were performed to investigate adiponectin expression in LCM. RT-PCR analysis of lymphocyte mRNA was carried out to look for expression of adiponectin at the transcript level. *Results.* Inclusion of LCM as 10% of the top layer of agar in a myeloid colony assay inhibited growth of CFU-GM by 52±11% (n=3), confirming the presence of the inhibitory activity. Western blot analysis demonstrated a distinct banding pattern in days 3-7 LCM corresponding to monomers, dimers, trimers and greater. This is consistent with adiponectin which circulates as a multimer of trimers. Characterisation of GIA at the transcript level confirmed that GIA is in fact adiponectin. The N-terminal collagenous domain, C-terminal globular domain and full length adiponectin were amplified by RT-PCR analysis and confirmed by sequencing. *Summary and conclusions*. Adiponectin is thought to be secreted exclusively from adipocytes and much of our current knowledge of this molecule relates to its metabolic functions. Our study provides evidence that adiponectin is also produced by lymphocytes and may play a role in the pathogenesis of neutropenia.

0440

ABSENCE OF LEF-1 TRANSCRIPTION FACTOR IN MYELOID PROGENITORS OF PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA RESULTED IN ABROGATED EXPRESSION OF NEUTROPHIL ELASTASE AND DEFECTIVE GRANULOPOIESIS

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Recently we have shown that Lymphoid-enhancer binding factor 1 (LEF-1) is a decisive transcription factor in granulopoiesis controlling proliferation, proper lineage commitment and granulocytic differentiation via regulation of its target genes C/EBPα, cyclin D1, c-myc and survivin. Expression of LEF-1 and its target genes was abrogated in myeloid progenitors of severe congenital neutropenia (CN) patients (Skokowa et al., Nat Med. 2006;12:1191-7). CN is a heterogeneous syndrome with two major subtypes: 1) autosomal dominant CN defined by mutations in ELA2 gene encoding neutrophil elastase (NE) and 2) autosomal recessive CN (including Kostmann syndrome) carrying HAX-1 mutations, both characterized by an early stage maturation arrest of granulopoiesis. Interestingly, in line with LEF-1 levels, ELA2 mRNA expression in myeloid progenitors as well as NE protein levels in the blood were severely reduced in CN patients irrespective of *ELA2 or HAX1* inheritance. ELA2 gene promoter is positively regulated by direct binding of LEF-1 or C/EBPα. Transduction of LEF-1-GFP lentiviral constructs containing cDNA of either full-length or dominant negative (dn) LEF-1 into U937 myeloid cell line led to significant upregulation of ELA2 mRNA and NE protein, similar as we have shown for C/EBPα. LEF-1 rescue of CD34+ cells of two CN patients resulted in increased NE levels and in granulocytic differentiation *in vitro* . Therefore, these data confirm the importance of LEF-1 in myelopoiesis and in the pathogenesis of CN. Absence of LEF-1 is a common decisive mechanism of the defective maturation program of myeloid progenitors in CN downstream of both, ELA2 or HAX1 mutations.

Presidential Symposium: six best abstracts

0441

PRIMARY ALLOGENEIC STEM CELL TRANSPLANTATION VERSUS BEST AVAILABLE DRUG TREATMENT IN CHRONIC MYELOID LEUKEMIA

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Early allogeneic hematopoietic stem cell transplantation (HSCT) has been proposed as primary treatment modality for patients (pts) with chronic myeloid leukemia (CML). This concept has been challenged by persisting transplantation mortality and improved drug therapy. In order to verify retrospective results, a randomized controlled trial was designed to compare primary HSCT and best available drug treatment in a cohort of 621 newly diagnosed CML pts in chronic phase. Assignment to treatment strategy was by eligibility for HSCT and genetic randomization according to availability of a matched related donor. Evaluation followed the intention to treat principle. 354 pts (62% male; median age 40 years, range 11-59) were eligible and randomized. 135 pts (38%) had a matched related donor of which 123 (91%) received a transplant within a median of 10 months (range 2-106) from diagnosis. 4 pts died before scheduled transplantation, 8 pts withdrew consent. 219 pts (62%) had no related donor and received best available drug treatment. Of these, 97 pts (44%) received a matched unrelated donor (MUD) transplant in 1st chronic phase and were censored at the time of transplantation. As 1st line treatment after randomization pts received interferon alpha based therapy. In the course of the study a total of 197 pts were switched to imatinib after failure of interferon alpha. Currently 31 (57%) of 54 living pts of the drug treatment group receive imatinib or 2nd generation tyrosine kinase inhibitors (dasatinib n=2, nilotinib n=1). With a median observation time of 8.9 (4.2-11.2) years median survival of all 621 pts was 8.1 years. During the first 8 years after diagnosis survival curves of drug treated patients were superior to those of transplanted patients reflecting transplant-related mortality. Beyond 8 years survival curves were no longer distinct. 5 (10) year survival was 62% (53%) for transplanted and 73% (52%) for drug treated pts, in the low risk group 68% (59%) for transplanted and 85% (62%) for drug treated pts, respectively. Survival was superior for drug treated pts up to the cutpoint of survival curves at year 8 (p=0.041) and during the study period up to 11 years from diagnosis (p=0.049), particularly so in low risk pts (p=0.027 to cutpoint, p=0.032 overall). Significantly higher proportions of complete cytogenetic remissions (91% vs 48%, p=0.002) and of major molecular responses (ratio BCR-ABL/ABL <0.1%; 81% vs 45%, p=0.001) were found in the transplant group indicating higher levels of residual disease in the group receiving drug treatment. On the basis of up to 11 years of follow-up the general recommendation of HSCT as 1st line treatment option in chronic phase CML can no longer be maintained. It should be replaced by a trial with modern drug treatment first. Exceptions may be patients´ preference, very low transplantation risk and economic reasons.

0442

DUPLICATION OF THE MYB ONCOGENE IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. T-ALL is an aggressive T-cell malignancy that is most common in children and adolescents. Leukemic transformation of thymocytes is caused by the cooperation of mutations that affect proliferation, survival, cell cycle, and T-cell differentiation. Molecular analyses have identified a large number of genetic alterations including deletion of CDKN2A (p16), ectopic expression of transcription factors, episomal amplification of NUP214-ABL1, and mutation of NOTCH1. *Aims.* In order to detect novel unbalanced genomic rearrangements in T-ALL, we performed micro array comparative genomic hybridization (array CGH) with a resolution of 1 Mb over the entire genome and with higher resolution around oncogenes and candidate oncogenes. *Results.* We identified a duplication of the MYB gene in 2 of 27 T-ALL cases using array CGH. The duplication was confirmed using quantitative PCR (Q-PCR) and Q-PCR analysis of DNA from diagnosis and remission confirmed that that the duplication was an acquired event. We used Q-PCR to screen additional cases and identified a duplication of MYB in 9 of 107 (8.4%) T-ALL cases and in 5 T-ALL cell lines (ALL-SIL, MOLT-4, P12-ICHIKAWA, CCRF-CEM and RPMI-8402). The commonly duplicated region in all patients and cell lines only covered the MYB gene, strongly suggesting that MYB is the critical gene deregulated by the chromosomal duplication. In support of this, the average expression level of MYB was found to be significantly elevated in T-ALL cases with MYB duplication compared to cases without the duplication. In T-ALL cell lines, MYB expression was variable, with MOLT-4 and RPMI-8402 showing high level MYB expression. The MYB gene encodes a nuclear transcription factor that is implicated in proliferation, survival and differentiation of hematopoietic progenitor cells. Proper levels of MYB expression are known to be important during hematopoietic cell development, and the Myb gene is a frequent target of retroviral insertions in myeloid, B- and T-cell leukemias in the mouse. Knock-down of MYB expression using specific siRNAs resulted in an irreversible differentiation of the RPMI-8402, MOLT-4, and ALL-SIL cells, but not of T-ALL cell lines without MYB duplication, as observed by changes in expression of the CD1a, CD3, CD4 or CD8 markers. Although the downregulation of MYB expression had almost no effect on the proliferation and viability of the cells, we observed that a combined inhibition of NOTCH1, another oncogene in T-ALL, and the downregulation of MYB expression resulted in a complete block of proliferation with a strong impact on cell viability and proliferation. These data suggest that NOTCH1 mutation and MYB duplication may cooperate in the pathogenesis of T-ALL. Most of the patients with MYB duplication, and all of the cell lines with MYB duplication indeed harbour mutations in NOTCH1. *Summary.* We identified a duplication of the MYB oncogene in approximately 8% of human T-ALL, and we show that MYB expression is critically required to block differentiation in T-ALLs with MYB duplication. A combined inhibition of MYB and NOTCH1 strongly affects proliferation and survival of T-ALL cell lines, establishing MYB as a novel target for therapy in T-ALL.

0443

THE JAK2 V617F MUTATION IS PRESENT IN A MINORITY OF HUMAN HEMATOPOIETIC STEM CELLS FROM PATIENTS WITH MYELOPROLIFERATIVE DISORDERS AND DOES NOT MODIFY THEIR SELF-RENEWAL PROPERTIES

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Background. The JAK2 V617F mutation is the most common genetic defect observed in hematological malignancies since it is observed in essential thrombocythemia (ET), polycythemia vera (PV) and primitive myelofibrosis (PMF). Whether it is the first genetic defect in these 3 myeloproliferative disorders (MPD) remains a matter of debate. However, all these malignancies are considered to arise from a defect in a hematopoietic stem cell (HSC). *Aims.* To investigate whether the V617F mutation occurs in a true stem cell and modifies its self-renewal and differentiation properties, we repopulated immune-deficient mice with human JAK2 V617F cells. *Methods.* After informed consent was obtained, $CD34^+$ cells isolated from the blood or bone marrow (BM) of 28 patients with JAK2 V617F-positive MPDs (14 PV, 9 PMF, 4 post PV-MF, 1 ET) were intravenously injected into sublethally irradiated and anti-CD122 antibody-treated NOD/SCID mice. 3, 6, and 12 weeks after transplant, immunophenotypic analysis on BM aspirates was performed to assess human cell engraftment. 15 weeks after transplant, mice were sacrificed and hematopoietic cells were used for secondary transplants. Before transplant and 3, 6, 12, 15 weeks after, cells were seeded in methylcellulose for colony assays and each harvested colony was genotyped to assess the presence of JAK2 wild type (WT) and JAK2 V617F alleles. *Results.* 10^5 to 10^6 $CD34^+$ cells were injected in one to 3 mice per patient's sample. Three weeks after transplant, the mean percentage of human cells ($CD45^+$, $CD45^-/CD36^+$, $CD45^-/CD36^-/GpA^+$) was 22±21% but decreased to 6±6% at week 6. This decrease of engraftment was

observed in all mice. Analysis of long-term reconstitution (12 and 15 weeks post-transplant) distinguished 2 groups of mice: 1) most of them (25 patients' samples) had a progressive reduction in human cell engraftment, with a majority of CD19+ cells, such as observed in mice repopulated with normal CD34+ cells. No engraftment was detected in secondary transplant. 2) On the contrary, a small number of mice (2 repopulated with PMF cells and 1 with PV cells) developed a MPD-like syndrome characterized by an increase in human cell engraftment, a majority of CD33+ cells and even some GpA positive cells. Genotypic analysis of human colonies demonstrated that the first group of mice contained a majority of JAK2 WT colonies. However, in this group, we could also observe some heterozygous colonies at 12 and 15 weeks after transplant, in 2 mice reconstituted with cells from 2 PV patients, confirming that the JAK2 V617F mutation is indeed present in HSCs. Genotyping of the second group of mice is still under investigation and the results will be presented. *Conclusions.* Using NOD/SCID mice we demonstrated that the JAK2 V617F mutation is present in only a minority of HSCs in MPDs and that it does not induce self-renewal advantage in human HSCs. We can hypothesize that the HSCs engrafted in mice that develop a MPD-like syndrome probably have an additional genetic event that modifies their stem cell properties.

0444

A MOLECULAR CLASSIFICATION OF LEUKAEMIA REVEALS MDS AS A DISEASE CONTINUUM WITH NON-LEUKAEMIA AND AML SUB GROUPS

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Robust gene expression signatures associated with distinct sub-classes of paediatric and adult leukaemias have been identified from microarray studies. Recently, the MILE (Microarray Innovations in LEukemia) study group has compared the accuracy of gene expression profiling to gold standard diagnostic workup for ~2000 patients within 16 acute and chronic leukaemia subclasses, MDS, and non-leukaemia as control group in 11 centres (ELN: 7, USA: 3, Singapore: 1). The overall cross-validation accuracy was very high for the leukaemia subclasses: >95%. Based on this data set a customized AmpliChip Leukemia microarray has been designed for leukaemia classification. However, only 49.1% of the 173 MDS samples included in the study were correctly called as MDS from their underlying gene expression profiles. The remainder were approximately equally split between an algorithm classification call of *non-leukaemia* (24%) and *AML* (24.6%). Our analysis showed that neither centre nor age were a factor in differentiating between *MDS*, *MDS with an AML-like signature* or *MDS with a non-leukaemia like signature*. WHO classification was highly correlated with the microarray classification result; specifically RAEB (I or II) was associated with *AML-like signature* ($p<0.0001$) whilst, RA/RARS was highly correlated with *MDS* or *non-leukaemia-like signature*. Furthermore, IPSS was significantly correlated with classification call ($p>0.0001$): 65% of patients with an IPSS score of Int-2 or above were classified as *AML*. Individually, the blast, karyotype and cytopenia contributions were highly significant ($p<0.0001$, <0.013 and <0.0001 respectively) when comparing *MDS like AML*, *MDS* and *MDS like non-leukaemi*a samples. Survival data (available for 122 of the diagnosed MDS patients) showed that MDS patients called *MDS like AML* had a trend towards shorter survival ($2p=0.2$) than those called *MDS* or *MDS like non-leukaemia*. The mapped molecular pathways and functions between these sub-groups may give an indication of the molecular steps involved in disease evolution and lead to a molecular redefinition of MDS. An external review of the morphology slides by two experts in the field has been completed with a few samples being reclassified or excluded from the MDS analysis: mainly as a result of being reclassified as true AML. The Amplichip Leukemia is also being used in the prospective Stage II of the MILE study to further determine the molecular differences within the expression based sub groups of MDS.

MILE Study Group on behalf of Workpackage 13 of the European LeukemiaNET (ELN)

0445

IDENTIFICATION OF THE CHROMATIN-REMODELING PROTEIN SATB1 AS A DEVELOPMENT-DEPENDENT LONG-RANGE TRANSCRIPTIONAL REGULATOR OF THE PU.1 GENE

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Expression of the transcriptional master regulator PU.1 is dependent on a highly conserved upstream regulatory element (URE) located many kilobases upstream of the transcriptional start site. The proximal promoter of PU.1 alone, without this enhancer, displays 100-fold reduced promoter activity in reporter assays and is not capable of driving PU.1 expression in transgenic mice. Targeted disruption of the URE reduces PU.1 expression by 80% and leads to acute myeloid leukemia (AML) in mice. However, the mechanisms mediating the long-range regulatory function of the URE are largely unknown. In this study, we identified a binding site of the chromatin remodeling special AT-rich sequence-binding protein 1 (SATB1) in the URE of PU.1. Utilizing EMSA and ChIP, we found that SATB1 binds to the URE in myeloid U937 cells *in vitro* and *in vivo*. Luciferase assays in stably transfected U937 cells demonstrated 7-fold reduced reporter activity upon disruption of the SATB1 binding site. When we transduced U937 cells or primary murine Lin-, Kit+ progenitor cells with a SATB1-IRES-GFP lentivirus, we found an upregulation of PU.1 expression in sorted GFP+ cells. We did not observe this effect in URE-/- progenitors indicating that the PU.1 regulatory function of SATB1 is mediated by the URE. 75% inhibition of SATB1 by stable transfection of a construct expressing a SATB1-directed siRNA into U937 cells led to a 3-fold decrease in PU.1 expression levels. This inhibitory effect was not seen in a myeloid cell line derived from URE-/- mice, again demonstrating that the regulatory function of SATB1 is dependent on the URE. To address the question at which stages during myeloid development SATB1 regulates PU.1, we sorted KSL-HSC (Kit+, Sca1+, Lin-), CMP (Lin-, Kit+, Sca1-, CD34+, FcgRII/IIIlow), GMP (Lin-, Kit+, Sca1-, CD34+, FcgRII/IIIhigh), and MEP (Lin-, Kit+, Sca1-, CD34-, FcgRII/III-) from SATB1 knockout mice and determined PU.1 expression levels by quantitative real-time RT-PCR. Interestingly, PU.1 expression levels were 88% and 80% reduced in SATB1-/- GMP and MEP, while KSL-HSC and CMP did not show significantly changed PU.1 levels. This finding indicates a stage-specific regulatory function of SATB1 during myelopoiesis. In conclusion, we have shown that the chromatin-remodeling protein SATB1 binds to the URE and acts as a development-dependent long-range transcriptional regulator of the PU.1 gene.

0446

THALIDOMIDE-DEXAMETHASONE VERSUS MELPHALAN-PREDNISOLONE AS FIRST LINE TREATMENT IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA: SECOND INTERIM ANALYSIS

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Background. Thalidomide-Dexamethasone (TD) is an active regimen both in patients with relapsing/refractory and in previously untreated patients with multiple myeloma. *Aims.* In the present trial we compare TD with standard Melphalan-Prednisone (MP) in previously untreated elderly patients with multiple myeloma. *Methods.* 276 patients have been enrolled so far (median age: 72 years, stage I: 12 (4%), stage II: 91 (33%), stage III: 173 (63%). Patients are randomized to Thalidomide 200mg/day and Dexamethasone 40 mg, days 1-4 and 15-18 (on odd cycles) and days 1-4 (on even cycles) or Melphalan 2.5mg/kg day 1-4 and Prednisone 2 mg/kg days 1-4, q 4-6 weeks. Thalidomide should be dosed up to 400 mg/day, if feasible. Patients achieving response or stabilization are randomized to maintenance treatment either with Thalidomide (maximal dose 200 mg/day)-Interferon alpha-2b (3Mega U, TIW) or Interferon alpha-2b (3Mega U/TIW). All patients are scheduled for monthly Zometa (4mg) during the entire period. Response is defined according Blade's criteria, plus nCR defined as IF positive CR and VGPR defined as >90% reduction in PP. Statistical results are given by intend to treat and per protocol analysis. *Results.* 197 patients are evaluable for response as yet. Best response to TD was: CR 7 (7%), nCR 20 (21%), and VGPR 13 (14%) PR 16 (17%), MR 10 (10%) yielding an ORR (CR-PR) of 58%. The respective results in patients on MP were: CR 4 (4%), nCR 10 (10%), VGPR 11 (11%), PR 25 (25%), MR 16 (16%), ORR 50% (ORR in TD vs. MP p=0.051). Analysis per protocol revealed an ORR of 72% in the TD and 52% in the MP group (p<0.01). Time to response and time to best response was significantly shorter in the TD (8, 11 weeks, respectively) compared to the MP group (10, 39 weeks, respectively; p<0.01, p<0.0047, respectively). There were more early treatment discontinuations in the TD arm (20 vs. 8, p<0.007). Patients on MP had statistically more frequently grade III-IV leukopenia, while for thrombocytopenia only a tendency for a higher incidence was noted (14% vs. 2%; p< 0.05, and 9% vs. 4%, p=0.252). Patients on TD had more grade II-III neuropathy (28% vs. 9%, p<0.001), psychological toxicity (18% vs. 7%, p<0.02), and a tendency for more skin toxicity (10% vs. 4%, p=0.09) compared to those on MP. Thromboembolic complications were seen in 9% of patients on TD and in 4% in those on MP (p=0.15). *Summary.* TD treatment was associated with a significantly shorter time to response and to best response, higher rate of CR-NCR, and a tendency for higher ORR in the intent to treat analysis. In the per protocol analysis, ORR was significantly higher in the TD arm. Patients on TD had more neuropathy and psychological toxicity, a tendency for more skin toxicity and thromboembolic complications, and a higher rate of early treatment discontinuations, while haematological toxicity was higher in patients treated with MP. Updated data on time to progression and survival for both groups combined will be presented at the meeting.

POSTER SESSION II

Allogeneic stem cell transplantation

0447

FAVORABLE OUTCOME FOLLOWING STEM CELL TRANSPLANTATION WITH UNMANIPULATED GRAFTS OF HLA 7-8/10 ALLELE MISMATCHED UNRELATED DONORS IN CHILDREN

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Background. Allogeneic hematopoietic stem cell transplantation (HSCT) is an efficient curative treatment for certain malignant and non-malignant diseases. Unfortunately in central Europe only 15% of the indicated children have an HLA-matched sibling available and in around 65-70% others HLA allele matched (9-10/10) unrelated donor (UD) could be identified in registries worldwide. For the rest of patients (15-20%) it is necessary to identify other alternative donors such as HLA mismatched family or unrelated donor or cord blood. Here we report our single-center clinical experience of HSCT using HLA partially mismatched (7-8/10) unrelated donors. *Patients and Methods.* 27 children (age range 0.4-18.1 years; median 9.0) were transplanted at our center between I/2001 and XII/2006 for malignant (n=20; 74%) and non-malignant disease (n=7) using unmanipulated grafts from UD mismatched in two or three HLA alleles. HLA allele mismatches were in patients and UD prospectively identified by PCR SSP four digits (A* 3x, B* 18x, Cw* 28x, DRB1* 3x, DQB1* 10x). No donor/patient pair was mismatched in more than one allele in loci A*, B* or DRB1*. The median interval between start of search till the day of graft infusion was 142 days (range 68-492). Standard myeloablative conditioning regimen was used in majority of patients. Median age of UD was 34 (range 22-49 years) with 14 males and 13 females. Serotherapy with rabbit ATG (Fresenius; 4x 10mg/kg/dose) and cyclosporine A (CsA) with standard short term methotrexate (MTX) were used as graft versus host (GvHD) prophylaxis. Primary grafts were peripheral blood stem cells (PBSC; n=16) and bone marrow (BM; n=11). Four patients later received five grafts (all PBSC) for graft failure (2) or leukemia relapse (2). Informed consent form was signed by parents and approved by local ethical committee. *Results.* Stable primary engraftment was achieved in 25/27 (93%) children, two with acute graft failure were successfully re-grafted using the same UD. 96% of patients achieved stable complete donor chimerism (VNTR/STR). Acute GvHD grade II was diagnosed in 21 (78%) of them, grade III-IV in 1 (3.7%). Extensive chronic GvHD was diagnosed in 7 of 26 evaluable patients (27%). Transplant related mortality (TRM) at day+100 is 3.7% with overall TRM 18.5% (mainly in patients with repeated transplants). Overall survival (OS) of the whole cohort is 74% with median follow-up 34.5 months (3-71 months). Altogether 7 patients died in median 289 days post-transplant (range 44-602), 2/20 (10%) died in a consequence of leukemia relapse 451 and 502 days post-transplant. OS is better in recipients of PBSC (81%) compare to 64% in those receiving BM, but this difference is not relevant due to small cohorts. *Summary and conclusions.* Combination of rATG/CsA/MTX for GvHD prophylaxis represents efficient modality even for patients transplanted with unmanipulated grafts from HLA markedly mismatched UD, enabling stable engraftment, good control of GvHD and full reconstitution of immunity. Furthermore this approach is feasible, not connected with unacceptable early or late TRM. No patient in this cohort died due to EBV related lymphoproliferative disease, one died due to CMV pneumonia.

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0448

A NOVEL MDR-DEPENDENT PHARMACOLOGICAL APPROACH FOE *EX VIVO* EXPANSION OF HEMATOPOIETIC STEM CELLS (HSCS) FROM HUMAN UMBILICAL CORD BLOOD (HUCB) FOR TRANSPLANTATION

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While HUCB is an attractive alternative source of hematopoietic stem cells (HSCs) for allogeneic transplantation (alloSCT), major disadvantage is its relative low number of HSCs. A rational approach, thus, involves

ex-vivo expansion of HUCB-derived HSCs. The multidrug transporter MDR1 (ABCB1) gene product (Pgp) and ABCG2 channel transporter reported being over-expressed in various stem cells relatively to their differentiated progeny. We, therefore, compared the expression level and activity of Pgp in HUCB-derived $CD133^+$ HSCs relatively to $CD133^-$ cells. Moreover, we reasoned that higher Pgp activity in these $CD133^+$ HSCs will make them more resistant to cytostatic agents as colchicine (COL) and thus its presence during the expansion process could be applicable to their selection and enrichment. Towards this end, we isolated $CD133^+$ HSCs from HUCB by CD133-immunomagnetic separation (MACS). Pgp-expression level was measured by flow cytometry using the Pgp-antibodies MRK-16, and its activity was measured by accumulation assay of the Pgp-substrate Rh123. We further analyzed the $CD133^+/Pgp^+$ and the $CD34^+/CD38^-/Pgp^+$ subsets during 8 weeks of standard cytokines based expansion in the presence or the absence of COL. Analyses of freshly isolated $CD133^+$ HSCs from various donors (n=6) indicated that the majority (>92%) of these HSCs express Pgp on the cell surface. Moreover, the Pgp is functional as the accumulation of Rh123 was approximately 260-fold lower relatively to CD133 negative cells and the Pgp inhibitor R-VRP inhibited the efflux of Rh123. Analyses after *ex vivo* expansion demonstrated a significant dose-dependent enrichment of $CD133^+$ cell fraction by COL. At optimal COL dose (2.5 ng/mL, the relative fold-enrichment of $CD133^+/CD34^+/CD38^-$ HSC was 5.2±2.3. At 8 weeks of expansion the $CD133^+$ cell number increased from 105 cells to $1.6\pm0.4\times10^9$ and $0.6\pm0.2\times10^9$ in the presence and absence of COL, respectively and thus the total yield of $CD133^+$ HSCs after expansion in the presence of COL was 2.9±0.5 fold higher than in its absence. The long exposure of CD133+ HSC to COL at the expansion process did not affect their ability to form hematopoietic colonies in semisolid cultures. In conclusion, we show that HUCB-derived HSCs over-express a functional Pgp that may be used as a novel tool for their expansion *ex vivo* towards clinical transplantation.

0449

PATIENT HSP70-HOM TG HAPLOTYPE IS ASSOCIATED WITH DECREASED TRANSPLANT-RELATED MORTALITY AND IMPROVED SURVIVAL AFTER SIBLING HLA-MATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Heat shock protein 70 hom (HSP70-hom) gene is known to play an important role in protein folding and immune responses. Therefore, HSP70-hom gene polymorphisms may act as important factors predicting prognosis in patients receiving allogeneic hematopoietic stem cell transplantation (HSCT).

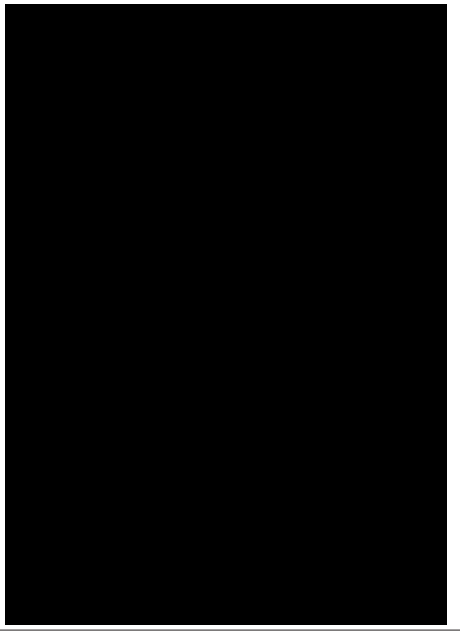

Figure 1. Overall survival and treatment-related mortality according to TG haplotype or non-TG haplotype.

Aims. The aim of this study is to evaluate the role of HSP70-hom gene polymorphisms in prognosis of patients receiving sibling human leukocyte antigen (HLA)-matched allogeneic HSCT.*Methods.* The HSP70-hom polymorphisms, T2437C and G2763A, were genotyped in 147 patients receiving sibling HLA-matched allogeneic HSCT, and individual diplotypes were estimated from genotype data of two HSP70-hom polymorphisms using the expectation maximization algorithm. *Results.* Patients with 2763GG or GA genotype showed longer overall survival compared with those with 2763AA genotype, and patients with TG haplotype (TG/TA, TG/TG or TG/CG) also showed longer overall survival compared with those without TG haplotype (TA/TA or TA/CG) (both G2763A genotype and diplotype, $p<0.01$). Moreover, 2437TT genotype was found to be protective for treatment-related death compared to 2437TC genotype, and TG haplotype (TG/TA, TG/TG or TG/CG) was found to be very protective for treatment-related death compared to non-TG haplotype (TA/TA or TA/CG) (T2437C genotype, $p=0.04$; and diplotype, $p=0.02$). *Conclusions.* Therefore, our results suggest that polymorphisms of the HSP70-hom gene play an important role in the prognosis of patients receiving sibling HLA-matched allogeneic HSCT.

0450

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT *IN VITRO* T-CELL DEPLETION FOR THE CHRONIC MYELOID LEUKEMIA: IMPROVED OUTCOMES IN PATIENTS WITH ACCELERATED PHASE AND BLAST CRISIS PHASE

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Backgroud. Allogeneic hematopoientic stem cell transplantation (Allo-HSCT) remains the only proven curative therapy for chronic myeloid leukemia (CML), but many patients have no suitably HLA matched related or unrelated donors. Recently, we developed a new method for haploidentical allo-HSCT without *in vitro* T-cell depletion, which could achieve comparable outcomes to HLA identical sibling transplantation. So good outcomes of HSCT from mismatched family donor for CML patients could be expected. *Methods.* Ninety-three patients, including 51 cases in CP1, 14 cases in CP2, 15 cases in AP and 13 cases in BC pre-HSCT, who underwent mismatched family donor HSCT were analyzed. Patients received the modified BU/CY2 regimen consisting of cytarabine, busulfan, cyclophosphamide, simustine and ATG. Both of donor G-BM (G-CSF immobilized) and G-PB were harvested and infused to recipients. All patients were given CsA/MTX and MMF for prophylaxis of GVHD. *Results.* All patients achieved full donor chimerism. The cumulative incidences of aGVHD is 64.52% (CI 55.49%-75.01%)?grade III-IV is 26.45% (CI 17.51%-39.95%). The cumulative incidence of total cGVHD was 61.79% (CI 49.33%-77.39%) and 28.93% (CI 19.25%-43.46%) for extensive chronic GVHD at 2 years after transplantation. The non-relapse mortality was 8.72% for 100 days, 20.72% for 1year and 20.72% for 2 years. 100d TRM of patients in CP1, CP2/CR2, AP, BC are 7.8%, 7.1%, 13.3%, 7.7%, 1 year TRM of patients in CP1, CP2/CR2, AP, BC are 28.3%, 16.92%, 13.33%, 7.69% respectively, there are no different among them ($p=0.622$). The probability of 1-year and 4-year LFS was 76.5%, 74.5% for CP1 patients; 85.7%, 85.7% for CP2/CR2 patients ; 80%, 66.7% for AP patients; 53.8%, 53.8% for BC patients ($p=0.221$). At present, all living patients who relapsed after transplantation achieved CMR. The probability of 4-year OS was 76.5% for CP1 patients, 85.7% for CP2/CR2 patients 73.3% for AP patients and 61.5% for BC patients ($p=0.7442$). Multivariate analysis showed that factors affecting transplantation outcomes were 3-4 aGVHD for LFS, OS, TRM, and the stage of disease at transplantation for relapse. *Conclusions.* For patients with CML without an HLA identical sibling donor, haploidentical family members can be an alternative donor for HSCT. In our HSCT protocol, survival of HSCT for CML in advanced stage was no worse than that in stable stage. The optimal time of HSCT for CML patients from MM-family donors need to be further studied.

0451

CHLORAMBUCIL AND FLUDARABINE AS A NEW PRE-TRANSPLANT CONDITIONING FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA : RESULTS OF *IN VITRO* AND *IN VIVO* EXPERIMENTS

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Background. CLL, incurable by standard treatments, may by potential-

ly cured by allogeneic hematopoietic stem cell transplantation. Since CLL affects predominantly older people, there is a need for some low-toxicity conditioning with, on the other hand, strong anti-leukemia activity. Since there are very encouraging results with busulfan + F conditionings in myeloid malignancies, and since the clinical study with the combination treatment with CHL + F was stopped prematurely for myelotoxicity, we hypothesized, that this CHL + F combination might have the potential as a good conditioning for high-risk lymphoid malignancies. *Aims.* To test the CHL + F combination *in vitro* and *in vivo* (rats) for anti-leukemia effects and for toxicity. *Methods. In vitro* cell viability testing: DHL-4 (mut-p53), DOHH2 cell lines (wt-p53), and three B-CLL primary cultures (two wt-p53 and one mut-p53) were used for WST-1 viability assay. Animal experiments: Male Wistar rats were used for all experiments. First, the maximal tolerated dose (MTD) of either drug was tested. For F, doses of 0.75 - 60 mg/kg/d were used, and for CHL, doses of 0.15 - 50 mg/kg/ were used, all administered for 5 days. Then, the combination treatment was tested: 1) F+CHL, 2) F followed by CHL, 3) CHL followed by F; all drugs were administered for 5 days. *Results. In vitro* testing: Cell lines testing did not reveal significant differences between simultaneous vs. sequential drug applications. The combination treatment of CLB and FLU caused a prominent decrease of viability in p53-mut cells compared to individual drugs alone both for cell lines and for B-CLL cells, which can have significant clinical importance. Animal experiments. For F alone, the MTD has not been reached. Clinically, the rats tolerated well even the highest doses. Moreover, no myelotoxicity was seen. However, histological examination found pneumotoxicity, hepatotoxicity, nephrotoxicity, and gastrointestinal toxicity. For CHLB alone, the MTD is about 40-50 mg/kg/d. Pneumotoxicity, nephrotoxicity, gastrointestinal toxicity, and mild myelotoxicity was seen. Combination treatment tested fixed dose of F (3 mg/kg/d) and three doses of CHL (1, 2, and 4 mg/kg/d). Clinically, the combination tolerated at best was F followed by CHL. Myelotoxicity was mild, usually affecting predominantly lymphocytes, and interestingly, was most pronounced in the clinically best tolerated regimen. *Conclusions.* Rats can tolerate extremely high doses of F and CHL. Based on these experiments, for further development, hopefully into the clinical usage, we could recommend administration of fludarabine, followed by chlorambucil. This combination will be further tested together with monoclonal antibodies and total lymphoid irradiation.

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0452

HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR HIGH RISK REFRACTORY MULTI SYSTEM LANGERHANS CELL HISTIOCYTOSIS (MS-LCH) IN CHILDREN

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Background/Aims. LCH is a heterogeneus proliferative disease varying between a reactive or a neoplastic process. The Histiocyte Society Working Group identified a *single system* LCH as usually associated with a favourable prognosis, while *multisystem* LCH as an extremely variable but aggressive clinical course. The LCH-II Study showed that response to initial therapy is a powerful prognostic predictor; MS-LCH patients non-responders after 6 weeks of initial therapy, have extremely poor prognosis with only 17% of survival at 3 years. The high rate of mortality in this latter subgroup justifies an early switch to salvage approaches; HSCT has been indicated as an alternative salvage therapy. Myeloablative conditioning regimens were associated with a high treatment-related morbidity and mortality while Reduced Intensity Conditioning regimen, recently reported by an Histiocyte Society Study, has an increased risk of non-engraftment and graft rejection. *Methods.* Here we describe four consecutive refractory MS-LCH paediatric patients diagnosed and treated at the Haematology Department of Bambino Gesù Children's Hospital. All patients were males with a median age at diagnosis of 9 months (4-25 months). All received first-line LCH therapy according to the study protocol LCH-II or LCH-III and all experienced severe disease progression, unresponsive to chemotherapy. All patients recived allogeneic HSCT at median of 38 months after diagnosis (10-72 months). Stem cell source was HLA-matched sibling donor in two cases and unrelated cord blood (CB) donor (1 and 2 HLA mismatched antigens) in the remaining two. Conditioning regimen included oral Busulfan (16 mg/kg) + Fludarabine (120 mg/m^2) in 4 days + Thyotepa (10 mg/kg) for 1 day. GvHD prophylaxis consisted of Cyclosporine, horse Antilymphocyte Globulin and Methylprednisolone. *Results.* Median time to neutrophil engraftment was 43 days (14-99 days), while platelets engraftment was 136 days (19-437 days). All patients reached full donor chimerism at day 25, 24, 25, 41 respectively, which persisted until the last follow-up. Three patients presented acute GvHD (two patients grade II, with skin involvement and one grade III, with skin and gut involvement). Long term follow-up showed a *peculiar* recovery from the underlying disease. In the post transplant period no additional therapy specific for LCH was administered to any patient. *Conclusions.* The literature reports enlist only 38 LCH patients who underwent HSCT. Therefore, even if limited to 4 patients, our prospective unicentric contribute could be significant to consider: HSCT is a good salvage approach for resistant LCH patients with MS organ involvement unrelated CB is a reliable alternative option in high-risk patients lacking sibling donor. By using an immune-myeloablative conditioning regimen, all patients obtained a full stable engraftment with no TRM even if a diffuse disease was present at moment of HSCT and regardless of the stem cell source . Acute GvHD did not exceed grade III. At the time of last follow-up (median 1470 days; range 990-1920), all patients are still alive in absence of active LCH disease (Lansky scale> 90%).

Table 1. HSCT characteristics and results.

Pts	HSCT/HLA mathc donor	Number of Stem Cell transplanted	Engrafment	a/c GVHD	F-up	LCH
1	MSD HLA Hydentical	NC/kg 3.578×10^8 CD34+/kg 14.4×10^6	PMN⇒g+33 PLTS⇒g+50 VNTR⇒Full donor+25	II grade (Skin)	58 m 1740 days Alive	NAD
2	MSD HLA Hydentical	NC/kg 7.6×10^8 CD34+/kg 9.6×10^6	PMN⇒g+14 PLTS⇒g+19 VNTR⇒Full donor+24	0	33 m 990 days Alive	NAD
3	U-CBT HLA 5/6 1-1 class Ag m.matched	NC/kg 5.5×10^7 CD34+/kg 7.5×10^6	PMN⇒g+29 PLTS⇒g+19 VNTR⇒Full donor+25	II grade (Skin Gut) Chronic (Skin Lungs)	33 m 990 days Alive	NAD
4	U-CBT HLA 4/6 1-1 class Ag m.matched	NC/kg 11.0×10^7 CD34+/kg 2.5×10^6	PMN⇒g+99 PLTS⇒g+437 VNTR⇒Full donor+70	II grade (Skin)	64 m 1920 days Alive	NAD

UCBT: Umbelical Cord Blood transplantation; MSD: Matched Sibling Donor; NAD: Non Active Disease

0453

CHRONIC KIDNEY DISEASE AFTER MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Because many recipients of hematopoietic stem cell transplantation now have long term survival, late complications become more important. Chronic kidney disease is a complication that is not well-established after stem cell transplantation. *Aims.* The aim of this study was to determine incidence of chronic kidney disease in recipients of allogeneic stem cell transplantation with a long follow up, and to determine baseline factors, complications and mortality associated with chronic kidney disease. *Methods.* We performed a single centre retrospective cohort analysis on 271 adult patients that received a hematopoietic stem cell transplantation between 1993 and 2004 and survived for more than 6 months. All patients had a myeloablative conditoning regimen that consisted of cyclophosphamide (60 mg/kg/day for two days), followed by total-body irradiation (600 cGy/day for two days) with partial shielding of the lungs (total lung dose 850cGy) and partial shielding of the kidneys (500 cGy/day for 2 days). Follow up was through February 2006. Primary outcome was the incidence of chronic kidney disease defined as a glomerular filtration rate (GFR) of < 60 mL/min/1.73 m^2, calculated using the Modification of Diet in Renal Disease (MDRD) study equation. *Results.* Chronic kidney disease developed in 62 (23%) of 271 patients. Severe kidney disease (GFR of < 30 mL/min/1.73 m2) developed in 8 (3%) of 271 patients, of which 2 patients needed dialysis. Factors associated with chronic kidney disease were lower GFR (p<0.0001, OR 0.98 95% CI 0.96-0.99) and higher age (p=0.020 OR 1.04 95% CI 1.01-1.08) at baseline, and the occurrence of hypertension after transplantation (p=0.014, OR 0.46 95% CI 0.24-0.86). Gender, diagnosis, type of transplant, mismatch, presence of high-risk malignancy, history of hypertension, previous autologous transplantation, acute renal failure, thrombotic thrombocytopenic purpura, sinusoidal occlusion syndrome, acute graft-versus-host disease, chronic graft-versus-host disease, cytomegalovirus reactivation and longer than 3 months cyclosporine use did not differ statistically between the groups with or without chron-

ic kidney disease in univariate or multivariate analysis. Mean follow-up of surviving patients was 7.0 years (range 1.7-13.1 years). Death occurred in 107 patients (40%) after a mean of 2.0 years (range 0.5-12.6 years) due to relapse (19%) or treatment related mortality (21%). There was no significant difference in survival between patients with and without chronic kidney disease. *Conclusions.* Chronic kidney disease is a common late complication of myeloablative allogeneic hematopoietic stem cell transplantation. Lower GFR and higher age at baseline and the occurrence of hypertension after transplantation are associated with development of chronic kidney disease. It is known that chronic kidney disease in general is a risk factor for cardiovascular disease. Decreased GFR is associated with high blood pressure, anemia, malnutrition, bone disease, neuropathy and decreased overall functioning and well-being. In order to prevent morbidity and mortality of chronic kidney disease, all recipients of allogeneic stem cell transplantation should be monitored lifelong for renal function and hypertension and be treated according to guidelines developed for chronic kidney disease in general.

0454

TRANSPLANT-RELATED MORTALITY AFTER ALLOGENEIC REDUCED INTENSITY CONDITIONING HEMATOPOIETIC STEM CELL TRANSPLANTATION: A STUDY ON INFLUENCE OF PARAMETERS BEFORE AND AFTER TRANSPLANT

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We studied the impact of demographic, pre and post-transplant parameters on transplant related mortality (TRM) in 141 allogeneic reduced intensity conditioning transplant (RICT). There were 52 female and 89 male recipients [median age: 51 yrs], 71 female and 70 male donors [48.5 yrs]. There were 83 sex-mismatched pairs (59%), 48 ABO incompatibilities (34 minor, 14 major) and 68% CMV seropositive pairs. 33 patients received bone marrow, 105 peripheral blood stem cells with a median $CD34^+$ cell number of 5×10^6 /kg (0.42-64) and a median number of $CD3^+$ cells of 197×10^6 /kg (0-761) and 3 cord blood cells from 121 related donors and 20 unrelated donors (17 HLA matched and 3 mismatched), 48% of cases were previously transplanted. For conditioning, 68 patients received Fluda+Busulfan+ATG with dose varying from 2.5 mg/kg to 12.5 mg/kg, 39 Fluda+TBI (2 grays), 6 Fluda+Treosulfan+ATG and 28 other combinations. The GVHD prophylaxis consisted of methotrexate+Ciclosporine (CsA) for 41 patients, CsA alone for 50, CsA+MMF for 39 and other combinations for 11. The diagnosis pre-transplant were 30 AML, 5 ALL, 13 CML and myeloproliferative disorders, 8 Hodgkin disease, 10 CLL, 16 MDS, 43 MM and 16 NHL; 40 were in CR (28%), 54 in PR (38%), 4 in stable disease and 43 in progressive disease (30%). The median interval between diagnosis and transplant was 20 months (5-300). We observed 10 graft failures, 51 patients (39.5%) developed acute GVHD≥II and 54 (42%) chronic GVHD (28 limited - 26 extensive). At the last follow-up, 73 patients (52%) relapsed and 97 patients had died (55 from TRM causes and 42 from relapse), 43 (30.5%) received DLI. The TRM at 1 year and 3 years was 24% and 34% respectively. We analyzed in multivariate analysis using a Cox proportional hazard model the relation between TRM and (1) demographic parameters: donor and recipient age, sex-matching (2) pre and post-transplant variables: number of previous transplant, ABO compatibility, HSC source, conditioning, ATG dose, status of disease and acute GVHD. We found a significant positive impact of disease status on TRM (HR=0.17 95%CI 0.03-0.77 p=0.02) and a significant negative impact of minor ABO incompatibility (HR=4.60 95%CI 1.25-16.65 p=0.02). This retrospective analysis showed that TRM in RICT for this high risk population is significantly influenced by disease status and minor ABO incompatibility which pointed out the importance of donor choice, transfusion rules and methotrexate addition in this case.

0455

REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION) AS SALVAGE TREATMENT FOR RELAPSING MULTIPLE MYELOMA (MM): A *DONOR* VS. *NO DONOR* COMPARISON

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The role of RIC allo-SCT in MM is still controversial. This single centre study aimed to evaluate RIC allo-SCT for relapsed MM, using a genetic randomization through a donor vs. no donor comparison. Between 2002 and 2005, 32 patients with relapsed or refractory MM, and with an identified sibling, were referred to our centre for HLA typing. In all, 19 patients (59%; donor group) had an HLA-identical sibling donor, while the remaining 13 pts (41%; no donor group) had no HLA-identical sibling donor. There were no significant differences between these two groups that were comparable as for demographic, disease and prognosis factors. Median age was 54 (range, 37-65), and all pts had previously failed auto-SCT. Pts from the no donor group received salvage therapy including thal., bortezomib, dex., and/or additional high dose chemotherapy. Among the 19 pts from the donor group, 18 (95%) could proceed to allo-SCT. With a median overall FU of 36 m., 11 patients (85%;95%CI:54-98%) from the no donor group had disease progression despite salvage therapy, and only 6 of them are still alive, of whom 5 (80%) in progressive disease at last FU. In contrast, only 5 pts (28%; p=0.001) from the donor group progressed after RIC allo-SCT. In the RIC allo-SCT group, 10 pts (56%;95%CI;33-79%) are still alive, with 4 pts being in CR, and 5 in PR or VGPR. Only one patient is currently experiencing disease progression and receiving salvage therapy. Interestingly, 11 pts (61%;95%CI,39-83%) from the RIC allo-SCT group showed objective disease response, usually concurrent to chronic GVHD. In all, 6 pts died from TRM for an overall incidence of TRM of 33% (95%CI,11-55%) in this population of heavily pretreated and relapsed or refractory MM population. In an intention-to-treat analysis, the KM estimate of progression-free survival was significantly higher in the donor group as compared to the no donor group (p=0.01;46%vs.8% at 3 y.; Figure 1). In all, these results compare favorably with those achieved using other standard non-allo-SCT salvage therapies for relapsed MM. Therefore, RIC allo-SCT from an HLA-identical sibling is a feasible and potential therapy that should be proposed for refractory or relapsed MM, since a potent graft-vs.-MM effect can be induced despite heavy pretreatments, allowing for significantly longer PFS. Also, the latter results are expected to be further improved with the systematic and early use of maintenance therapies after RIC allo-SCT.

Figure 1.

0456

REDUCED INTENSITY SIBLING ALLOGENEIC STEM CELL TRANSPLANTATION AND DONOR LYMPHOCYTE INFUSIONS IN SEZARY SYNDROME AND ADVANCED MYCOSIS FUNGOIDES RESULT IN DURABLE CLINICAL AND MOLECULAR REMISSIONS

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Background. Prognosis for tumour stage Mycosis fungoides (MF) and Sézary syndrome (SS) is poor with a median survival of approximately 5 years. Treatment for advanced stage disease is currently largely palliative, and allogeneic stem cell transplantation (SCT) with the potential of mounting graft versus lymphoma effect (GvL) could result in long-term complete clinical and molecular remission *Aims.* In this pilot study, we looked at the role of reduced intensity conditioned (RIC) HLA matched sibling allogeneic transplants on survival in advanced stage MF/SS. We

also looked the role donor lymphocyte infusions (DLI) for post-transplant relapse in the absence of clinically significant graft versus host disease (GVHD). *Methods.*METHODS Six patients were enrolled into our programme of HLA matched sibling allogeneic haematopoietic stem cell transplantation (HSCT) for Sézary syndrome and advanced cutaneous T-cell lymphoma (CTCL). Sézary syndrome n=3, tumour stage MF n=2, and the erythrodermic variant of MF n=1. All six patients were in advanced stage disease (stage IIIA or above) at time of transplant conditioning. All patients were conditioned with a reduced intensity regime using Campath-1H Fludarabine and Cyclophosphamide. Disease status pre and post-transplant was monitored clinically, radiologically and molecularly by PCR for TCR gene rearrangement. Upon disease relapse post-transplant, and in the absence of graft versus host disease (GVHD) a programme of escalating dose DLI with a starting dose of 10×10^6/kg CD3 positive T-cells was commenced. *Results.* Of the six patients who had a RIC SCT, five demonstrated a clinical complete remission (CR), four of those also experienced a molecular CR. One patient died in CR of transplant related mortality (invasive aspergillosis). One patient developed autologous reconstitution and died of disease progression while four patients experienced early relapse with cutaneous disease and the re-emergence of a T-cell clone (median time to relapse 82.2 days (range: 28-100). One patient developed acute GVHD after withdrawal of immunosuppression and entered a molecular CR. Three patients received DLI. All three developed acute skin GVHD, and regression of the relapsed disease occurred concurrently. All three remain alive (median 32 months, range: 12-24 months) in clinical and molecular CR. The minimum dose to induce GVHD and result in CR2 was 10×10^6/kg CD3 T-cells. *Conclusions.* RIC-SCT can result in clinical and molecular remissions post-transplant in the majority of patients. However, most patients experience early relapse. Reduction of immunosuppression and donor lymphocyte infusions induce GVHD which is followed by regression of the relapsed disease and subsequent clinical and molecular complete remission. We believe that this is clear evidence of a graft versus tumour effect in patients with cutaneous T-cell lymphoma.

0457

MULTI-DONOR STEM CELL TRANSPLANTATION (MDT) FROM HAPLOIDENTICAL MISMATCHED DONORS ENGRAFTMENT AND SAFETY DATA

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Introduction. The aim of stem-cell-transplantation (SCT) is to combine tumor cytoreduction and to replace host with donor cells. For patients with no matched sibling available for allogeneic SCT (approximately 70% of patients) an alternative treatment option is SCT from a relative carrying only one identical HLA haplotype (haplo-SCT), which may provide a donor for almost every patient in need at optimal timing. Mandatory T-cell depletion (TCD) increases the risk of graft rejection and decreases the intensity and efficacy of GVT effect. In umbilical cord blood transplant, in order to overcome the delayed engraftment, the possibility of using several cord-blood units to increase the stem cell dose was investigated. In the small number of multi-cord transplants reported, it was found that usually only one unit engrafted faster, while the others were rejected. We hypothesized that multi-donors may improve the engraftment, GVT effect and immune reconstitution following haplo-SCT. *Patients and Methods.* Eleven ultra high-risk patients (median age 38 years, 9-54, mostly after 1-2 previous SCTs) were included. Conditioning regimen was fludarabine-TBI (n=8) or TLI-CY (n=3) based. All grafts were T-cell depleted. *Results.* All nine evaluable patients engrafted but one of them later rejected the graft. The time for neutrophil engraftment (0.5 and 1.0×10^9/L) was fast, 11 days (9-23) and 12 days (10-17) respectively. Platelets engrafted earlier (20 and 50×10^9/L), 9.5 days (8-11) and 11 days (9-14) respectively. Of 6 evaluable patients, all converted to single donor hematopoiesis within a median of 33 days (21-49). In 2 patients in-which the leukemia persisted throughout the conditioning, there was a noteworthy disappearance of leukemic cells during the conversion to single donor chimerism. Despite major blood type differences between the recipient and the 2 donor, no hemolysis related side-effects were noted. Two patients developed GVHD (grade 2 and 3). *Conclusions.* MDT is safe and induces fast and durable engraftment of both myeloid and thrombocyte lineages in patients undergoing haplo-SCT. Further studies should follow this report.

0458

FEASIBILITY AND OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER A FLUDARABINE-BASED INDUCTION PROGRAM

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There are only limited data about allogeneic stem cell transplantation (HSCT) in elderly patients (pts). In the present analysis we report the feasibility and outcome of HSCT in elderly pts with acute myeloid leukemia (AML) after a fludarabine-based induction program. Between 2000 and 2006, 62 consecutive AML pts aged 50 years or older (range 50-78) were treated with fludarabine-based induction regimen [FLAI+Mylotarg 20/62 (32%), FLAIE 28/62 (45%), FLAI 14/62 (23%)]; the median age at diagnosis was 59 years (range 50-78), high risk at onset 45/62 cases (76%). Thirty-eight of 62 pts (61%) achieved complete remission after induction chemotherapy (death during induction was only 3%). Twenty-four of these 62 pts (39%) underwent HSCT. The median time from diagnosis to transplant was 6 months. Disease status at time of transplant: high-risk 21/24 cases (88%), advanced disease 10/24 cases (42%), complete remission 14/24 cases (58%). Median age 60 years (range 50-69). Donor status: sibling donor 50% (12/24), unrelated donor 37% (9/24), haploidentical donor 13% (3/24). Twelve of 24 pts (50%) received a reduced intensity conditioning regimen (RIC). Conditioning regimen: Fludarabine + Busulfan 4/24 (17%), Thyotepa + Cyclophosphamide + ATG 8/24 (33%), Busulfan + Cyclophosphamide + ATG 7/24 (29%) and other regimens 5/24 (21%). Outcome: all patients achieved engraftment. Acute GvHD was observed in 11/24 pts (46%) with 8 having grades I-II and 3 having grades III-IV. Data on chronic GvHD was available for 14/24 pts (58%); of those 1/14 (7%) developed extensive chronic GvHD. Transplant-related mortality occurred in 3/24 pts (12%). At the time of analysis, after a median follow-up of 8 months (range 1-37), 14/24 pts (58%) were alive and in complete remission while 10/24 (42%) have died (leukemia relapse 7/10 and TRM 3/10). One and two year probability of Overall Survival (OS) was 70% and 56% respectively. The pts transplanted in complete remission have a significantly better OS and DFS compared to those transplanted with relapsed or refractory disease (log rank=0,01). These preliminary results are encouraging and compare favourably with results after conventional chemotherapy in elderly pts with AML. Related and unrelated donor HSCT is feasible in elderly pts with AML, with outcomes that are similar to younger pts. Favourable outcome was observed expecially in those transplanted in complete remission and early in the course of disease. Besides our data confirm the efficacy of fludarabide-based induction regimen for AML pts older than 50 yrs (61% of complete remission rate) allowing the treatment intensification with HSCT in a high proportion of cases (39%, 24/62).

0459

IMPACT OF ALLOGENEIC STEM CELL TRANSPLANTATION IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA PATIENTS AND CORRELATIONS WITH PGP EXPRESSION

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Background. Patients with acute myeloid leukemia (AML) and normal karyotype represent about 40-50% of all AML cases. Despite being included in an 'intermediate' risk group, with a median 5-year overall survival (OS) of 35%, they display an heterogeneous clinical course. Different genetic abnormalities have been found to impact on the clinical course of these patients. We have recently demonstrated that Multidrug resistance (MDR) protein P-glycoprotein (PGP) is related with a reduced survival also in cytogenetically normal (CN) AML patients. Allogeneic hematopoietic stem cells transplantation (HSCT) is recommended in patients with unfavourable karyotype AML, while its role in CN AML cases is not yet established. *Aims.* We have retrospectively analyzed 111 patients with high-risk CN AML. We have evaluated the role of allogeneic HSCT in 51 patients who underwent transplant procedure 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. *Methods.*

One hundred eleven CN AML patients, considered at high risk according to initial clinical presentation (WBC >30×10^9/L, PGP MFI >6, secondary AML) or on the basis of poor response to induction therapy, were considered candidate to allogeneic HSCT. Fifty-one (46%) identified an HLA identical donor and underwent transplantation, while 60 patients without a donor received chemotherapy-based intensification. The two cohorts were comparable for WBC count and secondary AML, while the transplanted patients were younger (45 vs 60 years) and with a lower incidence of PGP-positive (27% vs 47%) and primary resistant (22% vs 42%) cases. HSCT was performed at a median of 7 months (range: 1-99) from diagnosis. Twenty-eight patients (55%) had a sibling donor, 23 an unrelated one. HSC source was bone marrow in 30 cases, peripheral blood in 21. Twenty-eight transplants were performed in first (n=23) or subsequent (n=5) remission. Conditioning regimen was myeloablative in 40 cases (78%), nonmyeloablative in 11. *Results.* Acute GvHD developed in 17 patients (33%), mostly in MUD recipients. Chronic GvHD occurred in 14 of 41 evaluable patients (34%). One year transplant related mortality (TRM) was 22% (11 cases). CR rate at engraftment was 90%. Sixteen of 21 patients transplanted with active disease obtained CR (76%). Relapse occurred in 17 patients, with no difference according to PGP status at diagnosis. Disease-free survival (DFS) and OS was influenced only by disease status at transplantation (p=0.027). 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. *Summary*. Allogeneic HSCT 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 therefore pursued in patients who display this high risk feature at diagnosis.

0460

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

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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 present the preliminary results of our study in which we are evaluating the dynamic expression of WT-1 in AML patients (pts) following Allogeneic bone marrow transplantation (BMT). The expression of WT-1 was measured using Real Time Quantitative RT-PCR with the specific TaqMan probe; the WT-1 expression was related to expression of the control gene ABL. The cDNA level of WT-1 was detected in bone marrow samples from 20 AML pts at diagnosis (11 males and 9 females), at the time of transplant and after the allogeneic BMT. Samples of diagnosis showed high WT1 expression levels in all cases with a mean of 5468 (SD 4025) copies of WT1/10000 Abl, median 4200 (range 658-13923) copies WT1/10000 Abl. At transplant 12 pts (60%) were in complete cytologic remission (CcR) and 8 (40%) had refractory or relapsed AML. Bone marrow samples from pts in CcR at BMT showed significantly lower WT1 expression levels (mean 74,3±126), compared to the samples from pts with relapsed or refractory disease (mean 5627±4165) (p<0,001). After BMT a rapid decline of mean and median WT1 expression levels was observed in all pts that maintained a condition of CcR, expecially in those that were in CcR at BMT (Figure 1A). After a median follow up of 7 mths from transplant, 3 out 20 pts relapsed (15%) and all of them had high expression levels of WT1 (Figure 1B). One of these pts died with leukemia and two were successfully reinduced with DLI±chemotherapy with a rapid reduction of WT1 levels.*Conclusions.* In our preliminary experience, taking into account the small number of cases, there was a complete concordance between WT1 levels and status of leukemic disease before and after BMT. WT1 (from bone marrow samples) may be useful as a non-specific leukemia marker (NSLM) for monitoring of MRD and as a possible predictor of AML relapse. Besides WT1 levels before and after BMT could help to measure the burden of neoplastic disease.

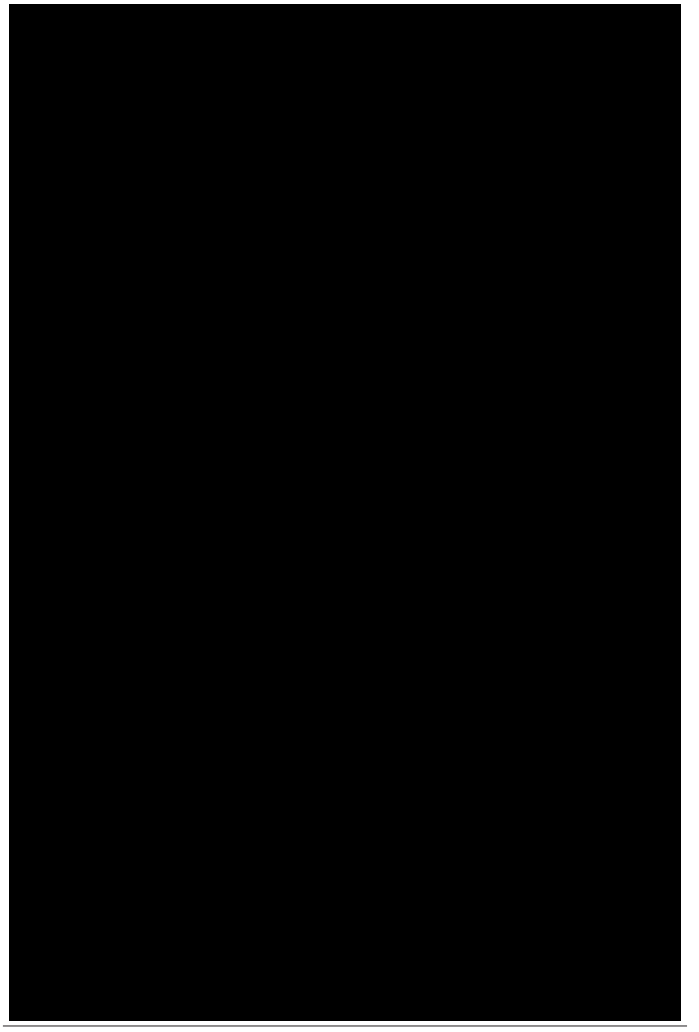

Figure 1. A) WT1 in pts transplanted in CcR that mantained CcR after BMT. B) WT1 in 3 pts t (one in CcR and two with active AML at BMT) that relapsed after BMT.

0461

BONE MASS IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION (SCT)

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Background and Aims. Organ transplantation is associated with bone loss. There are scarce studies on bone mass in patients undergoing SCT. The aims of the study were: 1 To determine the changes in bone mineral density (BMD) of patients receiving SCT. 2 To study the differences in BMD between allogeneic and autologous SCT. 3 To determine serum osteoprotegerin levels and to correlate them with BMD. *Patients and Methods.* Prospective study of 98 consecutive patients with hematologic malignancies undergoing SCT. BMD prior to SCT and at 6 and 12 months after SCT were measured by dual-energy x-ray absorptiometry (Lunar Prodigy, Madison WI) at the lumbar spine and the proximal left femur. Bone formation/resorption markers (N-terminal propeptide of human procollagen tipe I [P1NP]/ N-telopeptide cross-links of tipe I collagen [NTX]) were measured at baseline and after 3, 6 and 12 months. Osteoprotegerin was determined prior to SCT and at 6 months after SCT. *Results.* Mean (SD) age was 44 (12) yr, 45 females; 54 autologous SCT and 44 allogeneic SCT. Prior to SCT, 36 patients (37%) had low BMD (25 osteopenia and 11 osteoporosis). BMD values can been

observed in Table 1. Osteoprotegerin levels showed a non significant increase after 6 months, both in autologous (4.33 vs 5.35 pmol/L, p=0.093) and allogeneic SCT (5.16 vs 5.84 pmol/L, p=0,258) and there was no correlation with BMD. P1NP levels showed a significant increase after 3 months in autologous SCT group (80.02 vs 113.85 ng/mL, p=0.002) and a decrease in allogeneic SCT at 3 months (112.47 vs 81.23 ng/mL, p=0.033), 6 months (130.28 vs 87.40 ng/mL, p=0.029) and 12 months (123.07 vs 90.98 ng/mL, p=0.111). No changes in NTX were observed in these patients. *Conclusions.* Hematopoietic SCT (autologous and allogeneic) is associated with bone loss, specially in femoral neck. Patients undergoing allogeneic SCT showed a significant decrease in bone turnover markers, which are involved in the reduction of BMD. Changes in osteoprotegerin levels were not correlated with changes in BMD.

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Table 1. HSCT caracteristics and results.

0462

ROLE OF PRIMACY OF BIRTH IN HLA-IDENTICAL SIBLING TRANSPLANTATION

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Role of sibling primacy in HA-identical sibling transplantation. HLA-identical siblings are prime donors for allogenic hematopoietic stem cell transplantation (HSCT). Despite matching for MHC antigens, the risk for GvHD remains high. Fetomaternal microchimerism has been associated with better survival of maternal compared to paternal grafts in non-T-cell depleted, haploidentical HSCT. Fetomaternal and transmaternal sibling microchimerism have been associated with autoimmune diseases. In analogy, we hypothesized that pretransplant encounters of recipient cells with their later donors through fetomaternal trafficking should have an impact on outcome in HLA-identical sibling HSCT. This retrospective single-center cohort study analyzed overall survival (OS) transplant related mortality (TRM), relapse mortality (RM), and incidence and severity of acute and chronic GvHD after HLA-identical sibling HSCT. We defined based on birth sequence, three groups: Firstborn donor (FD), firstborn recipient (FR) and others (FO). 311 consecutive patients with complete information on sibling sequence, transplanted from 1980 to 2004 were included. Recipient and donor age and sex, diagnosis, stage of the disease, transplantation date and conditioning regimen, stem cell source (bone marrow vs. peripheral blood stem cells), graft manipulation (T-cell depletion), acute and chronic GvHD, date of relapse and of last clinical visit or death were recorded. 97 patients were firstborn recipients (FR) and 107 recipients received a graft from a firstborn donor (FD). 107 patients were neither firstborn nor received a graft from a firstborn sibling (FO) and served as a control group. FR patients were by definition older than FD patients, FO patients had more siblings. No other significant differences for pretransplant variables were observed. 187 (60.1%) patients were alive with a median follow-up of 8.9 years. OS at 10yrs was better in FR than FD patients (49.6% vs. 63.7%, p=0.014) and cumulative incidence of death from relapse was lower in FR. Cumulative incidence of grade II or higher aGvHD was 41.2% in FR vs. 57.0% in FD (p=0.035). FO showed intermediate *Results.* Chronic GvHD was not different among groups. Multivariate analyses strongly confirmed the findings of the univariate tests and excluded recipient/donor age or sibling number as reasons for the observed *Results.* We describe a significant impact of sibling primacy on the incidence of acute GvHD, relapse incidence and overall survival in HLA-identical HSCT: Patients who were born as a first child in a family had the best survival with a significant reduction in acute GvHD and relapse mortality. Possible mechanisms include fetomaternal and transmaternal sibling cell trafficking and tolerisation of the donor. If confirmed, birth order could be integrated into the donor selection algorithm in HLA identical sibling HSCT or matched unrelated donors might be preferred over a high-risk HLA-identical sibling in some cases.

0463

PALIFERMIN IN UNRELATED DONOR TRANSPLANT RECIPIENTS

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Background. Within a compassionate use program in Austria (Medical University of Graz and Medical University of Vienna) and Germany (University Hospital of Schleswig-Holstein, Campus Kiel and Martin-Luther-University, Halle) we retrospectively analysed data on 39 palifermin (keratinocyte growth factor, KGF) treated patients receiving an allogeneic hematopoietic stem cell transplantation (allo-HSCT) for hematologic malignancies. Since 24 patients had an unrelated donor we evaluated a possible acute graft versus host disease (aGVHD) protective effect of palifermin, as indicated in several animal models, in dependence on donor status. *Methods.* Palifermin was administered 60µg/kg per day for three consecutive days before the initiation of conditioning therapy and after graft infusion as previously published in autologous HSCT. The conditioning included TBI-based regimens in two-thirds of the cases, one third was treated with chemotherapy only. Baseline demographics and disease characteristics are listed in Table 1.

Table 1.

	unrelated donor	sibling donor
No. patients	24	15
Sex, no. (%)		
Male	17 (71)	9 (60)
Female	7 (29)	6 (40)
Median age, y (range)	41 (18-67)	42 (21-68)
Disease, no. (%)		
AML	12 (50)	9 (60)
ALL	2 (9)	3 (20)
CML	1 (4)	0
MDS	1 (4)	0
NHL	6 (25)	3 (20)
Hodgkin	1 (4)	0
other malignancies	1 (4)	0
Conditioning regimen, no.(%)		
Chemo + TBI	15 (63)	11 (73)
Chemo only	9 (37)	4 (27)

Results. Age, sex, disease and conditioning regimen allocation were balanced between patients transplanted with an unrelated or sibling donor. Adverse events - mainly skin rash, erythema, swelling of lips and tongue and taste alteration - were comparable in both groups, mild to moderate in severity and transient. One patient with a sibling donor died before day 30 due to sepsis. No statistically significant difference was observed in the incidence of grades 2 to 4 (38 vs 36%, p=ns) or grade 3 to 4 (30 vs 29%, p=ns) aGVHD on day 100 after allo-HSCT. Although the incidence of grade 3 and 4 oral mucositis was lower in unrelated donors (25 vs 40%, p=0.016) both groups showed a similar median duration of febrile neutropenia (3.7 vs 4.1days, p=ns). In order

to asses intestinal epithelial damage we prospectively measured serum citrulline levels - a marker of small bowel enterocyte mass - once weekly during the course of HSCT (timepoints: day-12, -6, 0, +7, +14, +21) in 11 consecutive palifermin treated patients receiving either an unrelated or related donor. In a mouse model administration of palifermin reduced aGVHD documented by preserved citrulline levels, indicating a reduced injury to the gastrointestinal tract. In our patients median citrulline serum concentration showed an expectable decline until day+7 (7.70 µM) - reflecting a maximal intestinal barrier injury - followed by an adequate increase at day+21 (14.86 µM) which is in accordance with untreated patients at our site (day+7: 9.02 µM, *p*=ns and day+21: 16.33 µM, *p*=ns) and a historical cohort receiving allo-sibling-HSCT following myeloablative therapy. This lack of preserved gut integrity shown by an equal decline of citrulline concentration may explain the absent reduction of aGVHD rates. *Conclusions.* Palifermin in unrelated HSCT shows a comparable safety profile to sibling donor transplants. However, we could not find a favourable effect of palifermin on the incidence and severity of aGVHD.

0464

THE DEVELOPMENT OF CELLULAR IMMUNITY TO ASPERGILLUS FUMIGATUS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Slow immune reconstitution after mismatched and match unrelated donor transplantation results in a high mortality from viral and fungal infections. Invasive aspergillosis (IA) is one of the major fungal infections after hematopoietic stem cell transplantation (HSCT), although new antifungal drugs have emerged. Part of the problem is the failure to make an early molecular diagnosis, as for CMV. Invasive aspergillosis incidence after HSCT is about 10%, variability depending on environmental conditions. The mortality rate of IA is about 50 to 80%. It has been previously reported that a Th1-type cellular immune response could favourably influence the IA evolution. *Aims.* We initiated a study of cellular immune reconstitution against Aspergillus fumigatus after HSCT where 70 patients have to be included. We present hereafter the results of the 41 one first patients. *Methods.* Cell phenotype analysis and proliferation capabilities against mitogens and Aspergillus fumigatus conidies were performed at day 60, 100, 180 and 360 after HSCT. Secretion of IL10 and IFNγ cytokines was also investigated. Finally, antigen and molecular monitoring of aspergillosis infection were performed. *Results.* Six patients presented antigenic or molecular evidence of infection but only one developed an IA. Proliferation response against Aspergillus fumigatus conidies improved regularly after day 60 to be completely recovered at day 360. IFNγ level seemed to decrease during the first year post-HSCT whereas IL10 level remained constant. The patient who presented an IA at day 15 after HSCT was first unsuccessfully treated with anti-viral drugs. He underwent two surgical procedures at day 110 and day 160 which finally resulted in negativation of biological evidence of aspergillosis and development of a proliferation response against Aspergillus fumigatus conidies at day 265. However, no cytokine secretion could be detected. *Conclusions.* The analysis of such a cohort is interesting to better characterise how immune reconstitution against Aspergillus fumigatus happens depending on transplantation conditions.

0465

FLUDARABINE PHOSPHATE-BASED REDUCED INTENSITY CONDITIONING FOR ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA, MYELODYSPLASIA AND CHRONIC MYELOID LEUKEMIA: UPDATE OF A NATIONAL DOSE- FINDING STUDY

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Objectives. We prospectively studied the role of ATG in a Fludarabine phosphate (F)-based non myeloablative conditioning regimen to control the development of graft-versus-host-disease (GVHD), to accelerate donor engraftment and to maintain graft-versus-leukemia (GVL) effects in patients with myeloid malignancies not eligible for classical allogeneic transplant. We also analyzed the outcome of the patients (pts) according to their age and to the status for disease before transplantation. *Population*. A total of 61 pts were enrolled in this phase I-II study. Pts were conditioned with F (30 mg/m^2/day for 4 days) plus cytarabine (2 mg/kg/d for 4 days) or cyclophosphamide (1g/m^2/day for 3 days). All pts received cyclosporine (3-5 mg/kg IV, daily) and ATG 10 mg/kg/day either for 4 days (n=13), or for 2 days (n=7), or for 2 days + mycophenolate mofetyl (n=38) or no ATG (n=3). Chimerism analyses were performed on d30, 45, 60 and 90. *Results.* 23AML, 22 MDS, 13 CML and 3 Myelofibrosis (MF) were enrolled in this trial. The median age was 56.5 (18-75) years. 22 pts (36%) had 60 y.o. or more. The median follow-up is 41 (13-81) months. Prior to transplantation, 51% of pts were in complete remission. 49% had persistent disease (PR, PD). Engraftment rate was 97%. The occurrence of Acute GVHD (aGVHD) grade II-IV at day 90 was very low in all groups (<20%). Chronic GVHD rates are similar in all groups (30%).The low number of pts /group does not allow to find a statistical difference between the different doses of ATG. At d90, full T-cell (>90%) donor chimerism was achieved in 85% of the pts. 3 years OS and EFS in all patients were respectively 55% and 44%. According to the age, OS at 3 years seems to be better in young pts (60.5%) than in pts above 60 y.o (39%), *p*=0.07. For CML, OS and EFS at 3 year were 80% and 60% respectively in young patients versus 0% in elderly pts. The comparison of high risk pts (not in CR) and pts in CR didn't show any difference in term of OS or EFS. Primarily in diseases very sensitive to the GVL effect (AML and CML), there were no significant differences in OS and EFS curves according to the status before transplant. In AML and MDS pts, a plateau in the EFS curve (respectively 43% and 40% /3 years) suggests a cure in these pts. *Conclusions.* Although this series is too small to draw definite conclusions, these observations suggest that in AML and CML pts, RIC transplantation can be a curative option whatever the status of disease before transplant. In MDS pts, the status before transplant remains a prognostic factor and in CML pts, age (above 60) also has a negative impact.

Acute myeloid leukemia - Biology II

0466

ROLE OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 IN THE CHEMOTACTIC MIGRATION OF ALL-TRANS RETINOIC ACID-TREATED PROMYELOCYTIC LEUKEMIC CELLS TOWARD ALVEOLAR EPITHELIAL CELLS

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Background. Although all-trans retinoic acid (ATRA) can treat acute promyelocytic leukemia (APL), it also causes retinoic acid syndrome with presentations similar to acute respiratory distress syndrome. Our previous study has demonstrated that IL-8 and GRO-α secreted from alveolar epithelial cells play an important role in the cell-cell interaction involved in the chemotactic transmigration of ATRA-treated APL cells toward alveolar epithelial cells.(Critical Care Medicine-10.1097/01.CCM.0000256844.38259.27). *Aims.* In this study, we further investigated the role of monocyte chemoattractant protein-1 (MCP-1; CCL2) involved in the chemotactic transmigration of ATRA-treated NB4 (ATRA-NB4) APL cells toward A549 alveolar epithelial cells. *Methods.* Co-culture and transmigration assay were used in this study. NB4 and A549 cells were separately cultured with ATRA and/or dexamethasone for 1-3 days. NB4 or ATRA-NB4 cells were then placed in an upper insert and co-incubated with A549 cells or their conditioned medium (CM) located in a lower plate. *Results.* We firstly determined the MCP-1 level in the CM by ELISA. Only A549 cells constitutively secreted MCP-1. However, ATRA treatment markedly enhanced the secretion of MCP-1 in both A549 and NB4 cells. Exogenous administration of MCP-1 also promoted the ATRA-NB4 transmigration. Neutralization of MCP-1 in CM of A549 cells with its specific antibodies reduced ATRA-NB4 transmigration by about 38%. Pretreatment of ATRA-treated NB4 cells with antibody directed against MCP-1 receptor (CCR2) significantly reduce the transmigration of ATRA-treated NB4 cells by about 45%. Dexamethasone did not affect the secretion of MCP-1 in untreated- or ATRA-treated NB4 cells. However, when dexamethasone was applied to A549 cells, MCP-1 secretion was suppressed in both untreated- or ATRA-treated A549 cells, and there was attenuation of ATRA-NB4 transmigration. *Conclusions.* MCP-1 secreted from alveolar epithelial cells also play an important role in the cell-cell interaction involved in the chemotactic transmigration of ATRA-treated APL cells toward alveolar epithelial cells.

0467

REPORT OF 38 PATIENTS WITH HYPERDIPLOID KARYOTYPE IN ACUTE MYELOID LEUKEMIA: A GROUPE FRANCAIS DE CYTOGENETIQUE HEMATOLOGIQUE STUDY

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Background. Large hyperdiploidy (49 or more chromosomes) is a rare abnormality in AML and no large series describing the feature of such population of patients are available. Applying the definition of complexe caryotypes, all patients with hyperdiploid AML fall in the high risk cytogenetic cohort. *Aims.* We report here the first large cohort of 38 patients with more than 48 chromosomes and without karyotypic structural abnormality to better define the incidence, the clinical and morphological characteristics, and the prognosis of this rare group of patients. *Methods.* 4884 cytogenetic studies performed in laboratories members of the GFCH were screened. This corresponds to AML patients (adults and pediatrics) diagnosed in 19 French and Belgium institutions during A retrospective and one year prospective survey (February 2005-February 2006). We were able to select 38 AML patients with hyperdiploid karyotypes without structural abnormality under conventional cytogenetic. *Results.* Large hyperdiploidy is rare in AML with an incidence inferior to 1% (0.78%). Sex ratio F/M was 2.2 and median age 65 years (ranging from 1 to 91 years). All AML FAB subtype was represented. Although all chromosomes are involved, some seems to prevail: gains of chromosome 8 (68%), 21 (47%) and 19 (39%) are the most frequent followed by gains of chromosome 14 (34%), chromosomes 10 and 13 (29% each) and chromosomes 4 and 11 (26% each). Interestingly, some chromosomes involved in hyperdiploid ALL (4, 6, 8 , 10, 14, 18, 21 and X) are also found in our hyperdiploid AML patients, suggesting that these gains are not lineage restricted. Monosomie was uncommon and we report only two X chromosome losses in women. A cryptic rearrangement of MLL was found in 15% of these patients leading them in a subcategory in the WHO classification with an unfavorable prognosis. Therefore detection of MLL rearrangement should be systematically search in AML without a well cytogenetically established prognosis. When we applied the most frequent definition of complexe karyotypes (3 or more abnormalities), all patients with large hyperdiploid AML fall in the unfavorable category. Among the 18 patients without ML rearrangement receiving an induction therapy, 16 (89%) reached CR and 6 (33%) were still alive after a 26 months median follow-up (10-56 months). The median time to relapse is 658 days for the 16 patients in CR including 3 patients censored at the time of bone marrow transplantation. *Conclusions.* Although this study was retrospective these results suggest that hyperdiploid AML may be better classified in the intermediate prognostic group when hyperdiploidy is the only cytogenetic abnormality.

0468

CTLA-4 EXPRESSED BY CHEMORESISTANT, AS WELL AS UNTREATED, MYELOID LEUKEMIA CELLS CAN BE TARGETED WITH LIGANDS TO INDUCE APOPTOSIS

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Background and Aims. We previously reported that about 80% of acute myeloid leukemia (AML) samples tested at diagnosis constitutively expressed CTLA-4. In this study, we compared CTLA-4 expression and function of leukemic cells from AML patients at diagnosis with those from AML patients resistant to conventional chemotherapy. We also explored the possibility of targeting CTLA-4 for apoptosis induction in chemoresistant AML cells. *Results.* AML cells either from untreated patients (n=15) or in chemoresistant phase (n=10) were analysed for CTLA-4 protein and transcript expression by flow cytometry and RT-PCR, respectively. CTLA-4 expression was similar in untreated and in chemoresistant samples and not associated with patients' clinical features. CTLA-4 expressed by chemoresistant AML cells was able to transduce an apoptotic signal on engagement with its recombinant ligands r-CD80/r-CD86 which induced an average of 71% and 62% apoptotic cells respectively, at highest concentration. Apoptosis was equally induced in untreated leukemic cells accompanied by cleavage of procaspase-8 and -3. *Conclusions.* this study provides the first evidence that killing of leukemic cells from AML patients may be obtained upon engagement of CTLA-4 with its ligands opening the way to a novel potential therapeutic approach based on triggering the CTLA-4 molecule to circumvent chemoresistance in AML.

0469

PROTEOMICS ANALYSIS TO IDENTIFY BIOMARKERS AND PHARMACOLOGICAL TARGETS IN ACUTE MYELOID LEUKEMIAS WITH FLT3/ITD MUTATIONS

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The Flt3 receptor tyrosine kinase is a critical mediator in the patho-

genesis of acute myeloid leukaemia (AML). Flt3-activating mutations have been associated with poor prognosis and decreased overall survival of AML patients. Among them, internal tandem duplications (ITD) of Flt3 are found in a high proportion of cases with acute myeloid leukemia (AML). These genetic aberrations may lead to the constitutive activation of the receptor, thus providing the molecular basis for a persisting growth stimulus. On the other hand, alterations of transcriptional regulators are also key events in leukaemogenesis. Constitutive activation of MAP/Akt/STAT/NF-κB signal transduction pathways have been described in AML and they are commonly attributed to Flt3 activity. With the aim of better understanding the molecular mechanisms accompanying the acquisition of Flt3 activating mutations, a comparative proteomic approach was undertaken on Flt3/ITD and wild-type Flt3 AML cells. Proteomic analyses of three AML samples showing Flt3/ITD and three samples with wild-type Flt3 were carried out using 2-DE and MALDI-TOF mass fingerprinting analysis. Proteins identified as more significantly altered between the different AMLs analyzed belonged to the group of suppressor genes, metabolic enzymes, antioxidants and signal transduction mediators. Among them, four identified proteins were found significantly deregulated in Flt3/ITD leukemic cells in comparison to wild-type Flt3 AML cells, including two up-regulated proteins (peroxirredoxin and catalase) and two down-regulated proteins (calreticulin and RhoGDI). These proteins were widely involved in differentiation and cell survival. Furthermore, several intracellular pathways and transcription factors also implicated in differentiation and cell survival such as ERK 1/2, p38 MAPK, Akt, STAT5 and NFkB were found constitutively activated in the Flt3/ITD AML samples. Employing methods of cell biology and proteome analysis tools, we examined the effects of an inhibitor of Flt3 phosphorylation, AG1296 (AG), on the proliferation/apoptosis characteristics of myelomonocitic leukemia-derived cells MV411, which carried the Flt3/ITD mutation. AG induced apoptosis by suppressing Flt3 activity, which was followed by p38 MAPK and Akt inhibition. The proteomic analysis revealed several proteins that were differentially expressed due to AG treatment namely, calreticulin, eIF-5a, nucleophosmin, protein disulfide isomerase, glutamate dehydrogenase, peroxirredoxin and RhoGDI, all of them involved in the regulation of cell differentiation, proliferation and apoptosis. The further investigation of the relationship between the altered protein expression found in FLT3/ITD AML cells and the constitutive activation of those intracellular pathways, will uncover new clues to understanding leukemogenic effects of FLT3 activating mutations. This study shows the power of proteomic profiling for the discovery of novel molecular targets and a better understanding of the actions of Flt3/ITD at the molecular level in AML cells. Supported by FIS 050910, FIS 041291, JA 0024/05, 0060/05 and FIJC-06/ESP

0470

LOW DOSE RAPAMYCIN IS NOT EFFLUXED BY P-GLYCOPROTEIN IN AML

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Background. A clinical trial of rapamycin in elderly patients with AML is currently underway in Nottingham, UK. A potential confounding factor to the clinical effectiveness of rapamycin is its reported interaction with the drug efflux pump p-glycoprotein. *Aims.* We investigated the effect of rapamycin on mTOR targets and on pgp in a single cohort of AML cells in order to determine whether the mTOR target-modulating dose of rapamycin is above or below the threshold for interaction with pgp. *Methods.* Three pgp positive cell lines - KG1, KG1a and TF-1 were used. MTOR targets p70 S6K(phosphothr389) and 4E-BP1 (phosphoser65) were measured by Western blotting. Pgp function was measured using the UIC2 shift assay and the rhodamine 123 accumulation assay with cyclosporin A and vinblastine as positive controls. *Results.*10 nM rapamycin induced a complete loss of phospho-p70 S6K in KG1 and KG1a cells. TF-1 cells had undetectable basal levels. At 10 nM, rapamycin also reduced phospho-4E-BP1 levels to 56% of control values in KG1a, 26% of control in KG1 and 39% of control in TF-1 cells. At this dose, rapamycin failed to induce a UIC2 shift, indicating that there was no interaction with pgp. 10 nM rapamycin also failed to increase rhodamine 123 accumulation, whether added concurrently or up to one hour before the rapamycin, indicating that pgp has no significant interaction with 10 nM rapamycin. Higher doses of rapamycin did induce a small increase in rhodamine 123 accumulation (by approximately 30% in all 3 cell lines at 100 nM). Similarly, at 100 nM there was an approximately 20% increase in UIC2 binding in KG1a and TF-1, but not KG1 cells. Many drugs are documented to upregulate pgp within 48 hours. 72 hours' incubation with 10 nM rapamycin did not upregulate pgp protein expression in the three cell lines. *Conclusions.* We conclude that pgp-mediated drug resistance is unlikely to be a significant factor in the response of AML cells to rapamycin.

0471

SEPT2 IS A NEW FUSION PARTNER OF MLL IN ACUTE MYELOID LEUKEMIA WITH T(2;11)(Q37;Q23)

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Background. Abnormalities of 11q23 involving the MLL gene are found in several hematological malignancies, including acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), and also in a proportion of patients with therapy-related leukemia after treatment with topoisomerase II inhibitors. *Aims/Methods.* Cytogenetic, fluorescence *in situ* hybridization, and molecular studies were used to identify a new fusion partner of the MLL gene in a patient with a diagnosis of therapy-related acute myeloid leukemia with a t(2;11)(q37;q23). *Results/Discussions*. Fluorescence *in situ* hybridization demonstrated a rearrangement of the MLL gene with the chromosome band 2q37. RNA and DNA analyses showed the existence of an in-frame fusion of MLL exon 7 with SEPT2 exon 3, with the genomic breakpoints located in intron 7 and 2 of MLL and SEPT2, respectively. Search for DNA sequence motifs revealed the existence of two sequences with 94.4% homology with the topoisomerase II consensus cleavage site in MLL intron 7 and SEPT2 intron 2. SEPT2 is the fifth septin family gene fused with MLL, making this gene family the most frequently involved in MLL-related AML (about 10% of all known fusion partners). *Conclusions.* We have identified a new MLL gene fusion partner in a patient with treatment-related AML presenting a t(2;11)(q37;q23) as the only cytogenetic abnormality. SEPT2 is the fifth septin that has been found fused with MLL in acute leukemia, but the precise role played by this family of genes in this disease remains incompletely known.

0472

PROGNOSTIC VALUE OF CYTOCHROME C RELATED ACTIVATION OF CASPASES IN PEDIATRIC ACUTE MYELOID LEUKEMIA

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Background. Deficient activation of apoptosis signaling pathways has been considered to be responsible for treatment failure in acute leukemia. Studies have been performed to analyze the expression of apoptosis molecules with respect to their prognostic value for treatment outcome but could not establish a correlation with response to treatment and prognosis. In a previous study we investigated functionally the importance of intact apoptosis signaling for treatment response in childhood lymphoblastic leukemia by analysis of two key apoptogenic events, caspase-3 activation and mitochondrial cytochrome c release. By combining both parameters, we identified the novel indicator CRAC (cytochrome c related activation of capase-3) which directly connects the extent of caspase-3 activation to cytochrome c release in single cells in an individual patient sample. Pediatric B-ALL patients with positive CRAC values were found to be good responding to initial treatment and showed a significantly higher probability of relapse free survival in contrast to patients with negative CRAC values (Meyer *et al.,* Blood, 2006, 107(11): 4524-31). *Aims.* Since the investigation of cytochrome c release and caspase activation and the calculation of its relation as expressed by the parameter CRAC is of prognostic value in childhood lymphoblastic leukemia we were interested in the significance of intact apoptosis signaling represented by this parameter in pediatric patients suffering from acute myeloid leukemia. *Methods.* Myeloid leukemia cells identified by surface staining in samples (cryopreserved bone marrow or peripheral blood) obtained at diagnosis from pediatric AML patients were analyzed for active caspase-3 and released cytochrome c flowcytometrically. The relation between these two key apoptogenic events was analyzed upon induction of apoptosis by factor withdrawal and the parameter CRAC was calculated for each patient sample. *Results.* In the samples investigated a heterogenous pattern of caspase activation and cytochrome c release was observed. Especially insufficient caspase activation in the presence of mitochondrial released cytochrome c was found in a number of samples resulting in negative values for the CRAC parameter. This indicates deficient apoptosis signaling in this subset of AML patient samples. 16 of the 34 AML patients (47,1%) showed negative CRAC val-

ues whereas 18 (52,9%) revealed a positive value for CRAC. This distribution is comparable to the pattern found in the analysis of acute lymphoblastic leukemia in our previous study (N=78, CRAC positive n=35, 44,9%; CRAC negative n=43, 55,1%). *Conclusions.* The analysis of cytochrome c related caspase activation (CRAC) identifies leukemia samples with deficient apoptosis signaling in a group of pediatric AML patients. The detection of such patients by assessment of the parameter CRAC may be used for additional treatment stratification in childhood acute myeloid leukemia.

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0473

FLT-1 ACTIVATION BY VEGF/PLGF INDUCES LEUKEMIA CELL MIGRATION VIA P38/ERK1/2 KINASE PATHWAY, RESULTING IN RHO GTPASES ACTIVATION AND CAVEOLAE FORMATION

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Vascular endothelial growth factor (VEGF) receptors -1 (FLT-1) and -2 (KDR) are expressed by subsets of malignant hematopoietic cells, where they signal in a paracrine and/or autocrine manner to induce cell survival, proliferation and migration. We recently demonstrated that acute lymphoblastic leukemia (ALL) migration in response to VEGF via FLT-1 modulates the onset of extramedulary disease, and thus has clinically predictive value. However the molecular mechanisms involved in leukaemia cell migration were not yet characterized and are the subject of this study. We demonstrate that FLT-1 activation by VEGF or PLGF stimulation results in a significant increase of AML migration, having a minor proliferative effect. This FLT-1 mediated migration of AML cells resulted in the formation of actin membrane protrusions (as assessed by phalloidin/FLT-1 immuno-staining and confocal microscopy) with concomitant increased ERK1/2 and P38 phosphorylation and activation of Rho-GTPases and PAK/Cofilin. Also, by immunoprecipitation studies, we have shown that PLGF promoted the recruitment of Hsp90 and its binding to FLT-1, actin and caveolin-1. While PLGF stimulation of AML cells induced the formation of caveolae-like structures, cytochalasin D treatment, used as control to block cell migration, was shown to block the molecular interactions between FLT-1, actin, Hsp90 and caveolin-1.Taken together, these data highlight some of the molecular mechanisms whereby VEGF/PLGF stimulation of FLT-1 on AML cells results in cell migration. As such, our data reveal important biological and mechanistic outputs that may be used to monitor leukemia responses to cytoskeleton-disrupting agents or anti-angiogenic therapies. Our ongoing studies are know focusing on a screening of kinases/phosphatases that affect VEGF production in acute leukemia cells, using a system of retrovirus-based vectors that achieve with high-efficient siRNA-dependent silencing in leukaemia cells.

0474

A RECURRENT IN-FRAME INSERTION IN CEBPA TAD2 REGION IS A POLYMORPHISM THAT DOESNT HAVE A PRONOSTIC VALUE IN AML. A STUDY FROM THE FRENCH ALFA GROUP

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Background. CCAAT/enhancer-binding protein-α (CEBPa) is a transcription factor strongly implicated in myelopoiesis through control of proliferation and differentiation of myeloid progenitors. In patients with acute myeloid leukemia (AML), this gene is often mutated (about 8%). Those mutations are in-frame or out of frame types and the consequences are a loss of function of CEBPa. Among those mutations, an in-frame insertion of 6bp in the TAD2 domain, resulting in a His-Pro duplication (HP196-197ins), was found in patients at diagnosis and remission time, letting think of a constitutive abnormality, that may induce AML susceptibility. Surprisingly this 6bp insertion was observed in healthy voluntaries too. Recently Delwel *et al.* have described this insertion as being a polymorphism. They report that 6bp insertion frequency in AML patients is between 3.2% (9/282) and 3.9% (12/305), while in non-leukemic blood samples, the frequency is 8% (22/274). Further, they didn't found any correlation with CEBPa gene expression signature and more, patients with insertion didn't show any particular expression profile. *Aim of study and Methods.* In our study we have investigated 325 AML patients enrolled in ALFA 98-02 protocol and 994 healthy voluntaries for the presence of this insertion by nucleotide sequencing, and we studied its prognostic value. *Results*. First, we looked for the frequency of this insertion between the healthy whole population and the AML cohort; we didn't found any difference between AML patients and healthy voluntaries: 6.5% (21/325) versus 6.6% (66/994) respectively. This data strongly suggest that this insertion is a polymorphism, but familiar molecular study should be performed to confirm this hypothesis. Second, we made a comparison between true mutation of CEBPa and this 6bp polymorphism. Patients who harbored only the polymorphism have heterogeneous cytogenetic finding (8 normal, 5 complex and 1 intermediate karyotypes, 4 CBF-AML and 4 unknown), while patients with a true abnormality of CEBPa gene (n=23) show significant association to normal karyotypes (p=0.03). Mutations of CEBPa are often associated to M1 and M2 FAB subtypes, but we didn't find an association to FAB subtype for the group of patients with 6bp polymorphism. Regarding other molecular prognostic factors, CEBPa is rarely associated to NPM mutations (p=0.03) while polymorphism doesn't show this particularity (p>0.2). Regarding the EFS, we didn't find any correlation between this polymorphism, true CEBPa mutations and others patients. However regarding the OS only the true CEBPa mutation patients presented a trend for longer survival. *Conclusions.* In conclusion, we confirm that HP196-197ins is a common polymorphism spread in normal population and AML patients, without any prognostic value in our cohort of AML patients.

0475

CAN HYPERACTIVE SIGNALLING THROUGH THE RAS PROTEINS INDUCE AML IN MICE?

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Background. Mutationally activated forms of the RAS proteins, especially the N and K isoforms, are implicated in the pathogenesis of several haematological malignancies, including acute myeloid leukaemia (AML). However, in AML, the mutations in RAS genes are always accompanied by other genetic abnormalities (e.g. MDR1). In mice, hyperactive signalling through the RAS proteins, e.g., via expression of endogenous oncogenic K-RAS or inactivation of the tumor suppressor NF1, results in myeloproliferative disorders (MPD). At this point, it is unclear if hyperactive RAS signalling can induce AML in the absence of other genetic abnormalities. *Aims.* To test the hypothesis that hyperactive signalling through the RAS proteins can induce AML in mice, without other genetic abnormalities. To accomplish this, we will determine if inactivation of Nf1 - which increases signalling through the N- and K-RAS proteins - will transform a K-RAS-induced MPD into AML. *Methods.* For these studies, we use Cre-loxP techniques in mice. Cre-induced activation of endogenous oncogenic K-RAS in bone marrow cells results in a rapidly progressing MPD with leukocytosis, splenomegaly, and tissue infiltration; without an increase in blasts in peripheral blood. Similarly, Cre-induced inactivation of the RAS-GAP NF1 results in increased levels of RAS-GTP, hyperactive RAS signalling, and a slowly progressing MPD; without an increase in blasts. In this study, we simultaneously activate the expression of oncogenic K-RAS and inactivate the tumour suppressor NF1 in myeloid cells. This approach allows us to determine if higher levels of RAS-GTP would result in transformation of MPD into AML with > 20% blasts in peripheral blood. *Results and Conclusions.* This is an ongoing project. By June, 2007, we will have all the necessary data that will allow us to accept or reject our hypothesis.

0476

INCREASED RISK AND EARLY ONSET OF ACUTE MYELOID LEUKAEMIA IN BRAZILIAN INDIVIDUALS WITH NAD(P)H:QUINONE OXIDOREDUCTASE 1 (NQO1) AND CYTOCHROME P450 A1 (CYP1A1) GENE DEFECTS

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Background. Acute myeloid leukaemia (AML) has been linked to chronic exposure to benzene and tobacco's polycyclic aromatic hydrocarbons (PAH). NAD(P)H:quinone oxidoreductase 1 (NQO1) is an enzyme that detoxifies benzene-derived quinones and reduces oxidative stress on hematopoietic cells. A C-->T substitution polymorphism at nucleotide 609 of the NQO1 gene has been linked to a decreased activity of the coded enzyme. On the other hand, PAH are bioactivated by the cytochrome P450 A1 (CYP1A1) enzyme. An A-->G substitution

polymorphism at nucleotide 4889 of the CYP1A1 gene leads to the production of an enzyme with increased activity in PAH metabolism. Moreover, environmental-related diseases resulting from exposure to benzene and tobacco-related diseases have been described as serious health problems in south-eastern Brazil. *Aims.* The aim of this study was to evaluate the influence of the NQO1 C609T and CYP1A1 A4889G polymorphisms for AML risk and its clinical manifestations in individuals of the south eastern region of Brazil. *Methods.* Genomic DNA from 133 AML patients and 133 age, gender and race-matched controls were analysed using the polymerase chain reaction (PCR) and enzymatic digestion. The differences between the groups were calculated by the chi-square or Fischer exact test. *Results.* Controls samples (X^2=3.69, p=0.07) but not patients samples (X^2=6.52, p<0.02) were in Hardy-Weinberg equilibrium at the NQO1 C609T locus. Both the patients (X^2= 2.54, p=0.12) and controls (X^2= 0.89, p=0.34) samples also confirmed the Hardy-Weinberg expectations at the CYP1A1 A4889G locus. The frequency of the NQO1 609CT+TT genotype was significantly higher in AML patients than in controls (41.4% vs 28.6%, p=0.04). Carriers of the NQO1 wild allele (T) were under a 1.7-fold increased risk of AML (95% CI: 1.01-2.89). The frequency of the combined variant genotype was higher in patients under 47 years of age than in older patients (63.6% vs 36.4%, p=0.03). Similar frequencies of the CYP1A1 4889AG+GG genotype were seen in patients and controls (43.6% vs 30.8%, p=0.10). Similar risks for the disease were seen in carriers and non-carriers of the variant allele (G) (95% CI: 0.92-2.60). However, the frequency of the combined variant genotype was higher in patients under 47 years of age than in older patients (63.8% vs 36.2%, p=0.02). *Conclusions.* These results suggest a role for the NQO1 609TT+CT genotype in increased risk for AML and early onset of the disease and the CYP1A1 4889GG+AG genotype in the early age of disease onset in individuals of south eastern Brazil. Financial support: CNPq

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HALOFUGINONE INHIBITS TGF-β/VEGF SIGNALING IN ACUTE PROMYELOCYTIC LEUKEMIA

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Acute Promyelocytic Leukemia (APL) is a distinct subtype of Acute Myelogenous Leukemia on morphologic, clinical and molecular bases. APL bone marrow biopsies showed increased microvessel density, which was reduced by all-trans retinoic acid (ATRA), suggesting that aberrant angiogenesis may be relevant to APL pathogenesis. In addition, NB4 cells, an APL cell line harboring the t(15;17) translocation, were shown to secrete high levels of the pro-angiogenic factors VEGF and bFGF. TGF-β is known to regulate VEGF production, and Lin, Bergman & Pandolfi have recently shown that PML is an essential regulator of the TGF-β pathway. Therefore, our aim was to determine if the increased VEGF production observed in APL was related to PML/RARα protein effect on the TGF-β signaling. We analyzed the effect of Halofuginone (HF), a low molecular weight alkaloid that has been shown to inhibit TGF-β, reduce fibrosis and tumor progression in a variety of experimental models. NB4 and NB4-R2 cell lines, this latter resistant to ATRA, were treated with increasing doses of HF (6.25, 12.5, 25, 50, 100 ng/mL) and 10-6M of ATRA. VEFG production was measured by ELISA in culture supernatants, and a significant reduction of its levels was detected in samples treated with HF at doses higher than 25 ng/mL or ATRA. Western blot analysis showed that smaller doses of HF (6,25 to 25 ng/mL) and ATRA induced TGF-β expression while higher doses (50 to 100 ng/mL) were found to reduce its expression. We then evaluated the expression of p21 and CDC25A genes, TGF-β target genes involved in cell cycle regulation. HF treatment resulted in inhibition and increase of p21 and CDC25A expression, respectively. Cell proliferation and apoptosis were accessed by flow cytometry using a immunofluorescent staining of incorporated bromodeoxyuridine and 7AAD labeling, simultaneously. In both cell lines, HF progressively inhibited cellular proliferation and induced apoptosis, after 24 hours of treatment, in a dose-dependent fashion. In NB4, there was significant cell growth inhibition with all the HF doses superior to 25 ng/mL (p<0.001). In addition, a 1.5 fold increase in apoptosis was seen with 100 ng/mL after 24 hour incubation (p<0.001). In NB4-R2, cell growth inhibition was observed with 50 and 100 ng/mL and apoptosis with 100 ng/mL of HF (p<0.001). Results also showed that the drug was able to block the cell cycle progression at G1/S transition. Simultaneously, progressive reduction of Bcl2 protein expression in samples of both cell lines treated with increasing doses of HF was seen by flow cytometry analysis. Our results indicate that HF inhibits TGF-β signaling and its consequent VEGF production and thus may revert APL aberrant angiogenesis. TGF-β inhibition resulted in p21 and Bcl-2 downregulation leading to block of cell cycle, reduction of cell proliferation and induction of apoptosis. These effects were independent of ATRA sensitivity. We hypothesize that although the disruption of TGF-β signaling itself is not sufficient to initiate malignant transformation, it may be a critical second step that contributes to leukemia progression. In this context, HF may have therapeutic potential in APL.

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SIGNAL REGULATORY PROTEIN-α AS A POTENTIAL NEW TREATMENT TARGET IN ACUTE MYELOID LEUKEMIA

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Background. Signal regulatory protein-alpha (SIRP α) is a transmembrane receptor selectively expressed on myeloid and neuronal cells. The broadly expressed cell surface CD47 molecule acts as a major extracellular ligand. The SIRP α cytoplasmic tail contains ITIM motifs that upon CD47 binding mediate the recruitment and activation of the cytosolic tyrosine phosphatases SHP-1 and SHP-2. SIRP α signaling negatively regulates many signaling pathways leading to reduced tumor migration, survival and cell transformation. Indirect evidence has suggested that SIRP α may be downregulated in acute myeloid leukemic (AML). *Aims/Results.* We studied SIRP α mRNA levels in a micro-array data set of 285 AML samples and found a strong association with AML subtypes. (Minimal/poorly) differentiated myeloblastic and promyelocytic AMLs expressed low SIRP α mRNA levels. mRNA expression was higher in myelomonocytic/monocytic AMLs (comparable to normal bone marrow). These findings were confirmed by Western blotting on representative AML cell lines and 25 AML patient samples. SIRP α protein expression was low in AML cell lines KG1a (FAB M0) and Kasumi-1(FAB M2), while HL60 (FAB M3), U937 and THP1 (FAB M5) cells expressed higher protein levels. In the patient samples, SIRP α protein expression was absent/low in FAB M1/2 cases and high in FAB4/5 cases.We investigated whether SIRP α down-regulation in specific AML subsets, such as t(8;21) FAB M2 AML, resulted from epigenetic promoter hypermethylation. Although exposure to demethylating agents and HDAC inhibitors did increase SIRP α expression in t(8;21) Kasumi-1 cells, only limited methylation of the SIRP α gene promoter in these cells and primary t(8;21) AML patient samples was observed. In addition, we studied the functional significance of SIRP α by retroviral reconstitution of Kasumi-1 cells and incubation with an agonistic SIRP α antibody (ED9; 10 μg/mL for 7 days). Daily cell counting and annexin V/7-AAD FACS staining demonstrated significant growth inhibition (p=0.02) and promoted apoptosis (p=0.005). No effect was seen in Kasumi-1 empty vector control cells. Finally, we determined the efficacy of ED9 in combination with 6 concentrations of cytarabine (ara-C; 0.625-0.00061 μg/mL), daunorubicin (DNR; 2.56-0.0025 μg/mL) and etoposide (VP16; 10-0.0098 μg/mL) in these Kasumi-1 cells, by 4-day MTT assay. Calcusyn analysis demonstrated synergism between ED9 and ara-C (CI=0.46±0.32), DNR (CI=0.74±0.06) and VP16 (CI=0.60±0.05). *Summary and conclusions.* In conclusion, SIRP α expression appears to be reduced in specific AML subsets. SIRP α -derived signals can directly control myeloid cell growth and induce apoptosis in AML M2 t(8;21) Kasumi-1 cells. Moreover, agonistic SIRP α triggering synergized with conventional chemotherapeutic agents. Our results create a rational basis for the design of anti-leukemic therapies targeting SIRP α.

0479

ARHGAP21 INTERACTS WITH FOCAL ADHESION KINASE AND IS OVEREXPRESSED IN LEUKEMIA CELLS

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Background. ARHGAP21 is a new protein recently described by us and characterized as a Rho-GTPase activating protein (Rho-GAPs), a negative regulator of RhoGTPase signaling pathways. RhoGTPases mediate many aspects of cell biology, including proliferation, apoptosis, survival, adhesion and actin cytoskeleton dynamics. Recently, ARHGAP21 was

identified as a new component of cell-cell junctions that controls α-catenin recruitment and is able to interact with α-catenin, ARF1 and ARF6, important proteins in the cytoskeleton assembly and adherent junctions. Cell-to-cell adhesion is one of the major factors that restrict cellular invasion and metastasis in malignant diseases. FAK is a non-receptor tyrosine kinase, and coordinates signals from integrins, cytokines, growth factor receptors, and oncogenes. In AML cells, FAK has been found to be overexpressed and associated with enhanced blast migration, increased cellularity, and poor prognosis. *Aims.* In the attempt to verify the role of ARHGAP21 in leukemogenesis, we aimed to verify the expression level of ARHGAP21 mRNA and protein in normal hematopoietic cells and in leukemia cells. In addition, we attempted to verify a possible interaction of ARHGAP21 with proteins involved in cell adhesion, as Focal adhesion kinase (FAK). *Methods.* Marrow aspirates were obtained from 5 normal donors, 37 patients with Acute Myeloid Leukemia (AML) and 9 patients with Acute Lymphoblast Leukemia (ALL). The National Ethical Committee Board approved the study and informed-written consent was obtained from all patients and donors. Total cells were submitted to RNA extraction and the expression level of mRNA was detected by real time RT-PCR. The relative quantification value of gene expression was calculated using 2'DDCT. Leukemia cell lines (Jurkat, Molt-4, K562, HL60) and normal peripheral blood mononuclear cells (PBMC) obtained from 5 normal donors were submitted to immunoprecipitation and Western blotting analysis to detected protein expression and protein interactions. Pull down assays using three different FAK-GST fusion proteins: C-terminal (residues 687-1054), FERM domain (residues 60-349) and catalytic domain (residues 390-696) were performed to confirm the interaction between ARHGAP21 and FAK. *Results.* ARHGAP21 mRNA expression was found to be significantly higher in AML and ALL samples when compared with normal hematopoietic cells (medians: AML: 3.01 vs 0.25, $p<0.0001$; ALL: 4.47 vs 0.25, $p=0.0010$; Mann-Whitney test). Western blotting analysis showed higher ARHGAP21 expression in acute leukemia cell lines in comparison to normal PBMC. Immunoprecipitation and Western blotting assays using anti-ARHGAP21 and anti-FAK antibodies detected the interaction between ARHGAP21 and FAK in protein extracts of Jurkat cells and normal PBMC. Pull down assays, using three different FAK-GST fusion proteins, showed that ARHGAP21 is associated with the C-terminal region of FAK in protein cell lysates of HL-60 and normal PBMC. *Conclusions.* In conclusion, our data show that ARHGAP21 is overexpressed in AML and ALL cells and is associated with FAK in leukemia cell lines and in normal PBMC. These findings give rise to the hypothesis that ARHGAP21 may be involved in leukemogenesis, aiming this gene as a candidate for anti-tumor therapy.

0480

THE FOUR AND A HALF LIM DOMAIN PROTEIN 2 (FHL2) INTERACTS WITH CALM AND IS HIGHLY EXPRESSED IN AML WITH COMPLEX ABERRANT KARYOTPES

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Background. The balanced chromosomal translocation t(10;11)(p13;q14) results in the CALM/AF10 fusion gene. This translocation is found in acute myeloid leukemia (AML), T-cell acute lymphoblastic leukaemia (T-ALL) and malignant lymphoma. The CALM/AF10 fusion gene has recently been shown to cause aggressive biphenotypic leukemia in a murine bone marrow transplant model. The CALM (Clathrin Assembly Lymphoid Myeloid leukemia gene) gene product is a clathrin assembly protein which plays a role in clathrin mediated endocytosis and trans Golgi network trafficking. AF10 is a putative transcription factor most likely involved in processes related to chromatin organization and has polycomb group gene like properties. Known FHL2 interactors are BRCA1, PLZF (promyelocytic leukemia zinc finger protein), SKI1, β-catenin and Runx2. Deficiency of FHL2 causes decreased osteoblast activity. High expression of FHL2 in breast cancer has recently been shown to be associated with an adverse prognosis. *Aims.* The CALM/AF10 translocation is associated with a variety of acute lymphoid and myeloid leukemias. To learn more about the CALM/AF10 translocation we searched for protein interaction partners of CALM. *Methods and Results.* In a yeast two hybrid screen the four and a half LIM domain protein (FHL2) was identified as putative CALM interacting partner. The CALM-FHL2 interaction was confirmed by co-transformation assay in yeast, GST-pulldown and co-immunoprecipitation. In co-localization studies with transiently expressed fluorescent protein tagged CALM and FHL2, both proteins showed cytoplasmatic localization. Expression analysis (Affymetrix based) in different AML subtypes showed a significantly higher expression of FHL2 in AML with complex aberrant karyotypes compared to AML with normal karyotypes or balanced chromosomal translocations like the t(8;21), inv(16) or t(15;17). Reporter assay is being performed to test transcriptional activities of FHL2 on CALM. *Summary.* The observation that FHL2 has been shown to modify the transcriptional properties of other proteins suggests that FHL2 might regulate the nuclear function of CALM and it is thus conceivable that FHL2 is playing role in CALM/AF10-mediated leukemogenesis by tethering the CALM/AF10 fusion protein to various nuclear transcription factor complexes.

0481

SULFASALAZINE SENSITIZES ACUTE MYELOID LEUKEMIC THP-1 CELLS TO CYTARABINE

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Introduction. In spite of the advances made in the last decades, the prognosis of primary and relapsed pediatric acute myeloid leukemia (AML) is still poor, 60% and 40%, respectively. Resistance to chemotherapy including cytarabine (ara-C), etoposide and daunorubicin is partially responsible for this poor response. Ara-C is the most effective agent for the treatment of AML. Aberrant expression of enzymes involved in the transport/metabolism of ara-C including down-regulation of human equilibrative nucleoside transporter (hENT1) and deoxycytidine kinase (dCK) and upregulation of cytidine deaminase (CDA) is correlated with ara-C resistance. It has been suggested that activation of the nuclear factor κ-B (NF-κB) pathway is also associated with ara-C resistance by inhibiting apoptosis and stimulating cell proliferation. *Aims.* The aim of our study was to investigate whether inhibition of NF-κB by sulfasalazine (SSZ), an anti-inflammatory drug and inhibitor NF-κB, could increase the sensitivity of AML cell lines to chemotherapy. *Methods.* Two human myeloid leukemia cell lines AML cell lines, one being relatively resistant to ara-C (THP-1 cells from relapse AML patient) and one being ara-C sensitive (U937 cells) were chronically exposed to SSZ for 1-2 months. Subsequently, the drug sensitivity was determined by MTT assay, mRNA expression profiles were analyzed by microarray analysis and protein expression was measured by Western blot analysis. *Results.* U937 cells showed marginally altered changes in sensitivity to ara-C, etoposide and daunorubicin after prolonged exposure to SSZ. In contrast, SSZ-exposed THP-1 cells showed a 200 fold increased sensitivity to ara-C (see Figure 1A) and nearly unchanged etoposide and daunorubicin sensitivity.

Figure 1. A) Ara-C sensitivity measured by MTT comparing wild type (wt) cells with SSZ exposed cells. (B) Western blot analysis of these cells showing deoxycytidine kinase (dCK) and β-actin (β-act) protein expression.

Subsequently microarray analysis revealed a slightly decreased hENT1, dCK and CDA mRNA expression in SSZ-exposed U937 cells while SSZ-exposed THP-1 cells showed an increased hENT1 and dCK

expression along with a decreased CDA expression. For dCK, these results were confirmed at protein level by Western blot analysis (see Figure 1B), for the other parameters this remains to be established. Finally, unbalanced protein levels of NF-κB p65 and IkB (markedly increased) relative to NF-κB p50 (unchanges) of the NF-κB complex were indicative for an inactive state of the NF-κB pathway. *Conclusions.* These data suggest that chronic SSZ exposure decreases the NF-κB activity, increases the proteins involved in ara-C transport/metabolism and thereby increasing the ara-C sensitivity. Whether the dCK upregulation is caused by the NF-κB inhibitory effect of SSZ or by other mechanisms of SSZ is yet to be elucidated. In general, these data indicate that treatment of AML might be improved by combination with the NF-κB inhibitor SSZ, especially in relapsed AML.

0482

ABROGATION OF THE DNA-DAMAGE RESPONSE IN AML VERSUS MDS CELL LINES

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Introduction. The increasing evidence that activation of the DNA-damage-response (DDR) pathway in preneoplastic lesions of solid neoplasms hinders tumorigenesis prompted us to evaluate the same hypothesis in cell line models representing different subtypes of MDS and AML. *Methods.* Myeloid cell lines corresponding to prognostically different subgroups of MDS/AML (P39, MOLM-13, MV4-11, and KG-1) were assessed for their capacity to undergo a DDR. Using the quintessential inducer of a DDR, the cell lines were gamma-irradiated (2, 5 and 10Gy) and their ability to arrest the cell cycle in G2/M and/or to undergo apoptosis were assessed by FACS-measurement. Concomittantly changes in the expression levels of crucial proteins conferring the DNA-damage response were evaluated by immunoblotting. *Results.* Thus we show, that - in contrast to the *de novo* AML-derived KG-1 cells - the irradiated MDS/AML-derived cell lines P39 and MOLM-13 exhibit an earlier and more pronounced arrest of the cell cycle in G2/M, as well as a higher percentage of apoptotic cells. Assessing the underlying molecular determinants we demonstrate that irradiated P39 and MOLM-13 cells retain the ability to activate crucial mediators of the DDR, whereas this ability is decreased in the AML cell line KG-1. In contrast to KG-1 cells, irradiated P39 and MOLM-13 cells exhibit an increased upregulation of the crucial cell cycle/checkpoint regulators Chk1-P-Ser317 and Cdk1-P-Tyr15. To investigate the functionality of the DDR-pathway, cell lines were γ-irradiated in the presence of the ATM-inhibitor KU-55933 and the Chk-1 inhibitor UCN-01. Whereas inhibition of Chk1 (and to a lesser extent inhibition of ATM) is able to hinder G2/M-progression in irradiated MDS cells, it remained without effect in the AML cell lines KG-1 and MV4-11. Noteworthy, whereas irradiated P39/MDS cells hindered to activate Chk1 decrease expression of Plk-1 - which not only governs mitotic entry and exit, but also functions as an oncogene - KG-1 cells maintained a high expression, comparable to that observed in non-irradiated cells. *Conclusions.* We demonstrate the different capacities of MDS and AML cell lines to activate the DDR and thus provide evidence, that the DDR-pathway is abrogated in AML as compared to MDS cells.

Acute myeloid leukemia - Clinical II

0483

TREATMENT FOR ELDERLY PATIENTS (>70 YEARS) WITH *DE NOVO* ACUTE MYELOBLASTIC LEUKEMIA (AML). STUDY OF EFFICACY AND OUTPATIENT FEASIBILITY

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Background. The results of therapy for elderly patients with AML is dismal. Several intermediate dose cytarabine schedules have been introduced in an attempt to reduce toxicity. *Aims.* To evaluate the outpatient feasibility, tolerability and effectiveness in terms of rate of complete remissions (CR), overall survival (OS) and leukemia-free survival (LFS) of an oral (fludarabine and idarubicin) and subcutaneous (cytarabine and filgastrim) combined treatment in elderly patients (>70 years) with *de novo* AML. *Methods and patients.* Elligible patients: 70 years of age or older with a newly diagnosed AML, ECOG 0-2 and able to attend weekly hospital visits on an outpatient basis at baseline. Treatment schemes: FAG: fludarabine 25 mg/m^2 po days 2 to 5, cytarabine 200 mg/m^2 sc days 2 to 8, filgastrim 300 mcg sc days 1 to 7. IAG: idarubicin 20 mg/m^2 po days 2 to 4,cytarabine 200 mg/m^2 sc days 2 to 8, filgastrim 300 mcg sc days 1 to 7. Schedule: FAG induction followed by FAG and IAG consolidation in case of CR achievement. In case of partial remission (PR), FAG induction was repeated followed by the same consolidations in case of CR. If disease proved resistant after 1 or 2 FAG inductions, 1 to 2 inductions with IAG were attempted, followed by 2 IAG consolidations in case of CR. *Results.* From april 2004 to december 2007, 26 patients have been included (target 30 patients). Median age 74 (range 70-77) years. 8 (50%) males. AML characteristics: poor risk cytogenetics 4 (25%), trilineage dysplasia 5 (31%), FLT3 internal tandem duplication 2 (12%). Outcome: induction related deaths 4 (25%), CR after 1 (6) or further (1) induction cycles 44%. Consolidation related deaths 1 (6%). Patients completing the scheduled treatment 5 (31%). After a median follow-up of 7 months 1-yr (95%CI) OS and LFS probabilities are 24% (0-50) and 51% (12-90). Toxicity: The median duration of thrombocytopenia (<20×10^9/L) and neutropenia (<1×10^9/L) was 2 weeks (range 1-5) per cycle. Fever and infections grade 3-4 appeared in 70% and 27% of cycles respectively. Other toxicities were mild. Thirty-two percent of cycles could be managed in a completely outpatient basis and median hospital stay per cycle was 3.5 days (range 0-21). *Conclusions.* Selected elderly patients with AML could be managed on an outpatient basis with the proposed protocol. Although hospital admission was necessary in two thirds of the cycles, mainly due to infections, hospital stay was considerably shortened compared with standard dose cytarabine schedules while effectiveness is not importantly hampered by the cytarabine dose reduction.

0484

MARROW OSTEOPONTIN LEVEL AS A PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA

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Background. Osteopontin (OPN) is a glyco-phosphoprotein expressed in osteoblasts, osteoclasts, vascular smooth muscle cells, nerve cells, endothelial cells, epithelial cells, and activated immune cells. Secreted form of OPN is widely distributed in the body fluid. It could function in cell adhesion, chemotaxis, angiogenesis, apoptosis prevention, and tumor cell growth. OPN expression has been detected in many solid tumors, and its overexpression in tumor tissues has been shown to be associated with poor prognosis. Plasma or serum OPN levels were higher in patients with lung, breast, prostate, liver, and ovarian cancer than in normal controls. In many tumors, OPN blood levels were suggested as a prognostic or diagnostic marker. *Aims.* Previous studies in hematological malignancies demonstrated that OPN was overexpressed in chronic myeloid leukemia (CML) and multiple myeloma (MM). OPN concentration increased significant in CML and MM patients, as compared to normal controls. In the present study, we investigated whether marrow OPN level could be a diagnostic and/or a prognostic factor in acute myeloid leukemia (AML) patients. *Methods.* We determined the marrow OPN level in 52 AML patients before chemotherapy and 20

healthy controls at National Taiwan University Hospital (Taipei, Taiwan). All the molecules were quantified using ELISA. Concentrations in the AML patient and healthy control groups were compared using Student's t test. The receiver operating characteristic (ROC) curve was constructed, and the area under the ROC curve (AUROC) was calculated. Survival status was investigated by using the Kaplan-Meier survival curve and log-rank test. Multivariate Cox regression analyses were also used to estimate prognosis. *Results.* The OPN level was significantly higher (p<0.001) in the AML patients than in the healthy controls, while mean value was 19.46 ng/mL (range: 1.46-83.55 ng/mL) in patient group and 4.25 ng/mL (0.72-37.60 ng/mL) in control group. The ROC curve was illustrated to differentiate AML patients from controls. With a cut-off value of 4.3 ng/mL, OPN displayed a good result (AUROC = 0.896, 95% confidence interval, 0.802-0.956, p<0.001). Of the 52 patients, 31 patients undergoing conventional induction chemotherapy were further subjected to survival analysis. Patients with lower OPN (< 26 ng/mL) displayed a longer survival time than those with higher levels in one-year analysis (p=0.006, 12.0 vs. 2.5 months). In addition, it also demonstrated a borderline significant in five-year analysis (p=0.067, 22.1 vs. 2.5 months). Multivariate Cox regression models revealed that patients with higher OPN level displayed poor prognosis, with a hazard ratio of 4.96 (95% CI, 1.40-17.57, p=0.014) in one-year analysis and 2.99 (95% CI, 1.06-8.44, p=0.040) in five-year analysis. *Summary and conclusions.* In the present report, we demonstrated marrow OPN level in AML patients was not only higher than healthy controls, but patients with high OPN level also had significantly poor prognosis in one-year and five-year periods. As a conclusion, marrow OPN level is closely associated with the clinical outcome of AML patients and may be valuable in disease prognosis.

0485

CP-4055 IN PATIENTS WITH HAEMATOLOGIC MALIGNANCIES - A PHASE I STUDY

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Background. CP-4055 (cytarabine 5'-elaidic acid ester) is a novel cytotoxic nucleoside analogue. Cytarabine is the backbone of therapy in leukaemia. CP-4055 has similar mechanism of action to cytarabine but, unlike cytarabine, it is independent of nucleoside transporters for cellular uptake. CP-4055 is currently in Phase II in solid tumours and the recommended dose is 200 mg/m^2/day in a day 1-5 q4 weeks (1-5 q4w) schedule. Myelosuppression was the main dose limiting toxicity (DLT). *Aims.* Determination of the maximum tolerated dose and the preferred infusion time in patients (pts) with haematologic malignancies. *Methods.* Pts received IV CP-4055 over 2 hours (Arm A) or 24 hours (CIV, Arm B) in a day 1-5 q3w schedule. The starting dose in Arm A was 300 mg/m^2/day and in Arm B 200 mg/m^2/day, with standard definitions of DLT for haematologic malignancies. Dosing increments were by 50% (or by 30% in case of grade 2 non-haematologic toxicities). *Results.* In the ongoing study, 22 pts [14 male, median age 61.5 yrs (range 34-77); ECOG PS 0-1: 18 pts, ECOG PS 2: 4 pts; AML 18 pts, MDS 1 pt; ALL 2 pts, CML-BP 1pt, mean 2 lines prior chemotherapy (range 0-7)] have been treated at 2 US and 1 European centres. Seven pts are ongoing, 6 pts withdrawn with minimal response and 9 pts discontinued due to progressive disease. No treatment-related serious adverse events (SAE) have been observed. No DLT has been observed and dose escalation is ongoing at doses 875 mg/m^2/day in Arm A and at 675 mg/m^2/day in Arm B. Safety: Nausea and vomiting were the most common possibly related AEs. One patient experienced rash possibly related to study drug. Efficacy: Two pts (both AML) with stable disease/mild myelosuppression received a third cycle with CP-4055. One of these pts was referred to stem cell transplantation. Twelve pts received 2 cycles of therapy of whom 4 pts are ongoing. *Summary and Conclusions.* Patients with haematologic malignancies tolerated CP-4055 well up to a dose of 875 mg/m^2/day in a d1-5 q3w schedule (2 hrs infusion). Myelosuppression has been observed with minimal toxicity. Accrual is ongoing.

0486

OPTIMAL POST-REMISSION THERAPY FOR FLOW-CYTOMETRY MINIMAL RESIDUAL DISEASE POSITIVE PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Autologous (AuSCT) and allogeneic stem cell transplantation (SCT) 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. *Aims.* Minimal residual disease (MRD) may be a useful tool to stratify AML patients into categories of risk which can benefit from differentiated post-remissional approaches. To address this issue, we analysed retrospectively, a series of patients affected with AML in whom flow-cytometry serial determinations of MRD were available, at established time-points (post-induction, post-consolidation, post-stem cell transplantation). *Methods.* 123 patients were entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61 yrs) or AML13 (age>61 yrs), all consisting in intensive induction and consolidation cycles, and, for patients aged <61 years, AuSCT or SCT. Median age was 52 years (range 18-78), no APL cases were included in the study. The *Maximally Selected Rank Statistic* (MSLRS) 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 test was specifically developed to find out the optimal cut-off for a given biological variable correlating with clinical parameters of interest. *Results.* The MSLRS indicated 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 (64% vs. 14%, p<.001) and RFS (68% vs. 13%, p<.001). Therefore, among these 123 patients, we enucleated 2 groups of patients which underwent stem cell transplant procedure, 53 AuSCT and 11 SCT. The two subgroups were balanced in terms of FAB categories, WBC count, karyotype and expression of MDR-1 phenotype; 34/64 (53%) were MRD$^+$ (9 SCT, 25 AuSCT) and 30/64 (47%) were MRD$^-$ (1 SCT, 28 AuSCT). Among the 53 patients submitted to AuSCT, MRD$^-$ had a significant better outcome than those MRD$^+$, both in terms of 5-years OS (68% vs. 24%, p=0.003) and RFS (68% vs. 11%, p<.001). In the SCT group the low numbers hampered any firm conclusion; however, when the category of post-consolidation MRD$^+$ patients was separately analysed, the use of SCT was associated with a better RFS (40% vs. 12%, p=NS), (Figure 1); we assume that the lack of statistical significance was merely due to the few cases in the SCT group. *Conclusions.* 1) the threshold of 3.5×10^{-4} at post-consolidation check-point is critical to predict disease 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 SCT is associated with a superior outcome, although a larger number of patients is required to confirm this assumption.

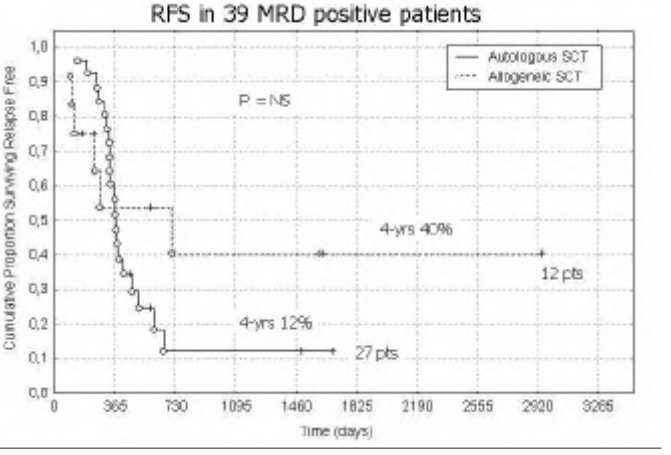

Figure 1. Duration of RFS according to type of SCT received.

0487

MUTATIONAL STATUS OF NUCLEOPHOSMIN (NPM1) AND FLT-3 GENES ARE STRONG PREDICTORS OF THE OUTCOME IN PATIENTS WITH CYTOGENETIC INTERMEDIATE-RISK ACUTE MYELOID LEUKEMIA UNDERGOING AUTOLOGOUS STEM-CELL TRANSPLANTATION

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The modality of post-remission therapy in AML is mainly based on cytogenetics. However, there are several cytogenetic categories, such as the intermediate-risk subgroup, that include patients with a highly heterogeneous prognosis. In this subset of patients, molecular markers such as mutations of NPM1 and flt-3 genes might be useful to determine prognosis more precisely. We analyzed the predictive value of these molecular markers in patients with AML receiving autologous stem-cell transplantation (autoSCT). Thirty-seven patients (age: 53, 15-66; 51% female) diagnosed with AML of intermediate-risk cytogenetics (normal karyotype, n=23) and submitted to autoSCT in first complete remission (CR1) during 1995-2006 were included in the analysis. Pre-transplant therapy was similar in all patients, consisting of one (n=32) or two cycles (n=5) of standard induction chemotherapy (ICE, n=8, IDICE, n=29) and one cycle of high-dose ara-C-based consolidation chemotherapy, according to three sequential trials (CETLAM protocols LMA-94, LMA-99, and LMA-2003). Conditioning regimen contained TBI in most cases (86%) and the stem-cell source was peripheral blood in all patients. Internal tandem duplication of flt-3 (flt-3 ITD) and exon 12 NPM1 mutations were studied in diagnostic samples by either PCR or RT-PCR following standard methods and visualized by means of Genescan analysis. After a median follow-up of 66 months (6-122), 12 patients relapsed after autoSCT, this resulting in a 5-year OS and LFS of 56±9% and 50±9%, respectively. According to molecularly-defined risk, three different subgroups of patients were considered: group 1 (NPMmut: n=12, 32%), constituted by patients with mutated NPM1 without flt-3 ITD; group 2 (NPM-neg: n=20, 54%), which included patients without NPM1 or flt-3 ITD; and group 3 (flt-3 ITD: n=5, 13%), defined by flt-3 ITD regardless NPM1 mutational status. The only variables with prognostic value for survival after autoSCT were flt-3 ITD, which conferred an adverse prognosis (5-yr OS: 62%±10% vs. 20%±18%, p=0.028), and molecular NPM1/flt3 category (5-yr OS: 90%±10% [group 1] vs. 48%±13% [group 2] vs. 20%±18% [group 3], p=0.02; see Figure 1). In conclusion, autoSCT is an effective post-remission strategy in patients with NPM1-mutated AML, whereas flt-3 ITD identifies a high-risk population who does not benefit from this procedure. For the subgroup of patients lacking NPM1 and flt-3 mutations, search of other molecular markers adding prognostic information is warranted in order to assess the role of different post-remission options.

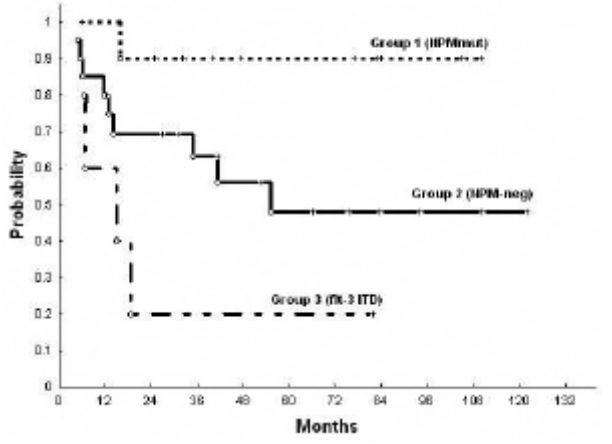

Figure 1.

0488

ANALYSIS OF THE ASSOCIATION OF ICAM-1 AND PECAM-1 POLYMORPHISMS AND THE DEVELOPMENT OF DIFFERENTIATION SYNDROME IN ACUTE PROMYELOCYTIC LEUKEMIA

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The use of all trans-retinoic acid (ATRA) is the base of treatment of Acute Promyelocytic Leukemia (APL) and constitutes the paradigm of differentiation therapy. In general, ATRA is well tolerated, but may be associated with a potentially lethal side effect, referred to as Retinoic Acid or Differentiation Syndrome (DS), which is characterized by fever, pleural and pericardial effusions, respiratory distress, weight gain and pulmonary infiltrates on chest radiography. The cellular and molecular mechanisms of DS are poorly understood, and involve changes in the adhesive qualities and cytokine secretion of leukemic cells during ATRA-induced differentiation. Since leukocyte extravasation is a key event in DS pathogenesis, we analyzed the association between the polymorphisms at exon 6 of ICAM-1 (K469E) and exon 3 of PECAM-1 (L125V) genes and DS development in APL patients treated with ATRA and anthracyclines. Bone marrow (BM) or peripheral blood (PB) specimens from 127 patients with APL were collected at diagnosis. PB samples from 248 healthy volunteers were used as controls. The genetic confirmation of the PML/RARα rearrangement was done by RT-PCR using the BIOMED-1 protocol. DS was diagnosed in 23/127 (18.1%) APL patients, at an average of 11.5 days after the start of ATRA (between the 4th and 20th days). We did not demonstrate any difference between the group of leukemic patients with and without DS regarding age, gender, white blood cells counts, hemoglobin value or platelets counts. All patients who developed DS presented respiratory distress associated with increased ground-glass opacity in chest radiographies and, other accompanying symptoms were: fever not attributable to infection (65.2%), generalized edema (37.5%), weight gain (37.5%) and impairment of renal function (8.6%). The genotype frequencies of ICAM-1 were GG 33.0%, GA 50.8% and AA 16.2% in APL patients and GG 8.0%, GA 54.6% and AA 37.4% in control group. For PECAM-1, these frequencies were CC 10.2%, CG 60.2% and GG 29.2% in APL patients and CC 22.3%, CG 57.0% and GG 20.7% in control group. These genotype frequencies of both groups did not deviate significantly from the predicted frequency using Hardy-Weinberg equilibrium. APL patients presenting the AA genotype at exon 6 of ICAM-1 had a significantly increased risk of developing DS with a relative risk of 2.6 (95% confidence interval: 1.2-5.6). The AA genotype frequency in APL patients with and without DS was, respectively, 33% and 12.6% (p=0.04). On the other hand, no significant association was detected between L125V polymorphism at exon 3 of PECAM-1 and DS development. Our results suggest that susceptibility to DS in APL patients may be influenced by genetic variation in adhesion molecules loci.

0489

PHARMACOKINETIC/PHARMACODYNAMIC CORRELATION WITH CLINICAL RESPONSES IN A PHASE 1 STUDY OF PATIENTS WITH RELAPSED/REFRACTORY ACUTE LEUKEMIAS TREATED WITH SNS 595

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Background. SNS-595, a novel cell-cycle active compound that disrupts DNA replication forks and triggers DNA damage responses, G2 arrest, and apoptosis, is in phase 1 and 2 clinical trials in hematologic and solid malignancies. Preliminary clinical, pharmacokinetic (PK) and correlative results from an on-going escalating-dose phase 1 trial of SNS-595 in refractory acute leukemias are presented. *Aims.* Objectives: 1) establish safety, tolerability and MTD of SNS-595 given on 2 schedules; 2) characterize PK; 3) assess clinical activity; 4) explore potential biomarkers of mechanism-based activity and patient stratification. *Methods.* SNS-595 IV dosing: Arm A: days 1, 8 and 15; Arm B: days 1, 4, 8 and 11; minimum cycle lengths were 42 days and 39 days, respectively. Starting doses were18 mg/m^2 (Arm A) and 9 mg/m^2 (Arm B), with escalation by cohort. PK samples and pretreatment blood and bone marrow aspirates were collected from all patients for exploratory analyses

of the level of DNA damage repair proteins DNA-PKcs, Ku70 and Ku80. Post-dose blood and bone marrow aspirate samples were obtained from a subset of patients to determine sensitivity to SNS-595 *ex vivo* as well as evidence for DNA damage, apoptosis, and cell-cycle response. H2AX phosphorylation, PARP cleavage, and G2 arrest were evaluated. *Results.* 21 patients (Arm A) and 14 patients (Arm B) were evaluable. All patients were consented and had refractory and/or relapsed disease (median 3 prior regimens (range 1-6)). Accrual and dose-escalation have continued at 60 mg/m^2 for Arm A and 30 mg/m^2 for Arm B. Diagnoses: AML 32 patients, ALL 3 patients. One dose-limiting toxicity, prolonged neutropenia, was observed. Non-dose limiting adverse events included nausea/vomiting, diarrhea, and mucositis; grade 4 neutropenic fever was observed in only 1 patient. SNS-595 PK were dose proportional (dose range characterized: 9-50 mg/m^2) and volume of distribution and clearance values were unchanged across the dose range. Evidence of DNA damage was observed in patients treated with <25 mg/m^2 SNS-595. Increased H2AX phosphorylation was observed 2-4 hours post-dose. H2AX phosphorylation was unaffected at 18 mg/m^2 (Arm A). Estimated EC90 levels (10-fold the for cytotoxicity in leukemic cell lines) of SNS-595 were present on average for at least 4 hours for the 25 and 50 mg/m^2 cohorts, but for less than 2 hours at the 18 mg/m^2 dose level. Patient bone marrow samples were sensitive *ex vivo* to SNS-595 induced cytotoxicity. Clinical responses were observed at 50 mg/m^2, including one patient with CRp. At this dose level, four out of six patients with relapsed and/or refractory AML had decreases in marrow blasts to <5%. Clinical activity was observed at a dose where EC90 concentrations were maintained on average for over 24 hr. *Summary and Conclusions*. SNS-595-induced DNA damage responses are observed in bone marrow at doses <25 mg/m^2. In patients with advanced leukemia, SNS-595 demonstrates promising clinical activity, including significant blast reductions (<5%) and a CRp at 50 mg/m^2, a dose level where EC90 concentrations of SNS-595 were maintained over 24 hr. An MTD has not yet been reached in either schedule.

0490

HIGH RELAPSE RATE OF ACUTE MYELOID LEUKEMIA WITH TRANSLOCATION (8;21) OR INVERSION (16) IN ELDERLY PATIENTS TREATED WITH CONVENTIONAL CHEMOTHERAPY

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Background. AML with rearrangements of CBF α or β (CBF-AML) are associated with a good prognosis when treated with intensive chemotherapy. Such treatments cannot be offered to elderly patients and previous reports (Schoch et al, Haematologica 2004) showed that aging was an independent adverse prognosis factor in CBF-AML patients. Nevertheless only few specific data are available on the characteristics and outcome of CBF-AML in the elderly. *Aims.* To describe the clinical characteristics of CBF-AML in the elderly and to analyze their outcome. *Methods.* A retrospective analysis was performed on 27 patients with t(8;21) or inv(16) older than 60 years who received conventional induction chemotherapy in our institution. CBF leukemia was confirmed by cytogenetics and/or transcript specific PCR. A predefined set of data was collected including medical history, disease related variables and treatments. A comorbidity index was calculated for each patient as described by Sorror et al (HCT-CI, Blood 2005). *Results.* Median age was 68 years (60-76). Eight patients (30%) were older than 70 years. Seven patients had t(8;21), 20 patients had inv(16) and 9 patients (33%) presented additional chromosomal abnormalities. 24 patients achieved CR after 1 or 2 induction courses (89% CR rate). There were 3 early deaths (11%) and 7 patients were referred to ICU (3/7 had a high HCT-CI). Post-remission therapy consisted of low dose maintenance chemotherapy (14 patients) or intensive consolidation (high dose cytarabine: 10 patients; HD Melphalan with autologous SCT: 4 patients). With a median follow-up of 14 months, 16 patients have relapsed (3-year cumulative incidence of relapse: 82%). Overall Survival and Leukemia Free Survival were respectively 22 and 18 months. There was a trend to a better OS for patients without initial high WBC count (p=0.1). No impact of other initial demographic or biological variables, post induction regimen or molecular minimal residual disease monitoring could be put in evidence. *Conclusions.* Elderly patients with CBF-AML must be offered standard induction which leads to high CR rate. Nevertheless, the majority of them relapse with conventional post-remission treatment and the administration of one cycle of HDAC did not seem to improve prognosis. Alternative strategies of post-remission therapy are thus warranted. New cytotoxic drugs as well as targeted molecules need to be investigated.

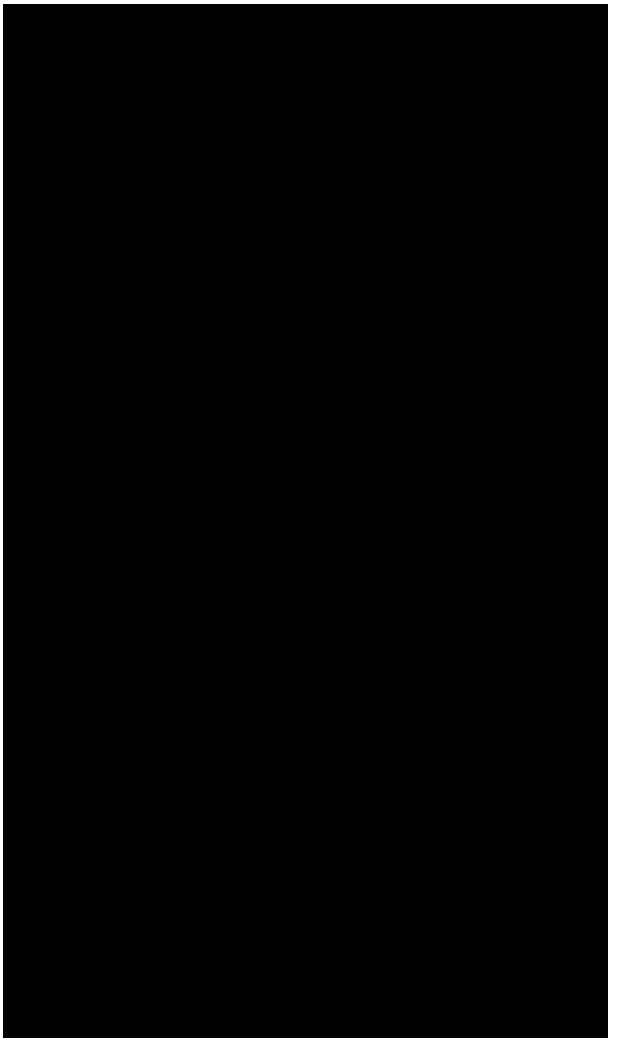

Figure 1. Overall survival and incidence of relapse.

0491

IMPROVED SURVIVAL IN ELDERLY ACUTE MYELOID LEUKAEMIA PATIENTS GIVEN CLADRIBINE IN COMBINATION WITH STANDARD REMISSION INDUCTION (DA 3+7) AND CONSOLIDATION TREATMENT (HD ARAC). SEVEN YEAR FOLLOW-UP OF PROSPECTIVE, COOPERATIVE PALG STUDY

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The aim of this study was to evaluate long-term outcome of acute myeloid leukemia (AML) patients treated within the PALG 1999 DAC vs. DA Study. Within 3 years (1999-2002) 400 patients, aged 18-60, were randomized 1:1 to the induction treatment DAC-7: daunorubicin 60 mg/m^2/d iv 1-3; cytarabine 200 mg/m^2/d ci d 1-7; cladribine (2-CdA) 5 mg/m^2 2h inf. iv d 1-5 and standard DA-7 regimen (the same therapy excluding cladribine). Patients achieving CR received two courses of subsequent intensive consolidation: 1) HAM (HD AraC, mitoxantrone) 2) HD AraC with or without cladribine in the DAC-7 or DA-7 arm, respectively. In case of PR after the first induction course the same regimen was repeated, Post-consolidation therapy was comparable in both arms with following proportions of autoHCT, alloHCT and maintenance: DAC-7 17%, 14%, 69%; DA-7 21%, 14%, 65%, respectively. As

previously reported, a single course of DAC-7 induction resulted in 17% higher CR rate compared to the DA-7 treatment (p=0,0008). The difference was particularly pronounced in patients: aged >40 years and with initial WBC >100×10^9/L. In the latter subgroup also the overall CR rate (achieved after entire induction program) was higher in the DAC-7 arm (71% vs. 43%). [Leukemia 2004;18:989-97] In the present report we analyzed seven-year long-term outcome (median follow-up 5 yrs) in the whole study population and in subgroups stratified according to age, initial WBC, cytogenetics, sex, FAB subtype, and preceding myelodysplasia. In the whole group the overall survival (OS) rate equaled 29,5% for DAC-7 and 24% for DA-7 arm (p=NS) and leukemia free survivall (LFS) 30% vs. 28% (p=NS), respectively. Of note, in patients aged >40 years, the therapy containing cladribine was associated with improved OS (26% vs. 14,5%, p=0.03), and LFS (28% for DAC-7 vs. 18,5% for DA-7, p=0.02). Other subgroup analyses revealed higher probability of the OS in patients with initial WBC ≤50 G/L assigned to DAC-7 compared to DA-7 arm (32% vs. 20,5%, p=0.04). The LFS rate equaled 35% and 27% (p=NS), respectively. In women receiving DAC-7 induction therapy in comparison with those treated in arm without 2-CdA reached higher OS: 29% vs. 19,5%, p=0,03, respectively. LFS in these subgroups was comparable: 25% vs. 22%, p=NS, respectively. We conclude that addition of cladribine to induction and consolidation therapy of AML improves long-term outcome in patients: older than 40 y, as well as in those with high tumour burden. The better outcome in older patients results mainly from reduced risk of relapse, whether that in cases with high WBC seems to be linked to a higher CR rate.

0492

INFANT LEUKEMIAS: TRANSIENT MYELOPROLIFERATIVE SYNDROMES IN CHILDREN WITH TRISOMY 21 MOSAIC

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Introduction. Children with Down syndrome have a 10- to 20-fold increased risk of leukemia and a 10%-incidence of transient myeloproliferative disorders (TMD). TMD may also occur in neonates without any stigmata of Down syndrome but with trisomy 21 mosaic. About 20-30% of DS-neonates with TMD developed myeloid leukemia of Down syndrome (ML-DS) during the first 4 years of life. TMD blasts are indistinguishable from ML-DS blasts in both morphology and immunophenotyping. In almost all patients with TMD or myeloid leukemia of Down syndrome mutations in exon2 or exon3 or the hematological transcription factor GATA1, leading to a truncated GATA1s, have been detected. *Materials and Methods.* We performed polymerase chain reaction (PCR) on exons 2 and 3 using primers that flanked each of the exons. DNA sequencing was done directly on purified PCR products. Each identified mutation was confirmed using sense and anti-sense primers. *Patients*. All three children were mature newborns without any obvious stigmata of Down syndrome. The clinical symptoms of leukemia occurred within the first weeks of life. Due to typical morphology and immunophenotype of the leukemic blasts (CD34/CD117/CD13/CD33/CD7/CD56/CD36/CD42b), a TMD was considered and no intensive anti-leukemic treatment initiated. Detection of GATA1 mutations in all children and the karyotyping of fibroblasts and hematopoietic cells, which revealed trisomy 21 mosaic, confirm the diagnosis of TMD. Due to clinical symptoms caused by the TMD, two children were treated with low-dose cytarabine (1 to 1.5 mg/KBW). All infants experienced complete remission within 6 weeks. During follow-up no evidence of relapse or development of a myeloid leukemia of Down syndrome was observed. *Results.* Table 1.

Table 1.

Patient	Sex/Age diagnosis	Symptoms	WBC/μL	Blasts (BM)	DNA source	Mutation	Consequence
P.M.	F/5 weeks	Leukemia cutis anemia, thrombocytopenia, hepatosplenomegaly	74,700	24%	blasts	IVS2+2 T>C	Splice mutant
K.V.	M/0	Pulmonal insufficiency, hepatosplenomegaly	130,000	13%	blasts	261 dup 156-261	Stop codon before Met84
A.S.	M/6 weeks	Anemia, thrombocytopenia, pericardiac effusion	40,600	65%	smears	328 del 12	Splice mutant

Conclusions. In newborns and infants, diagnosed with acute megakaryoblastic leukemia, the possibility of TMD and trisomy 21 mosaic should be considered. Analysis of the GATA1 mutation should be performed to prevent overtreatment. If clinical symptoms due to TMD occur, low-dose cytarabine is recommended. Due to the possible risk of developing myeloid leukemia, close follow-ups should be performed.

0493

CLAG-M IS HIGHLY EFFECTIVE SALVAGE REGIMEN IN PATIENTS WITH RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA. THE FINAL RESULTS OF MULTICENTER PHASE II STUDY OF THE POLISH ADULT LEUKEMIA GROUP (PALG)

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Objectives. Cladribine (2-CdA) in addition to cytosine-arabinoside (Ara-C) increases the accumulation of Ara-C-5' triphosphate which is responsible for the cytotoxic effect in leukemic blasts. Our preliminary results have suggested that addition of Mitoxantron (MIT) to 2-CdA, Ara-C and granulocyte colony-stimulating factor (G-CSF) CLAG-M improves the treatment results in refractory/relapsed patients with acute myeloid leukemia (AML). In this cooperative phase II study we evaluated the efficacy and toxicity of salvage regimen CLAG-M in refractory/relapsed AML patients with poor risk. *Materials and Methods.* The patients were qualified to high risk group if they represent: i) primary refractory AML, ii) AML in first relapse with duration of complete remission (CR) <6 months iii) second or greater relapse and iv) relapse after autologous or allogeneic stem cell transplantation (SCT). All patients were previously treated according to the Polish Adult Leukemia Group clinical research protocols. The patients received 2-CdA 5 mg/m^2 (days 1-5), Ara-C 2 g/m^2 (days 1-5), G-CSF (days 0-5) and MIT 10 mg/m^2 (days 1-3). In case of partial remission a second CLAG-M was administered. Patients with CR received consolidation courses based on high dose of Ara-C and MIT and allogeneic SCT if donor was available. *Results.* From November 2002 to November 2006, 112 patients (72 primary resistant and 40 relapsed) from 11 centers were registered. The median age was 43 years (range 20-66). CR was achieved in 63 (56%) patients 43 (38,5%) were refractory and 6 (5,5%) died early. Forty six out of primary resistant patients received daunorubicine (DNR) and Ara-C as the first line induction therapy (DA), 26 received additionally purine analogues: Cladribine (n=13; DAC) or Fludarabine (n=13; DAF). The CR rates after CLAG-M were 52%, 61% and 53% respectively (p=NS). Cytogenetic analysis was available in 89 patients. Two patients (2%) had a favourable karyoytype, 49 (55%) an intermediate, 26 (29%) an unfavourable and 12 (14%) were classified as unknown according to SWOG classification. The CR rate of 100%, 57% and 46% were achieved in favourable, intermediate and unfavourable cytogenetics respectively (p=NS). The only factors influencing the probability of achieving CR were WBC<30 G/L (66% vs. 41%; p<0.04) and LDH <500 U/l (65% vs. 47%; p<0,03). The hematological toxicity was the most prominent toxicity of this regimen. The overall survival (OS; 1 year) for the 112 patients as a whole and the 63 patients in CR were 42% and 62% respectively. In the univariate analysis WBC>30 G/L, lack of transplantation in CR and unfavourable cytogenetics were poor prognostic factors for OS (p<0.02; p<0,001 and p=0,051 respectively). In multivariate Cox regression model lack of transplantation in CR was the only factor influencing OS (p<0,01). *Conclusions.* CLAG-M is well tolerated and very effective salvage regimen in high risk refractory/relapsed AML. The toxicity is acceptable enabling most patients to receive futher treatment including transplantation procedures. SCT is the only factor influencing overall survival and should be treatment of choice.

0494

MONITORING OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA USING PERIPHERAL BLOOD AS AN ALTERNATIVE SOURCE TO BONE MARROW

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Background. Monitoring of minimal residual disease (MRD) by multiparametric flow cytometry (MPFC) can provide useful prognostic information in adult patients with acute myeloid leukemia (AML), when bone marrow (BM) is used. Recently, it has been reported that using

quantitative PCR, MRD is also detectable and measurable in peripheral blood (PB) of AML patients. *Aims.* Based on these premises, we assessed by MPFC the levels of MRD in PB and BM samples of 55 adult patients with AML to verify the feasibility of MRD detection in PB and its prognostic relevance. *Methods.* A total of 55 adult patients with AML were entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61 yrs) or AML13/AML15 (age>61 yrs), consisting in intensive induction and consolidation cycles, and, for patients aged <61 years, autologous or allogeneic stem cell transplantation. Median age was 47 years (range 21-73), all FAB subtypes were represented with the exception of M3 cases. We studied 55 and 53 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 5×10^{-3} (range 1×10^{-4}-1.64×10^{-1}) and 2.3×10^{-3} (range 1×10^{-5}-1.15×10^{-1}), respectively (r=0.84, p<0.001). After consolidation, the median value of BMRLC and PBRLC were 3.9x10-3 (range 2×10^{-5}-6.3×10^{-2}) and 3.4×10^{-3}(range 1×10^{-5}-1.34×10^{-1}), respectively (r=0.85, p<0.001). Using the maximally selected log rank statistics, we found that the cut-off of 1.5×10^{-4} PBRLC correlated with the disease outcome. In fact, 34 of 47 (72%) patients with PBRLC > 1.5×10^{-4} after induction had a relapse, whereas, the 8 patients with <1.5×10^{-4} did not (p<0.001). After consolidation, 42 patients had a level of MRD >1.5×10^{-4} and 32 (76%) had a relapse; 10 out of the remaining 11 patients, whose level of MRD was below 1.5×10^{-4}, 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- status after consolidation, whereas it was 11 months among those with a PB MRD$^+$ status 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). *Conclusions.* 1) MRD is detectable and measurable in PB of AML patients using MPFC; 2) MRD levels in PB are correlated to those measured in BM; therefore PB may be a complementary source for MRD studies in patients with AML; 3) PB MRD determination after consolidation therapy has a prognostic role; 4) combined BM and PB assessment might allow risk-category stratification to be improved, optimizing MRD monitoring in AML patients

0495

OUTCOME AND CHARACTERISTICS OF NEWLY DIAGNOSED PATIENTS WITH ACUTE MYELOID LEUKAEMIA. A COMPARISON OF MRC AML15 PATIENTS WITH ELIGIBLE NON-TRIAL PATIENTS IN A SINGLE CENTRE

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Background. St Bartholomew's Hospital is a major centre for the treatment of acute myeloid leukaemia (AML) and since June 2003 has been participating in the MRC AML15 trial. To date AML15 has recruited 2340 patients from 154 centres; St Bartholomew's is in the top ten centres for recruitment. Aims This analysis has been undertaken to determine what proportion of newly diagnosed patients who are eligible for the trial are actually entered, and to compare the presenting features and outcomes of those entered with those not. Results Since June 2003, 82 patients who were eligible for AML15 presented to our centre (age 17-60 years, median age 46.8 years, male:female ratio 1.3:1) Forty-nine patients (60%) were consented to the trial. The median age of trial patients was 50 years, median age non-trial patients 44.8 years. Twenty-nine (61%) of the male patients were randomised into the trial and 20 (57%) of the female patients. Sixty-five (79%) of the patients presenting were white Caucasian; 6 were African, 4 were African-Caribbean, 3 of South Asian origin and 4 were from other ethnic groups. Forty-one of 65 (63%) of the white Caucasians entered AML15, compared with 8/17 (47%) of the patients from the ethnic minorities. This trend was not statistically significant (p=<0.2). The trial and non-trial groups were matched for the cytogenetic risk status, except for patients with t(15;17). Fifteen of the eligible patients had t(15;17) at presentation, but only 6 (40%) were randomised into AML15. We also analysed the day of the week on which patients first presented to the hospital; only one of four patients who presented at the weekend (Saturday or Sunday), entered AML15, otherwise day of the week of presentation did not influence trial entry. Only 9/82 (11%) of patients were not invited to participate in the trial. One of these presented on the weekend; other reasons documented for not entering the trial included *too unwell to consent* (4 patients) cognitive impairment (3 patients), and communication difficulties (1 patient).Nineteen of the 73 patients invited to participate in the trial were not randomised. Fifteen (20%) patients declined.; 4 patients expressed uncertainty about consenting and 2 of these were judged to need urgent therapy and electively treated off trial, Two other patients who expressed uncertainty about randomisation had FAB type M3 and the physician elected therapy off trial. The reason for non-trial entry could not be established retrospectively in 5 patients. Survival of the cohort not entered into the trial is similar to the cohort that was entered. Conclusions A high proportion of eligible patients (89%) are invited to participate in AML15 at St Batholomew's hospital, and of these 60% enter the study. Recruitment is not affected by age, gender or ethnicity. There was a trend towards lower entry into the trial in patients with FAB type M3, due to physician bias. Outcome did not correlate with entry into the trial.

0496

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

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Infections are the major 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 the 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 + cytarabine + idarubicine + etoposide). During induction phase, 134/224 (60%) patients experienced a fever of undetermined origin (FUO), the incidence of Gram negative and positive sepsis was 14% (31/224) and 22% (49/224) respectively and 38/224 (17%) patients developed a possible/probable IFI. In 9/224 patients (4%) a proven IFI was found (6 Aspergillosis and 3 Candida). Taking into consideration the long lasting immunosuppressive effect of Fluda, we collected the data of the incidence of infections 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 Aspergillosis 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 infective complications in our series of AML patients favourably compares to the one reported by other Authors with induction chemotherapy not including Fluda.

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0497

SINGLE AGENT CLORETAZINE (VNP40101M) IN ELDERLY AML PATIENTS WITH UNFAVORABLE CYTOGENETICS: RESULTS FROM A PHASE II MULTI-CENTER STUDY

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Background. Outcomes for elderly patients with AML are poor, and it has been shown that elderly patients with unfavorable cytogenetics have a particularly poor prognosis. Additionally, a large number of these patients are considered unfit for intensive chemotherapy due to coexisting comorbidities, and are given best supportive care or low-dose AraC. However, no complete remissions are observed with this treatment and median overall survival is approximately 1 month (Burnett *et al.,* Cancer 2007). Patients with unfavorable cytogenetics who do receive cytotoxic induction treatment achieve complete response rates between 23-33% with very low 5-year survival rates (2-4%) (Grimwade et al Blood 2001, Rowe et al Blood 2004, Farag et al Blood 2006, Buechner et al JCO 2006). *Methods.* We present a subset analysis of patients with unfavorable cytogenetics in a Phase II study of Cloretazine® as a single agent (Vion Study CLI-033). Cloretazine® is a new alkylating agent that causes DNA cross-links and cell death. The study was designed for untreated patients with AML or high-risk MDS over the age of 60. Treatment consisted of Cloretazine® (VNP40101M) 600 mg/m^2 administered as a short IV infusion on day 1 (second cycle allowed) and an additional 400 mg/m^2 as consolidation for responders (CR or CRp). The primary endpoint was overall response (CR or CRp). Overall survival and relapse-free survival were also analyzed. *Results.* The study treated 128 patients of age 60 or older. Fifty-eight patients (45%) had unfavorable cytogenetics (-7, del5q, abnl11q, abnl9q, abnl20q, abnl13q, complex (≥ 3 abnormalities)). Of these 58 patients, 28 (48%) had secondary AML, 19 (33%) had *de novo* AML and 11 (19%) had high-risk MDS. Twenty-three (40%) patients in the unfavorable group had a complex karyotype. The response rate in patients with unfavorable cytogenetics was 26% (15/58). Response by diagnosis: 53% in *de novo* AML, 27% in MDS and 7% in secondary AML. Median overall survival in patients with unfavorable cytogenetics was 3 months (range 0.1-28). In patients achieving CR or CRp, median overall survival was 5 months (range 2-28) and relapse-free survival was 3.5 months (range 1-20). In non-responders, overall survival was 2 months (range 0.1-9). Twelve (21%) patients died within 30 days of receiving induction treatment. *Conclusions.* Cloretazine® has the ability to induce remissions in elderly AML patients with unfavorable cytogenetics. Survival in this group is improved compared to that reported for supportive care or low dose AraC. Cloretazine® may provide an important treatment option for poor prognosis patients with AML.

Autologous stem cell transplantation

0498

THE ROLE OF HIGH DOSE CHEMOTHERAPY AND PERIPHERAL BLOOD STEM CELL SUPPORT IN REFRACTORY TROPHOBLASTIC DISEASE AND NON-GESTATIONAL CHORIOCARCINOMA: A REVIEW OF EIGHT PATIENTS

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Objective. To evaluate the role of high dose therapy (HDT) with autologous peripheral blood stem cell support (ASCT) refractory advanced trophoblastic disease and non gestational choriocarcinoma. *Methods.* Eight patients (gestational trophoblastic neoplasia n =6, choriocarcinoma post complete hydatidiform mole n=1, possible non-gestational choriocarcinoma n=1 and germ cell tumour with choriocarcinoma differentiation n=1) were treated with HDT with ASCT. Median age was 33 years. All patients received a conditioning regimen of CarboPEC-Taxol (Paclitaxel 75 mg/m^2 on days -7, -5, and -3, Etoposide 450 mg/m^2 days -7, -5, and -3, Carboplatin 10 mg x glomerular filtration rate (GFR) +25 on days -7, -5,and -3, and Cyclophosphamide 60 mg/kg/day days -5, and -3). Four patients received this regimen as a tandem ASCT, receiving 50% dose of the conditioning regimen drugs with stem cell infusion twice, twelve weeks apart, to limit toxicity. All eight patients had multiply relapsed disease and had received more than two different prior treatments (median number of prior therapies was 3.5 range, 2-5) at time of ASCT. Two patients had cerebral metastases. At transplant four patients were in a partial remission (PR) and four had progressive metastatic disease. *Results.* The regimen was well tolerated. Seven patients showed an initial response immediately post ASCT, one patient demonstrated disease progression throughout. Five patients achieved a complete remission (CR) demonstrated by a reduction in serum tumour markers to normal (<4 IU/L), two patients achieved a good partial remission (PR) with a reduction of serum levels of β HCG to below 20 IU/L. Two patients died of transplant related causes (invasive fungal infection and gram negative neutropenic sepsis), a transplant related mortality (TRM) of 25%. All six surviving patients relapsed within three months (median DFS 0.99 months). Five patients died of progressive disease within fifteen months of transplant with a median overall survival of 8.97 months (range 7.29-15.21 months). Cerebral disease did not confer a worse outcome. One patient who relapsed immediately post-transplant with rising serum βHCG levels, remains alive at 41 months with undetectable βHCG levels despite no further treatment. *Conclusions.* Refractory trophoblastic disease has a poor outcome. High dose therapy using CarboPEC-Taxol with autologous stem cell rescue results in a short response, however, most patients experience early relapse. In young women this short period of remission may be of value, affording longer period of time spent with young families. It should be noted that those patients referred for HDT have been refractory to a number of chemotherapeutic regimens and had widespread disease. The question is whether an improved long-term survival might be seen with earlier high dose therapy and ASCT in CR2 or early progressive disease. Although this disease is not primarily of hematopoietic tissue, allogeneic transplant could be considered in this young age group, with a theoretical advantage of a graft versus tumour effect. We can offer no explanation for the patient who relapsed post-transplant entering a sustained remission, other than the development of an immunological anti-tumour response. If this were the case, then allogeneic transplant with donor lymphocyte infusion could have some role in the treatment of this aggressive disease. More information on the role of transplantation in trophoblastic disease is thus required before any conclusions can be drawn and treatment strategies considered.

0499

ENDOTHELIAL CELL MARKERS KINETICS FOLLOWING UMBILICAL CORD BLOOD TRANSPLANTATION IN ADULTS

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Background. Alloreactivity is supposed to induce modifications on the vascular endothelium. Complications occurring in the course of allogeneic haematopoietic stem cell transplantation, such as acute or chronic graft-versus-host disease (GVHD), have been related to endothelial cell alterations. Moreover, the incidence of GVHD is significantly different

following allogeneic bone marrow transplantation (BMT), peripheral blood stem cell transplantation (PBSCT) or umbilical cord blood transplantation (UCBT). *Aims.* Considering the different incidence of acute and chronic GVHD according to the transplanted type of haematopoietic stem cells and taking into account the supported xenotransplantation-related vasculopathy, we aimed to further clarify the vascular impact of the three aforementioned sources of haematopoietic stem cells. *Methods.* We studied 21 patients, 13 male and 8 female. Mean age was 42 years old (21-64). The underlying diseases were: 15 patients presenting an acute myeloid leukaemia, 3 patients with acute lymphoblastic leukaemia, 1 patient with multiple myeloma, 1 with bone marrow aplasia and 1 patient with chronic idiopathic myelofibrosis. Seven patients underwent BMT after myeloablative conditioning regimen (cyclophosphamide and busulphan or total body irradiation-TBI at 12Gy), 6 patients PBSCT following non-myeloablative preparative regimen (fludarabine and TBI at 2Gy) and 8 patients underwent UCBT following reduced intensity conditioning regimen (cyclophosphamide , fludarabine and TBI at 2Gy). We assessed the kinetics of plasma levels of 4 endothelial markers: soluble thrombomodulin (sTM), vWF-Ag, FVIII and the vascular endothelial growth factor (VEGF). Measurements were performed before the conditioning regimen, on days 0, 15, 30, 60 and 90, by using commercialised ELISA kits from Asserachrom (Diagnostica Stago, France). We used the Student paired t-test for statistical comparison. Differences were significant if $p<0.05$. *Results.* In BMT and PBSCT we observed a significant increase of sTM on days 30 and 60 respectively, in comparison to day 0 (141±42 ng/mL and 143±13 ng/mL respectively, $p<0.05$). No significant elevation of sTM, compared to pre-conditioning values, was shown in UCBT during the studied period. sTM in UCBT peaked on day 90 (119±54 ng/mL, $p>0.05$). Similarly, vWF-Ag was significantly increased on day 0 in BMT and PBSCT (217±66% and 214±93% respectively, $p<0.05$ compared to pre-conditioning values), whereas in UCBT vWF-Ag plasma levels remained almost unchanged during the studied period. FVIII was significantly elevated on day 15 in PBSCT (200±6%, $p<0.05$ in comparison to baseline values), but we did not observe any significant increase of FVIII, in comparison to pre-conditioning values, in BMT and UCBT. Finally, there was no significant modification of VEGF in the 3 types of transplantation. *Conclusions.* This is the first study investigating the kinetics of different endothelial cell markers following UCBT and comparing them to BMT and PBSCT. A significant difference was observed in the kinetics of endothelial cell markers, according to the source of stem cells. Cord blood cells seem to hardly affect vascular endothelium, as shown by the stability of sTM and vWF-Ag plasma levels in the course of the UCBT. The low impact of UCBT on the vascular endothelium could possibly be correlated to fewer transplant-related complications and less incidence of GVHD in this type of transplantation.

0500

HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMATOPOIETIC PROGENITOR CELL TRANSPLANTATION (AHCT) FOR NON-HODGKIN S LYMPHOMA IN PATIENTS OVER 65 YEARS OF AGE

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High-dose chemotherapy followed by AHCT is the treatment of choice for patients with relapsed NHL. Often this therapy is not offered to patients who are older than 65 yrs of age because of concerns regarding their ability to tolerate such aggressive therapy . We present a retrospective analysis of 99 consecutive pts who underwent AHCT for NHL at our institution from 6/96-3/06. All patients signed informed consent prior to AHCT. *Results.* Most common histologies were DLCL (55%), MCL (15%), and FL grades 1-3 (15%). Median age at diagnosis was 65 yrs (range, 47-81) and median age at AHCT was 68 yrs (range, 65-83). Seventy percent were males. Median number of chemotherapy regimens administered prior to AHCT were 2 (range 1-6). Median IPI score at the time of AHCT was 1 (range 0-4), 44% of pts had an IPI of > 1. Majority of patients (99%) had an ECOG PS score of < 2 prior to AHCT. Forty-four percent pts were in CR/CRu, 45% were in PR, and 11% had PD/SD. The preparative regimen comprised of BEAM (35%), BEAM/rituximab (53%), and Cy/TBI ± rituximab (10%). Median length of hospital stay was 23 days (range, 6-85) for AHCT with 38% requiring readmission within the first 100 days. Source of progenitor cells was HPC-A in 89%. Median $CD34^+$ cell dose infused/kg was 4.4×10^6 (0.08-32.2). All patients engrafted. Median time to reach an ANC of 500/mm^3 was 10 days (range 7-47). Ten patients never achieved a platelet count of 20,000/mm^3. For the remaining 88 pts the median time to reach platelet count of 20,000/mm^3 was 13 days (range, 6-375). Median number of PRBC units transfused were 4 (range, 0-50) and the median number of platelets transfusions required were 4 (range, 0-35). Grade 3-5 non hematologic RRT toxicity is summarized in Table 1. Cumulative TRM was 8% (95% CI 4-17) at 26 mths and 12% (95% CI 6-22) at 36 mths. At the time of this analysis the median median follow time among survivors was 26 mths (range, 1-115). The 3 yr OS for the entire cohort was 61% (95% CI 49-71). The 3 yr DFS was for 48% (95% CI 33-61), 68% (95% CI 36-87), and 63% (95% CI 22-87) for DLCL, MCL and FL respectively. On univariate analysis IPI > 1, LDH > normal, and having SD/PD at the time of AHCT were predictors of a worse OS. Disease status at transplant and LDH > normal remained significant predictors for OS ($p=0.002$) on multivariate analysis. Age, gender, histology, number of prior chemotherapy regimens, time from diagnosis to AHCT, and type of conditioning regimen were not significant predictors of OS. Most common cause of death was disease progression/relapse in 60% of patients. Eight patients developed sMDS/AML after AHCT. *Conclusions.* Patients over 65 yrs of age can undergo AHCT with acceptable toxicity and should be considered transplant candidates if they have chemosensitive disease and normal LDH at the time of AHCT.

Table 1. Non-Hematologic RRT Grades 3-5.

0501

EFFICACY AND SAFETY OF CYTARABINE-BASED REGIMENS FOR MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS IN MULTIPLE MYELOMA

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Background. Autologous stem cell transplantation (ASCT) represents one of the most effective treatments for multiple myeloma (MM). High dose cyclophosphamide is generally considered a standard choice for peripheral blood stem cells (PBSC) mobilization. Unfortunately, a small but significant proportion of patients can not mobilize an adequate number of PBSC or can not be treated with cyclophosphamide because of co-morbidities. Cytarabine has been shown as an effective mobilization therapy in highly pre-treated lymphoma patients. *Aim.* The aim of this study was to exploit the feasibility of PBSC mobilization with cytarabine-based regimens in MM patients. *Methods.* Twenty-eight patients with MM were treated with a cytarabine-based regimen, either an association of cytarabine 2 gr/sqm bid, cisplatin 50-75 mg/sqm, and dexamethazone 80-160 mg (DHAP) in 20 patients, or cytarabine alone (0.8 gr/sqm bid for 3 days) in 8 cases. For all patients chemotherapy was followed by a daily administration of G-CSF 5 micrograms/kg starting at day +3. Patients had a median age of 59 years (range 41-71), 21 were female, the median number of previous chemotherapy lines was 2 (range 1-5), 6 patients had previously received a melphalan-based chemotherapy, and 4 patients had previously received radiotherapy. Patients received a cytarabine-based regimen if they failed a PBSC mobilization with cyclophosphamide (10 patients) and/or if they had severe co-morbidities (23 patients), in particular cardiovascular disease (16 patients), renal failure (4 patients), previous radiation therapy for cancer (3 patients). *Results.* All patients mobilized PBSC with a median collection of 15×10^6 $CD34^+$/kg (range $0.5\text{-}53\times10^6$ $CD34^+$/kg). Ten patients, failing a previous mobilization with cyclophosphamide, collected 11×10^6 $CD34^+$/kg (range $7\text{-}43\times10^6$ $CD34^+$/kg). All the 6 patients who previous-

ly received melphalan-based chemotherapy collected a median number of 4.4×10^6 $CD34^+$/kg (range $0.5\text{-}19\times10^6$ $CD34^+$/kg). The cytarabine-based regimens were generally well tolerated. According to the NCI CTC version 3 scale, grade 3-5 toxicity was observed in two cases (7%): a patient reactivated Herpes Zoster infection, and a patient affected by a severe form of Churg Strauss vasculitis with kidney and lung involvement, and a long history of immunesuppression, died of septic shock. After PBSC collection, 4 (14%) patients received a single ASCT, 21 (75%) a double ASCT, one (4%) patient three ASCT, and 2 (7%) were not transplanted. All patients engrafted. The median time to neutrophil count $>500\times10^6$/L was 11 days (range 9-12) and to platelet count $>20\times10^9$/L was 10 days (range 0-14). *Conclusions*. Cytarabine-based regimens are a feasible option to collect PBSC in MM patients. Despite one (4%) chemotherapy related death, the treatment was well tolerated. While not active against MM, cytarabine-based regimens allowed the collection of an adequate number of PBSC to support a double ASCT in most patients, with a hematopoietic recovery comparable with other series.

0502

HUMAN UMBILICAL CORD BLOOD CELLS REGENERATE HEPATOCYTES BY FUSION IN A NON-MYELOABLATIVE SETTING

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Background. Many studies have shown that haematopoietic stem cells can regenerate hepatocytes. Human umbilical cord blood (UCB) is a rich source of hematopoietic stem cells and mesenchymal progenitor cells which might be used for tissue or organ repair. *Aims.* We evaluated the possibility and mechanism of hepatocyte regeneration by administration of human UCB cells following non-myeloablative conditioning after acute liver injury in an immunocompetent mouse model. *Methods.* Female C57Bl6 mice were administered toxic dose of acetaminophen. Six hours later, they were given fludarabine and cyclosporine followed by infusion of human UCB mononuclear cells. Surviving mice were sacrificed at two and four weeks post transplant. Immunohistochemistry, fluorescence in-situ hybridization (FISH) using centromere enumeration probe for human chromosome Y and FITC-labeled mouse pancentromeric probe, and polymerase chain reaction (PCR) analysis of hepatic DNA for α-satellite region of human chromosome 17 were used to confirm the presence of hepatocytes from human origin and the mechanism of regeneration. *Results.* Fifteen out of 24 mice received human UCB cells infusion after non-myeloablative conditioning survived beyond two weeks. Immunohistochemical analysis demonstrated the presence of hepatocytes expressing human hepatocyte antigen and human albumin in the hepatic sections of all but three of the surviving mice. FISH confirmed the presence of human Y chromosome in 0.5-20% of the hepatocytes. PCR analysis showed that 1-20% of the hepatic DNA was of human origin. Fusion of human cell with mouse cell was demonstrated by FISH (Figure 1).

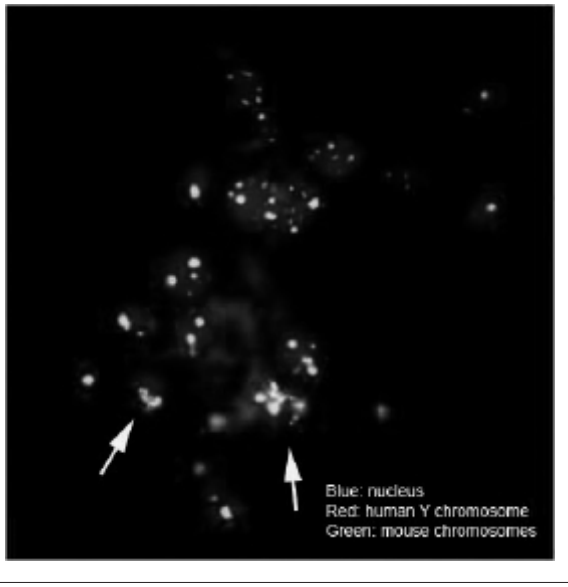

Figure 1. Cell fusion demonstrated by FISH.

Conclusions. Our data suggested that human umbilical cord blood can regenerate functional hepatocytes by fusion after acetaminophen induced acute hepatic injury in a non-myeloablative setting. This may be an effective approach in the management of patients with inherited diseases by using UCB cells for gene therapy.

0503

CLINICAL RESULTS OF A NEW DOSING STRATEGY OF IV BUSULFAN AS PART OF BUMEL HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CEL TRANSPLANTATION IN CHILDREN WITH HIGH-RISK SOLID TUMORS: REDUCED REGIMEN-RELATED TOXICITY AND VENO-OCCLUSIVE DISEASE

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Background. Oral busulfan (Bu) and melphalan (Mel) has been extensively used as a high-dose chemotherapy (HDC) regimen followed by autologous hematopoietic stem cell transplantation (ASCT) in pediatric patients (pts) with high-risk (HR) solid tumors. With the availability of IV Bu, a new dosing strategy based on body weight (BW) has been defined (Nguyen et al BMT 2004). We assessed prospectively this approach. Pharmocokinetic results have been reported in children and adolescents (Vassal et al BMT 2006) and we report here the final clinical results. *Patients and Methods.* 31 children (17 boys/14 girls), median age 4 y (range 0.7 to 14.9 y) and weight 14.5 kg (range 7.2 to 62.5), respectively were enrolled. Pts received IV Bu (Busilvex®) over 2 h at a dose of 1.0 mg/kg or 1.2 mg/kg or 1.1 mg/kg or 0.95 mg/kg or 0.8 mg/kg for <9 kg, 9- to < 16 kg, 16-23 kg, >23-34 kg, and > 34 kg BW, respectively. Mel 140 mg/m^2 was then administered followed by HSCT. Clonazepam was given as seizures prophylaxis. Indications for HSCT were : HR neuroblastoma (NB), n = 27:9 CR1/ CR2, 11 VGPR, 7 PR1/PR2, Ewing sarcoma (EW), n=4:2 CR1, 2 PR1. Regimen-related toxicity (RRT) was graded according to NCI-CTC 2.0. Kaplan-Meier EFS and OS were calculated. *Results.* No adverse effect was observed during IV Bu administration. Pts received 5.8×10^6 $CD34^+$/kg (range 3-34.8) with post transplant G-CSF in 27/31. Neutrophils ($>0.5\times10^9$/L) and platelets ($>50.0\times10^9$/L) recovery occurred at day 11 (range 10-15) and day 34 (range 11-133), respectively. Digestive toxicity (mucositis) was the main RRT: grade I-II and grade III occurred in 24 and 14 pts, respectively. Four pts (13%) had hepatic veno-occlusive disease (VOD) but none was severe. No early or late regimen-related death occurred. 15/31 pts had disease relapse/ progression after a median time of 9 months (2.6-40.4), and 11/31 pts died. With a median follow-up of 41.2 months (range 3.2-52.2) EFS and OS rates were as follows: 42±22% and 57±20%, for HR-NB, respectively; it was 38% and 67% for HR-EW, respectively. *Discussions/Conclusions.* Oral BuMel is currently the standard HDC regimen in pts with HR-NB but its use is limited by the toxicity, especially VOD (up to 40%). The current results suggest that the IVBu has similar impact on efficacy of the BuMel regimen but with reduced toxicity, especially VOD (13%). The IVBu schedule could be an useful alternative in the ongoing prospective randomized trial (HR-NBL-1/SIOPEN).

0504

THREE STRATEGIES OF IMMUNOABLATIVE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE SCLEROSIS PATIENTS

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During the last decade immunoablative therapy with ASCT has been used more often as a therapeutic option for MS patients. Among a number of unclear questions are the terms of conducting immunoablative therapy with ASCT. There are 3 strategies of immunoablative therapy with ASCT depending on the terms of disease process: early, conventional and salvage/late. We aimed to study the clinical and patient-reported

outcomes in MS patients after early, conventional and salvage/late transplantation. Forty-three patients with MS (secondary progressive - 22 patients, primary progressive -10, progressive-relapsing - 1, relapsing-remitting - 10) from 6 medical centers were included in this study (mean age - 32.0, range: 17-51; male/female - 18/25). Seven patients underwent early transplantation (EDSS 1.0-3.0), 32 patients -conventional transplantation (EDSS 3.5-6.5) and 5 patients - salvage/late transplantation (EDSS 7.0'8.5). Median EDSS at base-line was 6.0 (range 1.5-8.0). The median follow-up duration was 18 months (range 6-84 months). All of the patients had previously undergone conventional treatment. Neurological and quality of life (QoL) evaluation was provided at baseline, at discharge, at 3, 6, 9, 12 months, and every 6 months thereafter following immunoablative therapy with ASCT. MRI examinations were conducted at baseline, at 6, 12 months, and at the end of follow-up. FACT-BMT and FAMS were used for QoL evaluation. Notably, no transplant-related deaths or unpredictable severe adverse events were observed. All 29 patients (2 patients - early transplantation; 24 - conventional transplantation; 3 - salvage/late transplantation) with the follow-up longer than 1 year experienced a clinical stabilization or improvement. More than half of them improved: 8 patients showed significant improvement in EDSS (by more than 1.0 point), 4 patients improved by 1.0 point, and 4 patients - by 0.5 points on EDSS. Thirteen patients achieved stabilization. Two patients deteriorated to a worse score after 18 months of stabilization; 2 other patients progressed after 12 and 30 months of improvement, respectively. All of the patients with clinical stabilization and improvement had negative MRI scans. Out of 23 patients included in QoL analysis 21 exhibited improved QoL 6 months post-transplantation. In conclusion, immunoablative therapy with ASCT appears to be an effective treatment for MS both in terms of clinical and patient-reported outcomes. The data obtained point to feasibility of early, conventional and salvage/late transplantation in MS patients. Further studies should be done to investigate clinical and QoL response in MS patients receiving early, conventional and salvage/late immunoablative therapy with ASCT to better define treatment success.

0505

COMPARISON OF HIGH DOSE VS. LOW DOSE G-CSF FOR PERIPHERAL BLOOD STEM CELL MOBILIZATION: ANALYSIS OF A CASE CONTROL SERIES

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Objective. PBSC mobilization typically requires administration of multiple daily doses of G-CSF to achieve target collections of $CD34^+$ cells. A decrease in patient visits to the clinic may result in reduced overall costs and improved patient convenience. We report the effect of high-dose G-CSF on $CD34^+$ cell yields, days to myeloid and platelets engraftment and number of aphaeresis collections for autologous stem cell transplants (SCT) as compared with a historical control. *Methods.* Eighty-six patients underwent peripheral stem cell collection for various malignancies for stem cell transplantation during 2001 (n=31) and 2002 to 2006 (n=55). In 2001 G-CSF 10 mcg/kg/day was administered for 5 days followed by aphaeresis beginning on the 6th day. In 2002 to 2006, G-CSF 20mcg/kg/day was administered for 2 days followed by aphaeresis beginning on the 3rd day. The target $CD34^+$ cell count was 5×10^6 cells/kg (Coulter flow method) and no chemotherapy was used for mobilization. *Results.* The mean number of $CD34^+$ cells collected among patients receiving low dose G-CSF 10 mcg/kg/day for 5 days was 5.66×10^6/kg (range 4.17-9.24) compared to 7.61×10^6/kg (range 4.16-25.05) among patients receiving high dose G-CSF 20 mcg/kg/day for 2 days. All patients achieved target $CD34^+$ stem cell yields. The median percentage of $CD34^+$ stem cell on the first day of collection i.e. on day 6 for low dose G-CSF group was 0.40% compared to 0.30% for high dose G-CSF group. The median number of nucleated cells on the first day of collection for low dose G-CSF group was 19.9×10^8 cells/kg compared to 15.4×10^8 cells/kg for high dose G-CSF group. The mean number of aphaeresis per stem cell transplant in patients receiving low dose G-CSF was 3.3 compared to 2.9 in patients receiving high dose G-CSF. There was no difference between the low dose and high dose groups in terms of mean days to myeloid engraftment 11.8 vs.12.1 (p=0.26). However the mean days to platelet engraftment was better in patients receiving high dose G-CSF than those receiving low dose G-CSF, 14.5 vs.22.9 days (one tail p=0.09). Finally no differences in adverse effect were noted between the low dose and high dose groups. *Conclusions.* The results of our trial conclude that 20 mcg/kg/day x 2 days vs. 10mcg/kg/day x 5 days of G-CSF result in improved $CD34^+$ stem cell yield, decreases aphaeresis, equivalent time to myeloid engraftment and much earlier platelets engraftment. This resulted in decreased patient travel and clinic time. Early platelets engraftment will reduce hospital stay and platelets requirement

0506

THYMIC FUNCTION IN PATIENTS CANDIDATES TO AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Signal joint T cell receptor excision circles (sjTRECs) have been reported to be a clinical marker to evaluate the thymic reservoir after immunosuppression treatments. *Aims.* The aim of the study was to evaluate the impact of different features such as: age, type of lymphoma, number of first line chemotherapy (CT) cycles, time from the end of first line CT to the enrolment (TECT), HIV infection and T subpopulations. *Methods.* We studied the sjTRECs levels in a mono-institutional series of a cohort of 26 HIV-positive and 29 HIV-negative patients (pts) with relapsed or refractory lymphomas, candidates to ASCT considering important biological and clinical characteristics and virological and immunological parameters. Peripheral blood samples were collected in ethylene diamine tetraacetic acid, from all pts before the induction CT. PBMCs were isolated by Ficoll/Hypaque density gradient centrifugation and cryopreserved as dry pellet at -80°; a Real Time PCR method was used to measure sjTRECs. *Results.* The overall study subjects showed lower sjTRECs levels than healthy donors (p<0.01), but no differences in the sjTRECs content have been observed between HIV-negative and HIV-positive pts (536 vs 401 TRECs/106PBMCs respectively) as well as in the T cell naïve count. We found a significant correlation between the sjTRECs decay and the increase of age (r=-0.32, p=0.02), CD4 and CD8 naïve cell count and the sjTRECs level; on the contrary we did not observe any significant correlation between CT cycles number TECT, lymphoma type in both subgroups. HIV-positive viremic pts showed significant lower values of sjTRECs level than aviremic pts. *Conclusions.* Our analyses suggest that *de novo* T cell generation is partially maintained in lymphoma pts candidates to ASCT and could contribute to restore the immune function after transplantation. Chemotherapeutic treatments seem to induce a similar influence on thymic output, despite their intensity and surprisingly HIV infection is not a detrimental factor on the thymic reservoir at the time of lymphoma relapse, a good control of HIV replication seems to preserve the thymic reservoir.

0507

IMPACT OF INFUSED LYMPHOCYTE DOSE ON THE OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. It has previously been reported that the number of infused lymphocytes influences the outcome of patients with multiple myeloma (MM) undergoing ASCT, with patients receiving a lymphocyte-rich graft experiencing longer relapse free and overall survival. This could be because re-infused lymphocytes play a role in disease control, or because patients with lymphocyte-poor grafts have intrinsically worse disease, and/or have received more prior therapies. We sought to address these issues by carrying out a similar analysis of MM patients undergoing ASCT, and including in our analysis a third group of patients who received CD34-selected grafts. *Patients and Methods.* We analysed 135 patients (52 female, 83 male, median age 57 years, range 38-71) who underwent high dose melphalan and ASCT with unselected peripheral blood stem-cell grafts. The median number of infused lymphocytes was 1.05×10^9/kg of body weight (range 0.05-5.37). Patients were split into two groups, group A (those who received $<0.5\times10^9$ lymphocytes/kg, N=46) and group B (those who received $>0.5\times10^9$ lymphocytes/kg, N=89). We compared transplantation outcome of these patients with a third group C (n=48), of patients who received immunomagnetically selected autologous $CD34^+$ cells. *Results.* Time to neutrophil and platelet engraftment was comparable in all groups, however, group A patients had significantly longer hospitalization period, as compared to both, group B and -C patients (26.4±10.9 vs. 22±5.5 days, p=0.002, vs. 25.3±9.4 days for group C, p=0.0129). Common bacterial and viral infections

were similar among the 3 groups. However, episodes of varicella-zoster and CMV reactivation, as well as other opportunistic infections were more frequently encountered among patients of groups A and C (16/46 and 19/48 respectively), as compared with patients of group B (14/89, ×2: $p<0.001$). After a median follow-up time of 32 months, 17 patients from group A (37%), 53 from group B (59.5%) and 11 from group C (22.9%) are alive. Median relapse-free survival was 12.3 months in group A, 27.5 months in group B ($p=0.016$) and 20.6 months in group C (group A vs C, $p=0.097$, group B vs C, $p=0.711$). Similarly, median survival from SCT was 26.4 months in group A, 52.1 months in group B ($p=0.033$) and 45.3 months in group C (p: not significant for both comparisons). Median overall survival was 38.5 months for group A, 54.5 for group B and 49 months for group C. *Conclusions.* Lower lymphocyte dose in the graft predicts for a poorer outcome in patients with MM undergoing ASCT. This poor outcome is not seen in patients receiving a CD34-selected graft, suggesting that a lower lymphocyte dose is a reflection of patient disease state, and/or previous treatment, and that the lymphocytes infused in the graft play little part in disease control.

0508

COMPARISON OF A COMBINED APPROACH WITH AUTOGRAFTING FOLLOWED BY NON-MYELOABLATIVE ALLOGRAFTING VERSUS TANDEM AUTOGRAFTING IN MULTIPLE MYELOMA PATIENTS

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Multiple Myeloma (MM) is an incurable disorder despite the numerous attempts to find new therapy approaches. The better understanding of its molecular pathogenesis has led to the development of effective novel therapeutic agents like thalidomide and the proteasome inhibitor bortezomib. High-dose therapy (HDT) with stem cell transplantation and novel targeted therapies represent two approaches to overcome resistance of multiple myeloma cells to conventional treatments. The novel agents have shown impressive activity in relapsed/refractory MM patients. Autografting (AutoSCT) results have been compromised by high relapse rate while conventional allografting (AlloSCT) by excessive transplant-related mortality (TRM) and toxicity. Reduced intensity conditioning for transplant (RICT) has been shown to achieve remissions in MM patients. High-dose therapy/AutoSCT followed shortly thereafter by RICT allogeneic transplant might improve outcomes in MM as compared to AutoSCT or conventional AlloSCT used alone. As we have previously reported, we compared two retrospective cohort of advanced stage MM patients who underwent tandem AutoSCT (HDT consisted of melphalan 200 mg/m^2) or AutoSCT followed closely by RICT (patients with HLA-matched siblings) (AutoSCT+RICT). The two groups were matched for pre-transplant therapy, disease status at transplant, time from diagnosis to transplant. The median age was 56 years and 51 years in the AutoSCT and AutoSCT+RICT groups, respectively. All patients had received a median of 4 (range, 3-6) prior chemotherapy regimens. The median time from diagnosis to the first transplant was 6 months (range, 5-60) in AutoSCT group and 9 months (range, 7-42) in AutoSCT+RICT group, respectively. In the AutoSCT+RICT group, after a median interval of 80 days from AutoSCT, a dose-reduced regimen consisting of fludarabine (30 mg/m^2) and 2 Gy TBI followed by peripheral blood stem cell allografting from matched related donors was given to the patients in the attempt to induce a graft-versus-myeloma effect. Graft-versus-host-disease prophylaxis consisted of CyA/MTX. We have evaluated toxicity, engraftment, chimerism, GvHD, and response to RICT allograft after cytoreductive autografting. In the AutoSCT+RICT group the complete remission rate was higher (50%) than in AutoSCT group (14%); beside the risk of disease progression was reduced. The complete response in AutoSCT+RICT patients was obtained after achievement of full donor chimerism and the development of GvHD. Since the first clinical signs of response were noted between 70 and 120 days and the maximum response between 160 and 200 days after RICT, those responses should be considered immune-mediated. TRM has been 0% in both groups. The double AutoSCT+RICT protocol provides rapid engraftment with complete donor chimerism, reduces the risk of transplant-related organ toxicity, and induces high remission rates. Our results are encouraging and demonstrate that RICT following an AutoSCT mediates a potentially curative graft-versus-myeloma effect and it delays the incidence of disease progression, even though multicentric studies are required.

0509

CD34+ CELL MOBILISATION WITH INTERMEDIATE-DOSE CYTARABINE (ARA-C) IN PATIENTS WITH LYMPHOMA: AN EFFECTIVE BUT TOXIC REGIMEN

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Background. Peripheral stem cell mobilisation can be difficult in patients with lymphoma, especially after treatment including purine analogues or in heavily pre-treated patients. Intermediate-dose Ara-C (ID Ara-C) has been shown to be an effective mobilisation regimen in patients with chronic lymphocytic leukaemia (CLL) who have previously received fludarabine and campath (Montillo et al, Leukaemia 2004). *Aims.* To analyse the toxicity and efficacy of ID Ara-C as a mobilisation regimen in patients with lymphoma. *Methods.* Twenty-five patients with lymphoma (median age: 51 years, range: 20-74) were sequentially treated with ID Ara-C. Histological diagnoses were as follows: 10 patients, Hodgkin's lymphoma (HL), 12, follicular lymphoma (FL) and 3 other Non-Hodgkin's lymphoma (NHL). The median number of previous lines of chemotherapy was 1 (range: 1-4) for HL, 3 (range: 2-5) for FL and 2 (range: 2-3) for other NHL. Prior therapy included fludarabine in 5 patients. Five patients had previously failed stem cell harvest with other mobilisation regimens. ID Ara-C comprised Ara-C: 800 mg/sqm bd x 6 followed by G-CSF: 5°g/kg/d to the end of harvesting. *Results.* All patients required hospital admission, for neutropenic fever (18 patients, 5 of whom required second-line antibiotics) or neutropenic surveillance (7 patients), the median hospital stay being 9 days (range: 5-14). Platelet transfusion was required at least once in all patients, with a median of 3 units being transfused per patient. The median number of RBC units transfused was 2. Harvesting was successful in all the patients, with a median CD34$^+$ yield of 11.4×10^6 cells/kg (range: 1.96-112), at a median time of 15 days (range: 14-35) after start of Ara-C. The median CD34+ yield in patients who had received fludarabine was 11.4, whereas it was 3.77 for 11 patients who had received 3 or more previous lines of chemotherapy. *Conclusions.* ID Ara-C is a very effective mobilisation regimen for patients with lymphoma, including heavily pre-treated patients and those who had received purine analogues. However, given its considerable and universal haematological and, therefore, clinical toxicity and the consequent need for hospitalisation with its attendant costs, it should be reserved for patients in whom difficulties in harvesting are predictable in view of their previous treatment.

0510

EFFECTS OF RITUXIMAB ON PERIPHERAL BLOOD STEM CELL MOBILIZATION AND ENGRAFTMENT IN B CELL NON HODGKINS LYMPHOMA

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Treatment with rituximab is widely used for B cell non Hodgkin's lymphomas (B-NHL). However, its effects on peripheral blood stem cell mobilization are not completely known. We retrospectively evaluated 52 consecutive B-NHL (30 follicular and 22 diffuse large cells) patients responding to first-line chemotherapy (CHOP or R-CHOP), but failing to achieve complete remission (CR). In this group we compared the mobilization characteristics and engraftment kinetics of 24 patients receiving and 28 not receiving rituximab six months before PBSC mobilization. Patients mean age was 37 years (range 17-60). 44 patients (84.6%) had stage III-IV disease. 34 patients (65.4%) had bone marrow involvement; systemic B symptoms were present in 30 patients (57.7%). At the time of PBSC mobilization 32 patients (61.5%) were considered to be responsive (complete remission, partial remission or sensitive relapse) and 20 (38.5%) not responsive (refractory relapse or refractory to therapy). Mobilization chemotherapy consisted of a high dose cytarabine containing regimen (DHAP) in all patients. The median $CD34^+$ cells collected was 5.8×10^6/kg in patients receiving rituximab vs 7.2×10^6/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/mcl, occurred in 2 patients (8.3%) in the rituximab group and 3 (10.7%) in the non rituximab group. All patients were transplanted using myeloablative chemotherapy conditioning regimen (BEAM); G-CSF was administered subcutaneously from day +3 at a dose of 5 microg/kg body weight/day. Comparison of the two groups showed no statistical significant difference between median days to absolute neutrophil $>0.5\times10^9$/L and platelet $>20\times10^9$/L counts after autologous stem cell transplantation, and no differences in incidence and severity of infections, days of fever or duration of antibiotic treatment between groups. In conclusion, the use of rituximab six months before PBSC does not affect the ability to collect an adequate number of PBSC for autologous stem cell transplantation in B-NHL. Further studies are warranted in larger populations to determine the impact of rituximab on collection, engraftment and survival.

Chronic lymphocytic leukemia and related disorders - Biology II

0511

THE HIGH FREQUENCY OF T REGULATORY CELLS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) IS DECREASED BY THALIDOMIDE AND FLUDARABINE TREATMENT

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Background. The control of a rather slowly progressing B-cell chronic lymphocytic leukemia (B-CLL) by the immune system is not yet fully understood. Several mechanisms underlay the immunosuppressive status of CLL patients including the excess of T regulatory lymphocytes (Tregs). The forkhead family transcription factor FOXP-3 is critically important for the development and function of Tregs. *Aims*. In current study we wanted to define the expression of FOXP-3 on $CD4^+CD25^{high}$ T lymphocytes in the peripheral blood mononuclear cells (PBMC). Additionally, we wanted to characterize the influence of Tregs on immune responses against tumor and viral antigens in the complex system of PBMC. We also tried to define the influence of thalidomide (THAL) alone as well as combined with fludarabine (FLU) on Tregs subpopulation of CLL patients. *Methods*. In 60 previously untreated patients with B-CLL the subpopulation of $CD4^+CD25^{high}FOXP3^+$ T cells that phenotypically correspond to Tregs was identified. Immunosuppressive function of Tregs was evaluated in an enzyme-linked immunosorbent spot (ELISpot) assay to estimate T-cell immune responses against HLA-A2 restricted epitopes derived from tumor associated antigens (TAAs) of survivin, fibromodulin and RHAMM as well as viral peptide derived form infuenza matrix protein. *Results.* The frequency of Tregs was significantly higher in B-CLL patients compared to healthy volunteers (HVs) (12.1% vs. 1.9%, p=0.0001). The progressive increase of Tregs percentages were noted in advanced stages of disease, mean: 9.5% in stage A, 13.4% in stage B and 15.4% in stage C according to the Binet classification. Higher frequencies of Tregs correlated with decreased T cell responsiveness against viral and tumor antigens. Significantly lower secretion of IFN-γ was observed in patients with higher percentages of Tregs (more than 11% of the CD4 cells). In 80% CLL patients treated with THAL + FLU regimen significant reduction of circulating Tregs after THAL was observed. The combination with FLU resulted in further decrease of Tregs in 12 of 15 patients. After THAL therapy the mean reduction was higher in the population of Tregs compared to whole lymphocyte population (41.2% vs. 19.9%, p=0.046). Higher frequencies of Tregs were observed in CLL patients with higher levels of TNF-α serum levels (r2=0.45, p=0.001, n=20). *Conclusions*. Tregs presented in high frequencies in B-CLL constitute the crucial immunosuppressive mechanism among the mononuclear cells of the peripheral blood. The strategies combining THAL are very promising, since THAL might target not only the CLL cell population but also Tregs and therefore restore the $CD8^+$ T cell function. Increased TNF level might promote Tregs proliferation. Nontheless, the results of our study suggest that TNF is unable to block suppressive activity of Tregs in B-CLL

0512

B CHRONIC LYMPHOCYTIC LEUKEMIA CELLS EXPRESS THE ORPHAN RECEPTOR TYROSINE KINASE ROR-1

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Background. Ror receptors are cell-surface receptors participating in signal transduction, cell-cell interaction, regulation of cell proliferation, differentiation, cell metabolism and survival. Ror-1 is a member of the RTKs family of orphan receptors related to muscle specific kinase (MUSK) and Trk neutrophin receptors. The human Ror1 is highly expressed in heart, lung, and kidney but to a lesser extent in placenta, pancreas and skeletal muscles. In B-CLL a 43.8 fold increase of the orphan receptor tyrosine kinase (RTK) gene (Ror-1) expression has been observed previously. *Aims.* To study the expression of Ror-1 in B-CLL.

Methods. Several read-out systems were used in this study including RT-PCR, flow cytometry, Western blotting and phosphorylation assay. *Results.* Ror-1 was expressed at the gene and protein levels in leukemic B cells of all B-CLL patients (n=100). Ror1 was not expressed in leukocytes from healthy individuals, enriched B cells, T cells, and granulocytes but weakly in normal tonsil B cells. A strong activation signal (PMA/Ionomycin) induced expression of Ror1 in normal lymphocytes. Five different variants of the Ror1 protein were identified, ranging from 68 to 105 kDa (68, 85, 96, 102, and 105). The 102 and 105 kDa isoforms may represent the native Ror-1 molecules and were differentially expressed in progressive and non-progressive disease. The 68, 85, and 96 kDa isoforms were auto-phosphorylated but not the 102 and 105 kDa variants. *Conclusions.* The ectopic expression of auto-phosphorylated Ror1 in CLL, indicate a potential role of this RTK in the pathobiology of B-CLL.

0513

GENE EXPRESSION COMPARISON BETWEEN B CELLS EXPRESSING HIGH AND LOW LEVEL OF ZAP-70 MRNA REVEALS DISTINCT PROFILES, POTENTIAL THERAPEUTIC TARGETS AND NEW PROGNOSTIC FACTORS FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease characterized by a highly variable clinical course based on the IgVH mutational status currently considered as one of the powerful prognosis factors. Because of the complexity of this analysis, several surrogate genes of this mutational status have been found in the recent years, among them Zap-70 seems to be a reliable prognostic factor. *Methods.* We compared three predictive markers (Zap-70, lipoprotein lipase and CD38 expression) in term of treatment-free (TFS) and overall (OS) survival and finally Zap-70 (zeta-associated protein 70) was chosen because of its strong association with the IgVH status and high prognostic values. We developed a quantitative real time PCR to measure Zap-70 mRNA expression in a cohort of 100 patients and to classify patients with high and low Zap-70 expression. Gene expression profiles of high (Zap-70^{high}, n=7) and low (Zap-70^{low}, n=7) mRNA expression were then compared using Affymetrix U133 plus 2.0 genechips representing more than 47000 transcripts. *Results.* Among genes differently expressed ($p<0.05$), only genes with a fold change upper of 1.5 and with a false discovery rate (FDR) lower than 10% were selected. Zap-70, the first gene of the list, was followed by the phosphodiesterase 8A (PDE8A, $p<0.0001$), integrin α 4 (ITGA4, $p<0.0001$) and some genes of Fc receptor like family (FCRL, $p<0.0001$). These genes were confirmed in an extended patient cohort (n=80) and were able to separate the patients in term of TFS indicating their relevant clinical predictive power. *Conclusions.* CLL cells expressing high and low level of Zap-70 mRNA are characterized by a distinct gene expression profile that reveals new potential therapeutic target, new prognostic factors and genes implicated in cellular activation, adhesion and migration.

0514

A COMPARATIVE ANALYSIS OF PROTEIN EXPRESSION PROFILES IN PERIPHERAL BLOOD CHRONIC LYMPHOCYTIC LEUKEMIA CELLS AT DIAGNOSIS AND UPON DISEASE PROGRESSION

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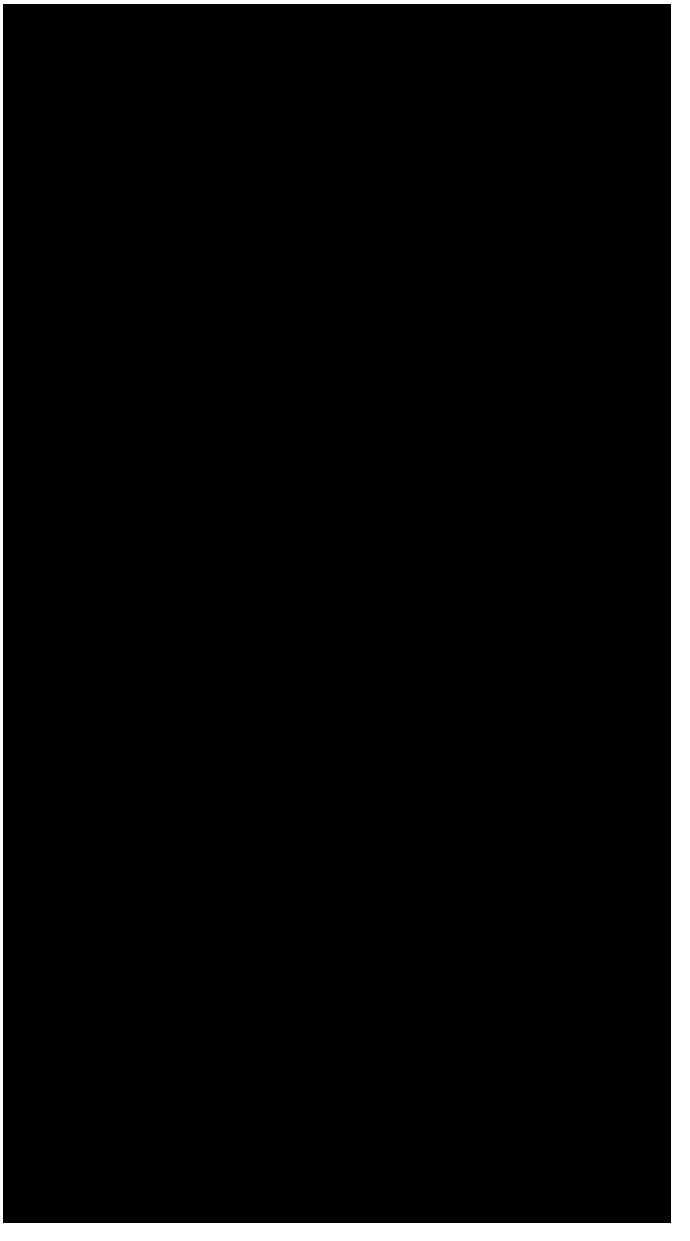
Background. CLL has a heterogeneous clinical course. Half of the patients diagnosed in early stage eventually progress and require treatment. Although some genetic abnormalities such as del(17p) have been associated with disease progression, the mechanisms underlying such a phenomenon are not well-known. *Aims.* To compare protein expression profiles in CLL cells at diagnosis and after disease progression in patients with early, low-risk CLL. *Methods.* We studied 6 patients in early stage and without poor risk cytogenetics (i.e., +12, 11q-, 17p-) for which leukemic cells obtained at diagnosis and after disease progression were available. To make sure that patients had low-risk disease those progressing or receiving therapy within the first 2 years after diagnosis were not eligible for the study. Cell purity of samples at diagnosis and at progression was assessed (mean $CD5^+/CD19^+$ cells 90% and 91%, respectively). Protein expression profile was analyzed by using a Fluorescence 2-D Difference Gel Electrophoresis (DIGE). Image analysis and statistical quantification of relative protein levels were performed using DeCyder V. 5.0 software (GE Healthcare) and the identification of differential spots by MALDI-MS analysis. *Results.* Different protein expression patterns were observed between diagnosis and disease progression both in the whole group of patients (Table 1) and in paired samples. Proteins undergoing significant changes in their expression levels have been involved in the ubiquitin-dependent degradation pathway, carbohydrate metabolism, and RNA cytoplasmic transport. In addition several of the proteins identified, such as EF-2, HNR, annexin A1 and nucleophosmine, have a role in cell proliferation and human oncogenesis. *Conclusions.* This study identified changes on the expression of a group of proteins that are part of important oncogenic pathways in CLL progression. The analysis of the exact role of these proteins in CLL disease progression deserves further investigation.

Table 1.



0515

TELOMERASE AS A NEW PROGNOSTIC FACTOR IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Activation of telomerase reverse transcriptase (hTERT), the catalytic rate-limiting component of the telomerase complex, is essential for unlimited cell growth and plays a critical role in tumorigenesis. *Aims.* The aim of the present study was to determine hTERT gene expression in B-cell Chronic Lymphocytic Leukemia (B-CLL) and to evaluate its prognostic value. *Methods.* We investigated hTERT gene expression in B-CLL and evaluated its prognostic value. Because hTERT mRNA undergoes alternative splicing as a regulatory mechanism of hTERT, real-time PCR assays to quantify either all hTERT transcripts (AT) or only the full length (FL) transcript encoding the functional protein were developed. *Results.* hTERT transcripts were quantified in 134 B-CLL cases, and compared them with other prognostic markers including IgVH mutation status, CD38 and ZAP-70 expression. hTERT-AT levels strongly correlated with hTERT-FT levels(r=0.743, p<.0001); both inversely correlated with the percentage of IgVH mutation (p<0.005) and were significantly higher in unmutated IgVH than in mutated B-CLL cases (p=0.004 and p=0.001, respectively). From ROC curve analyses, the hTERT values which best discriminated between the unmutated and mutated IgVH cases were 150 and 40 copies for hTERT-AT and hTERT-FL, respectively. Using these cut-off values, there was a significant difference in the survival of patients with high or low hTERT levels (p<0.0001). Interestingly, hTERT identified different groups of patients with poorer or better prognosis in IgVH mutated and unmutated cases; the unmutated cases with low hTERT level had an overall survival close to the mutated cases with high hTERT level. *Conclusions.* This work identifies hTERT as a new prognostic marker in B-CLL, and it may be used to identify previously unrecognized patient groups with the same IgVH mutation status and different disease outcomes.

0516

CORRELATION BETWEEN CD4+FOXP3+ REGULATORY T CELLS AND ZAP-70 EXPRESSION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Although several immune abnormalities are recognized in CLL patients the relationship between the activated T cells and the B -CLL cells is poorly understood. T-regulatory cells (Treg) are a distinct subset of $CD4^+/CD25^{high}/FOXP3^+$ T lymphocytes essential for down-regulating immune responses to self antigens . Tregs also suppress activation of both $CD4^+$ and $CD8^+$cells in an antigen independent manner and their reported increase in numbers have been considered as another mechanism for reducing immunity. The activation of T and B-CLL cells described in CLL patients indicates that both activated T cells and Tregs may be involved in the pathogenesis of CLL. Expression of ZAP-70 in B-CLL cells is regarded as a negative prognostic factor in CLL. This tyrosine kinase mediates TCR signaling and is structurally homologous to Syk, playing a role in BCR signaling. We reported that high ZAP-70 expression in B-CLL cells correlated with high ZAP-70 expression in T cells, implying elevated T-cell activation in these CLL patients. This positive correlation between ZAP-70 expression in B-CLL cells and T-cells in CLL may imply dual activation , involving T-cell/B -CLL cell interaction and may suggest that this crosstalk may affect clinical outcome in these patients (1) Additionally, an increase in the percentage of $CD4^+CD25^+$ Tregs in the blood of CLL patients was recently reported (2). *Aims.* The aim of this study was to determine whether there is a correlation between Tregs and ZAP-70 expression in B-CLL cells and T cells in CLL patients. *Methods.* Blood samples were collected from 32 newly diagnosed untreated CLL patients and healthy individuals. The diagnosis of CLL was based on standard morphologic and immunophenotypic criteria. Quantitative analysis of the intracellular levels of ZAP-70 was performed by flow cytometry MESF (3). CD4, CD25 and the transcription factor FOXP3 in the blood of CLL patients and viability of the lymphocyte populations (7AAD staining) were measured by flow cytometry. *Results.* $CD4^+/FOXP3^+$cells and $CD4^+CD25^{high}/FOXP3^+$ cells display highly significant elevations in CLL patients compared to healthy individuals, similar to the previously reported increase in $CD4^+/CD25^{high}$ cells(2). Both the $CD4^+/FOXP3^+$ cells and $CD4^+CD25^{high}/FOXP^{3+}$ were proportionately higher in ZAP-70+ patients than in ZAP-70- patients (7.1% vs 4.8%, p=<0.05), showing a correlation of Treg increase with ZAP-70 expression. ZAP-70 was also elevated in T cells of patients with high ZAP-70 in the B-CLL population. The percentage of $7AAD^+$ T cells in patients, was the same as that in normal blood (3%-5%). Moreover, no difference was seen in the viability of the Treg populations from $ZAP\text{-}70^+$ or $ZAP\text{-}70^-$ patients, indicating no difference in the viability of T cells in CLL patients. *Conclusions.* The number of $CD4^+CD25^+FOXP3^+$ Tregs is increased in CLL patients. We report for the first time that this is more prevalent in the cells from patients with elevated ZAP-70 levels, showing a correlation between ZAP-70 expression and the numbers of Tregs. These findings indicate that dual T and B-CLL cell activation may involve a simultaneous proliferation of Treg cells in CLL , which could regulate the immune response in these patients.

0517

IL-21 AND IL-15 EXERT OPPOSITE EFFECTS IN CHRONIC LYMPHOCYTIC LEUKEMIA (APOPTOSIS VERSUS SURVIVAL) AND ACTIVATE DIFFERENT SIGNALING PATHWAYS

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Background. Cytokines secreted in the microenviroment may influence natural hystory of B cell chronic lymphocytic leukemia (B-CLL). CLL B cells are prone to undergo apoptosis when cultured *in vitro*, but their survival can be extended when leukemic cells grow in contact with stromal cells present in the cultures. We recently reported that IL-21 induces apoptosis of CLL B cells, in particular after CD40-CD40L interaction which significantly upregulated IL-21R. IL-15, a cytokine with structural homology to IL-21, instead, enhances survival and induces proliferation of CD40-activated CLL B cells. *Aims.* We studied differential activities of IL-21and of IL-15 on CD40-activated CLL B cells. We further tried to identify differential signaling pathways triggered by the two cytokines leading to apoptosis or, on the contrary, to survival. *Methods*. Annexin-V/PI staining and subsequent cytofluorographic analyses were performed to determine the percentage of CD40-activated CLL B cells that undergo apoptosis or, on the contrary, that are rescued from apoptosis following cultures with IL-21 or with IL-15. CLL samples were in parallel studied by Western Blot to detect phosphorylation of JAK and STAT molecules after IL-21 and IL-15 treatments. Activity of IL-15, with or without JAK inhibitor or moAbs against β/γ chains of the IL15R, was also evaluated in proliferative assays. *Results.* IL-15R, the heterotrymeric complex consisting of IL-15R α chain, IL-15/IL-2R β chain (CD122), and the common IL-15R/IL-21R γ chain (CD132), was upregulated on CLL B cells after CD40 activation and, according to higher expression of the receptor, survival and mitogenic effects induced by IL-15 were stronger on activated than on resting cells. When, however, IL-15 and IL-21 were added in combination, IL-21counteracted IL-15 survival effects. In line with the observed opposite effects exerted by the two cytokines, the pathways of signaling induced by IL-15 and IL-21 showed relevant differences. JAK-3 was activated by both IL-21 and IL-15 while JAK-1 activation was evident only after IL-21 treatment. In addition STAT-1 was tyrosine-phosphorylated by IL-21 and not by IL-15, while STAT-5 was preferentially activated by IL-15, and STAT-3 preferentially phospsophorylated by IL-21. Proliferation of CLL B cells, induced by IL-15, was blocked by addition of antibodies against β/γ chains of the IL-15R or of JAK3 inhibitor (AG490). In agreement with proliferative data addition of AG490 and of anti-β/γ chains of the receptor counteracted the IL-15-survival effect on anti-IgM-induced apoptosis of CD40-activated CLL B. *Summary and Conclusions.* IL-15 and IL-21 show antagonistic effects on CD40-activated CLL B cells. Opposite effects of IL-15 and of IL-21 can be related to activation of different signaling molecules such as phosphorylation of JAK-1 and of STAT-1 by IL-21 only. IL-15, which can be present at level of bone marrow (stromal cells) and of germinal center (follicular dendritic cells), can have a role in trafficking and in expansion of the malignant B cell clone. Deeper understanding of the pathways leading to proliferation or, on the contrary, to apoptosis could be helpful in designing novel therapeutic strategies.

0518

SERUM AND CELLULAR EXPRESSION OF THROMBOPOIETIN IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA IN COMPARISON WITH IMMUNOGLOBULIN HEAVY-CHAIN MUTATION STATUS

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We analyzed the correlation between well-established clinical (i.e., Rai substages, β2-microglobulin, LDH) or biological parameters of prognostic relevance (i.e, mutational status of the immunoglobulin heavy chain variable region [IgVH], ZAP-70- and CD38-expression) and serum levels of thrombopoietin (TPO) in a series of 71 previously untreated Binet stage A B-cell chronic lymphocytic leukemia [CLL]B-cell CLL patients. Serum levels of TPO did not correlate with peripheral blood lymphocytosis (p=0.928), Rai substages (p=0.516), platelet count (p=0.572), hemoglobin level (p=0.228), LDH (p=0.144) and β2-microglobulin (p=0.520). The same applied when correlation with ZAP-70 (p=0.562), CD38 (p=0.258) or mutational status of IgVH (p=0.0794) were sought. After a median follow-up time of 32 months (range, 2-180 months) 28 (39.4%) out of 71 patients experienced a need for chemotherapy. Kaplan-Meier estimates of time to first treatment [TFT] , plotted after setting a cutoff at the median value for TPO (i.e., 46 pg/mL), failed to demonstrate any statistical difference between two groups [Hazard ratio (HR),1.42; 95% Confidence interval (CI), 0.67-3.15; p=0.342]. Similar results were found for a cut-off corresponding to 25th and 75th percentile, respectively. Gene expression of purified cells from 60 B-CLL patients were profiled using high density oligonucleotide microarrays. Normalized expression values for TPO gene transcipts denoted a very low expression in B-CLL cells. Furthermore, we wondered whether the pattern of distribution was homogeneous among patients with different biological profile. The analysis carried out after stratifying patients according to mutational status of IgVH showed a relatively higher normalized expression values of TPO gene transcript in patients with mutated CLL (p=0.01; Mann-Whitney test). Our results indicate that in early B-cell CLL clinico-biological profile including among other parameters TPO does not provide a useful insight into the complex interrelationship of prognostic variables. As a matter of fact, TPO may not replace the need for the determination of IgVH mutational status, as suggested by MD Anderson group (Blood 2006,108:1001-6). Finally, TPO gene transcipts is low in B-CLL cells although patients with mutated CLL display a relatively increased expression.

0519

THE CLINICAL SIGNIFICANCE OF MEMBRANE CD26 (DIPEDTIDYL PEPTIDASE IV) EXPRESSION IN B-CLL

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Background. CD26 (dipeptidyl peptidase IV) is a type II transmembrane glycoprotein with prolyl oligopeptidase activity and diverse biological functions including T-cell activation, chemokine regulation and is a receptor for adenosine deaminase (ADA). The role of CD26 in tumour cell transformation and development appears multi-faceted. Loss of CD26 expression has previously been associated with malignant transformation in melanoma. Conversely, CD26 appears to be differentially expressed in T-cell malignancies and has been associated with reduced survival and shorter complete remissions in T-cell NHL. CD26 is also expressed on normal B-cells. However, the significance of its expression in malignant B-cell disorders has not been elucidated. *Aims.* The aim of this study was to assess the clinical and biological significance of membrane-associated CD26 expression in B-CLL. *Methods.* Two hundred and thirty-two B-CLL patients were recruited for this study. Information on age at diagnosis, Binet clinical stage and time to treatment was available on all patients. Membrane expression of CD26 was assessed by 2-colour flow cytometric analysis of CD19 positive cells using anti-CD26PE. A value of ≥15% positivity was assigned to define a sub-group of patients (high CD26) with a relative increase in surface CD26 expression. IgVH mutational status and gene usage were determined using multiplex BIOMED-2 primers (InVivoScribe Technologies) and protocol and by sequence analysis. Interphase FISH analysis was performed to screen for common cytogenetic aberrations. *Results*. A statistically significant increase in the proportion of males was detected in the high CD26 sub-group (80% versus 58.3% in the low CD26 sub-group, p<0.0001). In addition, increased CD26 expression was associated with advanced stage disease (31.6% versus 19.9%) requiring chemotherapeutic treatment (47.4% versus 29.1%). No association was observed between CD26 membrane expression and age at diagnosis nor with cytogenetic abnormalities commonly observed in B-CLL. However, somatic hypermutation of the IgVH gene was significantly more frequent in the low CD26 sub-group (p<0.0001). Preliminary results also indicate that IgVH 4-34 gene usage is associated with low CD26 membrane expression. In addition, IgVH 3-21 gene usage (n=19) was not detected in the high CD26 sub-group. *Summary/Conclusions* In our B-CLL cohort, increased CD26 B-cell membrane expression was associated with the male gender, un-mutated IgVH gene status and more advanced Binet stage requiring treatment, irrespective of age upon presentation. Interestingly, CD26 expression has previously been associated with unfavorable prognosis in T-cell malignancies and poor response to 2'-deoxycoformycin, an inhibitor of the CD26 ligand ADA. In contrast, CD26-positive hairy cell leukaemia responds favorably to 2'-deoxycoformycin treatment. The significance of CD26 expression in B-cell malignancies remains to be fully elucidated. The possibility that it may be linked to responsiveness to treatment with the ADA inhibitor 2'-deoxycoformycin is intriguing and warrants further investigation.

0520

EXPRESSION OF INHIBITOR OF APOPTOSIS PROTEIN (IAPS) FAMILY AND ITS ANTAGONISTS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS IN RELATION TO THE DISEASE ACTIVITY

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Apoptotic mode of the cell death is a major regulatory process in all complex organisms. The slow accumulation of malignant cells in chronic lymphocytic leukemia (CLL) suggests that the disease is caused by a defect in apoptosis regulation. Apoptosis is executed through the activity of caspases, cysteine proteases which are regulated by a number of pro- and anti-apoptotic proteins. One such checkpoint is the control of caspase activation by relatively new family of apoptosis inhibitor of apoptosis proteins (IAPs). IAPs and their antagonists were poorly explored in CLL up to date. Majority of them were not investigated in this disease yet. Therefore, we aimed to perform a complex analysis of their expression in relation to the clinical course of the disease. Fifty six patients with CLL were included to the study. Patients were divided into two groups: with stable (N=31) and progressive disease (N=25). Expression of IAPs (cIAP1, cIAP2, XIAP, and survivin) and their inhibitors (Smac/Diablo and Htra2/omi) in CLL cells were investigated using flow-cytometry, after careful comparative studies by Western blot method. Protein expression was expressed as the mean fluorescence ratio (MFI). Leukemic cells from all patients studied were examined before treatment. Results were compared with several disease-related parameters, such as clinical stage according to Rai, ZAP-70, CD38 or β-2-microglobulin concentration. The secondary end-point of the study is to compare the results with outcome in the examined group. Patients with progressive disease (PD) showed significantly lower expression of two IAP antagonists, Htra2/omi and Smac/Diablo, in comparison to those with stable disease (SD). Median MFI levels for Htra2/omi and Smac/Diablo were 113.4 vs. 156.2 and 97.3 vs. 218.2, respectively (p=0.038 and 0.034, respectively). Lower expression of these pro-apoptotic proteins in the PD group before treatment, may suggest inhibition of CLL cells apoptosis in these patients. Moreover, PD patients showed a distinct trend toward higher expression of anti-apoptotic survivin (median MFI 69.3 vs. 29.8; p>0.05). Consequently, expression of Smac/Diablo correlated negatively with survivin levels (R=-0.44, p=0.0007). Among disease-related parameters Smac/Diablo correlated negatively with clinical stage according to Rai (R=-0.039, p=0.0.003) and ZAP-70 expression (R=-0.32, p=0.042). On the other hand, in patients with SD we found higher level of XIAP and cIAP2. Median MFI for XIAP and cIAP2 in SD vs. PD patients were 71.4 vs. 33.0 and 62.5 vs. 28.3, respectively (p=0.011 and 0.008, respectively). Moreover, cIAP2 correlated positively with β-2-microglobulin concentration (R=-0.42, p=0.015). Actually, the follow up of the study group is ongoing in terms of correlation between examined protein expression and patient fates, including outcome in those required treatment. In conclusion, this is the first comprehensive study on IAPs and their antagonists expression in CLL. The data suggest the connection between expression of these apoptosis-regulating proteins and activity of the disease. However, our results indicate also that these correla-

tion are very complex. Further studies on IAPs/IAP antagonists system in CLL to elucidate these mechanisms and to assess its potential prognostic usefulness seem to be warranted.

0521

THE EFFECT OF THE B-CLL MICROENVIRONMENT ON CD38 EXPRESSION BY THE MALIGNANT CLONE

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In order to determine whether microenvironment derived signals might influence CD38 expression in B-cell chronic lymphocytic leukemia (B-CLL), we compared levels of this molecule in peripheral blood (PB), bone marrow (BM), splenic red pulp (RP) and splenic white pulp (WP). In 35 paired BM and PB samples, significantly higher CD38 expression was observed on BM B-CLL cells compared to PB (27% vs 19%, p=0.009). Five splenic samples infiltrated with B-CLL were examined by confocal immunofluorescence microscopy. In three cases with detectable CD38 expression, a significantly higher percentage of CD38 was found expressed by tumor cells in the WP, an area containing Ki67$^+$ tumor cells and T-cells, compared to RP. We therefore hypothesized that non-malignant cell derived signals within the leukemic microenvironment might be responsible for the level of CD38 expression in B-CLL. To test this theory, we examined the effect of co-culture of B-CLL cells with T lymphocytes using an in-vitro system aimed at mimicking the tumor microenvironment. B-CLL cells were cultured at a 4:1 ratio with CD3/CD28 bead activated autologous T-cells. CD38 expression by B-CLL cells increased significantly in 15/15 cases over the 6-day culture period, an effect most marked with cell to cell contact. Proliferation of tumor cells was also induced and was more marked in cases with higher initial levels of CD38. A parallel reduction in apoptosis was also observed. Immunofluorescence microscopy of B-CLL lymph node showed that Ki67$^+$ tumor cells were significantly more likely to be in close contact with activated CD4$^+$ T-cells than Ki67$^-$ cells (p<0.0001 for all cases assessed). In 8 lymph nodes examined, 6 had detectable CD38 staining and in these cases the Ki67$^+$ tumor cells expressed CD38. We next examined CD31 expression, constitutively expressed on endothelial cells and the only known ligand for CD38. Assessment of the vascularity within infiltrated lymph node showed significantly increased amount of CD31 expressing endothelium on cases with detectable CD38 compared to negative cases (p=0.0078). High power confocal views showed areas containinh Ki67$^+$ tumor cells in close contact with both T-cells and vessels. Strongly CD38$^+$ tumor cells were also detectable in such areas. Our *in vitro* model and other findings show that CD38 expression in B-CLL is dynamic and CD38 levels predict for B-CLL proliferation. Furthermore, the level of CD38 expression may be influenced by both activated T cells and CD31 expressing endothelium. Expression of this molecule in the peripheral blood may therefore serve as a surrogate marker for the extent of proliferation and survival signals provided by non-malignant cells in the leukaemic microenvironment, and give a biological explanation for why CD38 is an independent prognostic factor in the disease.

Figure 1.

0522

CLLU1 EXPRESSION DISTINGUISHES B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA FROM OTHER B-CELL LYMPHOPROLIFERATIVE DISORDERS

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Background. Distinction of B-cell chronic lymphocytic leukemia (B-CLL) from other B-cell lymphoproliferative disorders, especially from leukemic forms of mantle cell or marginal cell lymphoma, can be a challenge, and the correct diagnosis has important prognostic and therapeutic implications. Recently, a novel gene called CLL upregulated gene1 (CLLU1), has been shown to be exclusively upregulated in B-CLL cells. Six transcripts of the CLLU1 gene have been identified, one of which has been shown to be of prognostic significance in B-CLL when overexpressed (Buhl AM, *et al.* Eur J Haematol 2006). Low expression levels of CLLU1 have been detected in single cases of other B-cell lymphoproliferative disorders and in normal blood cells. *Aims.* Our study aimed to assess the specificity and sensitivity of the CLLU1 expression and its utility in distinguishing B-CLL from other leukemic B-cell lymphoproliferative disorders. *Methods.* CLLU1 expression levels were measured by quantitative RT-PCR in a total of 96 patients with lymphoproliferative disorders including B-CLL (n=71), mantle cell lymphoma (n=14), diffuse large B-cell lymphoma (n=5), follicular lymphoma (n=5) and marginal cell lymphoma (n=1). In addition, CLLU1 expression of healthy controls (n=17) was measured and a range for the normal expression was defined. *Results.* Overexpression of CLLU1 was observed in 60/71 cases of B-CLL (85%). 15% of B-CLL cases and 32% of B-cell lymphomas exhibited CLLU1 expression within the normal range. Interestingly, in the majority of the lymphoma patients (17/25), no CLLU1 expression was detectable. Our results demonstrate a specific expression pattern of CLLU1 in B-cell lymphoproliferative disorders: The expression of CLLU1 was high in patients with B-CLL (Median: 185.2, range: 0.08-18879), low in patients with B-cell non-Hodgkin lymphoma (Median: 0.0001, range: <0.0001-8.7) and intermediate in healthy controls (Median: 0.53, range: 0.02-3.18). All three groups differed significantly from each other (p<0.01). CLLU1 overexpression was highly specific for B-CLL (specificity: 0.96, sensitivity: 0.85, positive predictive value: 0.98, negative predictive value: 0.68), whereas absent CLLU1 expression was highly predictive for B-cell non-Hodgkin lymphoma (specificity: 1, sensitivity: 0.68, positive predictive value: 1, negative predictive value: 0.91). Clinically, high CLLU1 expression levels have been shown to identify patients with an unfavorable disease. In our cohort of B-CLL patients, high CLLU1 expression was significantly associated with known bad prognostic markers such as unmutated IgVH (p<0.01), high expression of CD38 and ZAP70, and the unfavorable cytogenetic aberrations del(17p) and del(11q), confirming the prognostic value of this molecular marker. *Conclusions.* CLLU1 overexpression is highly specific for B-cell chronic lymphocytic leukemia, and lack of CLLU1 expression is significantly associated with B-cell non-Hodgkin lymphomas. Thus, analysis of CLLU1 expression by RT-PCR is a simple novel diagnostic tool that enables the distinction of B-CLL from other B-cell lymphoproliferative disorders, especially in cases where known diagnostic parameters fail to establish the diagnosis.

0523

DNA REPAIR INHIBITION IN RESTORING CHRONIC LYMPHOCYTIC LEUKEMIA LYMPHOCYTES SENSITIVITY TO FLUDARABINE AND RITUXIMAB

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Background. Combined application of cytotoxics and monoclonal antibodies not always enhances therapeutic activity in leukemia cells. Modified DNA repair contributes to resistance development in chronic lymphocytic leukemia (CLL) lymphocytes. Majority of cytotoxic drugs, including fludarabine (Frn), influence DNA synthesis and repair. Rituximab (Rtx) has multiple mechanisms of inducing *in vivo* cytotoxicity and direct apoptotic signaling is one of them. Lack of appropriate response to Frn and Rtx can be associated with enhanced DNA repair in peripheral blood lymphocytes. Usage of DNA-dependent protein kinase (DNA-PK) inhibitor vanillin (Vnl) may contribute to Frn and Rtx resistance overcoming. *Aims.* To investigate CLL cell response *in vitro* to the action of Frn and Rtx under the DNA repair inhibition with vanillin. *Methods.* MTT assay was used to evaluate cell sensitivity to purine nucleoside analog and monoclonal antibody (in concentrations similar to therapeutic), vanillin (in nontoxic concentration to normal lymphocytes) *in vitro*. According to lymphocyte viability, cell samples were divided into groups with different sensitivity to Frn and Rtx: sensitive (cell viability less than 35%), intermediate (35-70% viable cells) and resistant (cell viability more than 70%). *Results.* Vnl not significantly enhanced Frn activity in CLL cells irrespective of lymphocyte response to Frn (shown in table). In cells with high and intermediate sensitivity to Rtx negligible protective effect of Vnl was detected. On the contrary, in Rtx resistant CLL lymphocytes combined action of Vnl and Rtx significantly suppressed cell viability twice as much as a monoclonal antibodies alone. In Frn resistant group strong correlations between cell sensitivity to Vnl and Frn were observed (r=0.83-0.93, p<0,05), whilst no significant correlation was detected between Rtx and Vnl action (p>0.05). Correlation analysis confirmed unidirectionality of CLL cells response to Van and Frn, and lack of interdependence between Van and Rtx action. Cell sensitivity and clinical data dependence was studied. No significant correlations were observed in cell susceptibility to drugs and peripheral blood $CD5^+$ and $CD20^+$ cell number. Apoptosis marker bcl-2 level was also measured. Intrinsic bcl-2 level *ex vivo* didn't correlate with cell sensitivity to Frn, Rtx, and Vnl. Most likely Frn and Rtx don't directly influence bcl-2 pathway. Consequently endogenous bcl-2 lymphocyte level can not be used as strong prognostic factor for cell sensitivity. *Summary and conclusions.* Vanillin is an effective agent to overcome Rtx resistance in CLL lymphocytes, but it doesn't influence Frn activity. DNA repair inhibition is one of the possible pathways that leads to lymphocyte sensitivity restoration to Rtx, where as Frn unreceptiveness overcoming is achieved with DNA-PK inhibitor suggesting another pathways of Frn resistance formation.

Table 1. Viability modification by vanillin in cells with different sensitivity to Frn and Rtx.

Cell sample	Viable cells (%) under the influence of		Cell viability modification under the influence of Vnl	Viable cells (%) under the influence of		Cell viability modification under the influence of Vnl
	Frn	Vnl+Frn		Rtx	Vnl+Rtx	
Sensitive	21.0	13.4	7.6 ↓	9.3	15.5	6.2 ↑
Intermediate	48.7	40.2	8.5 ↓	53.3	59.5	6.2 ↑
Resistant	75.1	70.0	5.1 ↓	86.4	42.5	43.9 ↓↓↓ *

↓ - cell viability decrease; ↑ - cell viability increase; *- difference is significant, p<0.001

0524

EFFICACY OF FLUDARABINE IN A NOVEL NOD/SCID XENOTRANSPLANTATION MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) represents the most frequent leukemia in the western world. Although improvements in therapeutic strategies has been achieved in recent years, this desaese still remains incurable. Therefore novel therapeutical stragegies to improve patient outcome need to be established. However, preclinical *in vivo* drug testing, a crucial prerequisite for the developpent of such novel strategies in CLL, so far still is hampered by the lack of a suitable animal model. We have previously reported reliable engraftment of CD19/CD5/CD23/CD45 positive human B-CLL cells in the spleens of NOD/SCID mice (NSS-CLL cells) following i.v. and/or i.p. transplantation of 1×10^8 peripheral blood derived mononuclear cells from CLL patients (PB-CLL). On histology, in the majority of the samples, NSS-CLL cells formed focal aggregates which also contained various amounts (range:0-50%) of human $CD45/CD3^+$ T cells (ASH 2005; #52). *Aims.* We now have further characterized these NSS-CLL cells. Additionally, drug testing experiments were performed to determine if this novel model may be suitable to preclinically investigate therapeutic effects of established treatment strategies in CLL. *Methods.* For further characterization of NSS-CLL cells, a panel of surface and intracellular marker molecules like Ki67, Survivin, CD100, CD10, CD11c, CD127 and CD49d was utilized. Drug testing experiments in this model were performed using fludarabine (Flud), a chemotherapeutical agent with proven activity against CLL. For theses latter experiments 1×10^8 PB-CLL were injected i.v. into the tail vein of NOD/SCID mice. Starting two weeks after transplantation the animals were allocated to receive therapy with either Flud or no therapy, respectively. Flud was given at a dosis of 35mg/kg ip. for 5 consecutive days. Four weeks after transplantation animals were sacrificed and BM and spleens were analyzed for the presence of CLL cells using multicolor flow cytometry. *Results.* Concerning the further characterization of NSS-CLL cells, immunohistological analysis showed positive staining for the proliferation marker Ki67 in 18.5±3.5% of these cells. Further, markers related to proliferation/activation of B-CLL proliferation center cells such as survivin (mean MFI: 5.5±0.8 vs. 2.3±0.2; p=0.028; n=6) and CD100 (semaphorin 4d; mean MFI: 3.3±0.5 vs. 1.1±0.2; p=0.043; n=5) were significantly up-regulated in NSS-CLL vs. PB-CLL cells. In contrast, no expression of human CD10, CD11c, CD127 or CD49d was detected in B-CLL cells derived from either PB- or NSS-CLL cells, while both sources uniformly stained positive for CD40. With regard to 8 individual drug testing experiments a significant lower amount of 2.2±1.2 vs. $11.4\pm2.4\times10^5$ (p=0.003) CLL cells could be recovered from the spleens of treated animals when compared to the control group. Thus, treatment with Flud resulted in a relative reduction of total amount of CLL cells recovered from the murine spleen of 85.5±8.1% (p=0.003). *Summary.* NSS-CLL cells display certain similarities of CLL proliferation centers like expression/upregulation of proliferation/activation markers and therefore this model could serve as a valuable tool to investigate CLL tumour biology. In addition the model allowed for reliable *in vivo* drug testing of a conventional therapeutic agent and thus argues for a preclinical evaluation of this new model also to investigate novel chemotherapeutic strategies.

0525

CD74 IS A NOVEL SURVIVAL RECEPTOR IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Previous studies have shown that chronic lymphocytic leukemia (CLL) lymphocytes express relatively large amounts of CD74 mRNA compared to normal B cells. We have recently demonstrated in a murine model that CD74 stimulation with anti-CD74 antibody leads to an induction of a signaling cascade resulting in NF-κB activation, entry of the stimulated cells into the S phase, elevation of DNA synthesis, cell division, and augmented expression of BCL-XL. These findings therefore demonstrated that surface CD74 functions as a survival receptor. *Aims.* In the current study we aimed to determine whether activation of cell surface CD74 in B-CLL cells leads to induction of a signaling cascade resulting in cell survival.

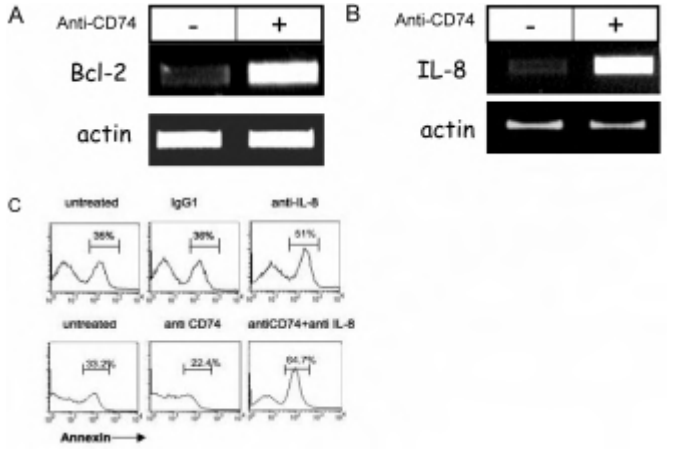

Figure 1. A) CD74 stimulation induces IL-8 and Bcl-2 expression in CLL B cells. Cells were incubated in the presence or absence of anti-CD74 antibody, Id2, a control antibody or MIF for 18 h. RNA was purified and levels of IL-8, Bcl-2 and actin mRNA were analyzed. The results presented are representative of 12 CLL patients. B) The conditioning medium of the cells was collected, and the level of IL-8 was assessed by ELISA. The results are representative of 3 independent experiments. C) IL-8 secreted following CD74 stimulation regulates B-CLL cell survival. CLL cells were incubated in the presence or absence of an agonistic anti-CD74 antibody, anti-IL-8 or a control antibody (c-jun) for 48 h. Cells were stained with annexin V and analyzed by FACS. The results presented are representative of 7 CLL patients.

Materials and Methods. B cells were purified from the peripheral blood of CLL patients in different stages, in accordance with the IRB of our hospital. CD74 stimulation was achieved using an agonistic anti-CD74antibody or MIF (a natural occurring ligand of CD74). IL-8 expression and function was determined by RT-PCR, western blot, ELISA and Annexin V staining. *Results.* In all the cells obtained from CLL patients there was a significantly increased expression of cell surface CD74, as compared to normal B cells. Activation of cell surface CD74 initiated a signaling cascade that resulted in increased expression and secretion of Interleukin 8, as well as over-expression of bcl-2 (see panels A, B in Figure 1). Stimulation of CD74 led to increased cell survival, as seen by annexin staining, whereas blocking of IL-8 led to increased apoptosis (panel C in Figure 1). *Conclusions.* Our data show that over-expression of CD74 in CLL is an important survival mechanism, operational from the very early stages of the disease, and inherent in all further stages. This survival mechanism thus appears to be an early and significant event in the pathogenesis of the disease. Our findings open prospects for novel therapeutic strategies aimed at interruption of this survival pathway.

Chronic myeloid leukemia - Biology

0526

OVER EXPRESSION OF THE ABC-TRANSPORTER BCRP, MRP1 AND MDR1 IN CML PATIENTS AT DIAGNOSIS, WHO RESPOND AND WITH ACQUIRED RESISTANCE TO IMATINIB MESYLATE

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IM is widely used for the treatment of CML inhibiting the BCR-ABL tyrosine kinase activity. Although, 90% of the patients respond to IM, resistance to this drug has been seen mainly due mutations in ABL tyrosine kinase domain. Recently, it was reported that IM is a substrate for the ABC-transporters BCRP (breast cancer resistance protein), MRP1 (multidrug resistant protein 1) and MDR1 (P-Glycoprotein). We studied 29 patients (9 CP, 17 AP, and 3 BC) who initiated treatment with IM after 49 mo (±10 mo) from diagnosis and develop resistance 31 mo (± 4mo) after beginning of therapy. To compare the expression of the resistant group, 34 CML patients at diagnosis (23 CP, 5 AP, and 2 BC) and 29 good responders to IM (22 CP, 5 AP, and 2 BC) with a mean time from diagnosis to initiation of IM of 29 mo (±6,6 mo). To detect the expression of those ABC-transporters RNA was extracted and cDNA was synthesized and amplified through real time PCR applying SYBR Green as a dye and reported as the 2- Ct where it is assumed that the amplification efficiency of the target gene (BCRP, MRP1 or MDR1) and the internal control gene (ABL) are the same. Over expression of BCRP was observed in 65.5% of the resistant group (4 CP, 12 AP, and 3 BC) when compared to 11,8% of patients at diagnosis (2 CP, 1 AP, and 1 BC), and to 10,3% of patients that respond to IM (3 CP) (Kruskal-Wallis test, $p<0.05$). MRP1 expression was detected in 51,7% in the resistant group (4 CP, 9 AP, and 2 BC), 10,3% in the responder group (1 CP and 2 AP), and no expression was detected in the group at diagnosis (Kruskal-Wallis test, $p<0.05$). For the MDR1 expression no difference was seen between the 14/29 resistant (4 CP, 9 AP, and 1 BC) patients and the 9/29 responder to IM (6 CP, 2 AP, and 1 BC), but when compared to the 6/34 patients at diagnosis (3 CP and 3 AP) a statistical difference was noted (Kruskal-Wallis test, $p<0.05$). Over expression of BCRP was more frequent in AP of the resistant group and MRP1 expression was not detected at diagnosis. For MDR1 expression no difference was seen among the distinct groups and at different phases. These data confirms that over expression of BCRP and MRP1 may contribute to imatinib resistance leading to a reduction in intracellular concentrations of IM. MDR1 expression could not be considered as a mechanism of resistance to IM in CML.

0527

THE POLYCOMB GROUP BMI-1 GENE IS A MOLECULAR MARKER FOR PREDICTING PROGNOSIS OF CHRONIC MYELOID LEUKAEMIA

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Background. Despite a consistent molecular abnormality, the BCR-ABL oncogene, CML exhibits marked clinical heterogeneity, and various attempts have been made to determine prognosis for individual patients at the time of diagnosis in chronic phase (CP). The PcG gene BMI-1 plays an essential role for the self-renewal of both hematopoietic and neuronal stem cells, as well as cancer stem cells. Coexpression of Bmi-1 and other proteins from the PcG confers a higher degree of malignancy. *Aims.* Because Bmi-1 regulates the proliferation of both normal and leukemic stem cells, we examined whether Bmi-1 might serve as a biomarker to predict disease aggressiveness and progression from CP to more advanced phases. *Methods.* Two independent cohorts of CML patients were studied: 1) patients in CP whose nucleated cells were collected by leukapheresis before start of treatment, and for whom complete follow-up was available (n=64); 2) patients with cryopreserved cells collected at CP or blast crisis (BC). Expression of BMI-1 and other genes of interest was assessed by Q-RT/PCR using the ABI *Assays-on-demand* TaqManTM probe-and-primer reagents according to the manufacturer's instructions. *Results.* BMI-1 expression levels in $CD34^+$ cells were significantly lower in CP than in more advanced stages (accelerated phase and BC) of CML ($p=0.006$). The same significant difference

held true when BMI-1 expression was compared in unfractionated CML-derived PBMCs (p<0.0001). In order to gain insights into the mechanisms underlying BMI-1 upregulation in CML, we also assessed the expression of E2f-1, a transcription factor that was shown to directly regulate Bmi-1 activity. We found that PBMCs from CML patients (all disease stages) displayed significantly higher levels of E2F-1 as compared to healthy controls (p=0.001). Furthermore, we uncovered a significant difference in BMI-1 levels between patients with an *indolent* (survival for over 7 years prior to the onset of BC), or an *intermediate* (survival between 3 and 7 years without developing BC) clinical pattern as compared to those who had an *aggressive* (development of BC within 3 years of diagnosis) clinical evolution (p=0.01). Patients displaying a low BMI-1 expression level at diagnosis had significantly longer survival than other patients. (p=0.005). When BMI-1 was included in a Cox multivariate survival analysis model (together with the previously established prognostic markers, CD7, ELA-2, PR-3, and other relevant demographic and clinical parameters), the combination of low BMI-1 and high PR-3 expression levels was found to be a strong independent marker associated with significantly longer overall survival (p=0.001; RR=0.20, 95%CI; 0.08-0.54). *Conclusions.* Our observations suggest an important role for BMI-1 in CML pathophysiology and prognosis. Genetic alterations impairing E2F-1, BMI-1 and their downstream targets may render hematopoietic cells refractory to the induction of differentiation. From the clinical standpoint, our findings demonstrate that BMI-1 can serve as a novel biomarker to predict prognosis in CML, particularly in conjunction with the expression level of immune-related proteins such as Pr-3. Therefore, the prospective screening for BMI-1 expression in combination with other molecular markers can help refining CML disease staging and prognosis towards optimizing therapeutic interventions, including perhaps Bmi-1-targeted inhibitors.

0528

EXPRESSION OF PRO-APOPTOTIC BID AND BIK GENES IS DECREASED IN CHRONIC MYELOID LEUKEMIA AND LOW LEVELS OF BIK MRNA ARE ASSOCIATED WITH LACK OF RESPONSE TO IMATINIB TREATMENT

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Background. Chronic myeloid leukemia (CML) is a myeloproliferative disease in which survival of Bcr-Abl leukemic cells is mediated by modulation of pro-apoptotic and anti-apoptotic molecules. Fully understanding the basic apoptotic pathway and its regulation in Bcr-Abl cells might unveil novel targets for manipulation, which may be translated into novel therapies. *Aim and Methods.* The present study investigated the expression of six important pro-apoptotic genes, members of Bcl-2 family, in CML. bax, bad, bak, bid, bik and bimel gene expression was evaluated in 15 healthy controls (8 men, 7 women; median age 49y [range 25-72]), and in 71 CML patients (36 men, 35 women; median age 52y (range 23-73); 20 in chronic phase [CP], 15 in accelerated phase [AP], 9 in blastic phase [BP], 20 in complete cytogenetic remission [CCR], and 7 imatinib-refractory [IR]). Gene expression was assessed in peripheral blood mononuclear cells by quantitative real-time PCR, and results were given as relative expression (amplicon ratio: investigated gene/β-actin housekeeping gene). Kruskall-Wallis post-test followed by the Dunn test for multiple comparisons was used to look for statistical differences in gene expression between controls and CML patients (taken as a single group), between controls and each of the five above-mentioned CML subgroups, and between the different CML subgroups. *Results.* Median bik levels were: 0.46 in controls, 0.14 in the whole CML group, 0.11 in CP, 0.10 in AP, 0.14 in BP, 0.28 in CCR and 0.01 in IR patients. bid expression was 6.7 in controls, 1.6 in the whole CML group, 1.0 in CP, 1.7 in AP, 1.5 in BP, 2.9 in CCR and 1.0 in the IR subgroup. Significant reduction in bik (p<0.001) and bid (p<0.001) mRNA was observed in the CML group (taken as a whole) in comparison to controls. In addition, in subgroup analyses, a significant decrease of bik expression was also found in IR patients when compared to other CML subgroups and to controls (p<0.001 for all comparisons). No significant differences between controls and CML patients (neither taken as a whole nor as subgroups) were verified regarding bax, bad, bak and bimel gene expression (p>0.05 for all comparisons). *Conclusions.* Our data indicate that altered expression of bid and bik is a feature of CML, and encourage further exploration of the role played by these two pro-apoptotic genes in contributing to the apoptosis resistance phenotype observed in CML. Finally, the finding of bik down-regulation specifically in the subgroup of IR-CML patients suggests that bik may be an attractive therapeutic target to be investigated in CML refractory to imatinib.

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0529

ASSOCIATION BETWEEN GSTM1, GSTT1 AND GSTP1 GENETIC POLYMORPHISM AND RISK TO CHRONIC MYELOID LEUKEMIA

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Background and Aim of the study. Glutathione S- Transferases (GSTs) are a polymorphic group of enzymes involved in phase II detoxification of wide range of xenobiotics & chemotherapeutic agents. Polymorphisms associated with genes coding for glutathione S-transferase enzymes are known to influence metabolism of different carcinogens and have been associated with incidence of various types of cancer. Humans are polymorphic in their ability to detoxify intermediates, which in theory may explain differences in leukemia risk as a result of exogenous exposure. Allelic variants of these genes may result in less effective or absent enzymatic detoxification and thus increased susceptibility to Chronic Myeloid Leukemia (CML). The aim of our study was to investigate the role of GSTM1 & GSTT1 null genotype & functional Ile 105 Val polymorphism in GSTP1 as genetic risk factor for CML. *Methods.* DNA was isolated by standard proteinase K and phenol-chloroform method. Multiplex PCR was carried out to determine GSTM1 & GSTT1 null genotype. β globin was used as an internal positive control. PCR-(RFLP) was performed to investigate the GSTP1 allelic variants. PCR products were separated using 2% agarose gel. The relationship between GSTM1, GSTT1 and GSTP1 genotypes and risk of CML was assessed by means of chi square test. The odds ratio (OR) with 95% confidence limits was calculated by logistic regression. *Results.* The frequency of GSTM1 and GSTT1 null alleles in the controls was 24.7% (26/105) and 8.5% (9/105), respectively. A higher frequency of these genotypes was reported in CML patients, which was 30% (24/80) and 20% (16/80), respectively. In CML patients the frequency of GSTP1 homozygosity for the Ile105 wild-type allele, heterozygosity (Ile105/Val105), and homozygosity for the Val105 mutant allele is 79% (59/75), 19% (14/75), and 3% (2/75) respectively. Likewise in controls the frequency of GSTP1 homozygosity for the Ile105 wild-type allele, heterozygosity (Ile105/Val105), and homozygosity for the Val105 mutant allele is 86% (67/78), 13% (10/78), and 1% (1/78) respectively. *Conclusions.* The study provides valuable evidence based data from Indian population to the knowledge of GST polymorphism. There was no difference in the frequencies of the GSTM1 null genotype and the combined GSTM1 and GSTT1 null genotypes between patients and controls in the study. A higher frequency of heterozygosity of (Ile105/Val105), and homozygosity for the Val105 mutant allele in CML patients as compared to controls is reported, which was 22% and 14% respectively. Though the frequency for homozygosity for Val 105 mutant allele was not statistically significant but Odds Ratio = 2.27 projects two fold higher risk with CML patients carrying this mutation as compared to controls. However, statistical significance was found with GSTT1 null genotype frequency in CML patients as compared to controls [16/80 (20%) vs 9/105 (8.5%); OR=2.67, 95% CI: 1.03 - 7.01]. It projects a 2.67-fold increased risk for CML in individuals with GSTT1 null genotype as compared to those possessing both alleles of the gene.

0530

CCN3 REDUCES CLONOGENIC CAPACITY OF BCR-ABL+ CELLS

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Background. Chronic Myeloid Leukaemia (CML) is characterized by expression of the constitutively active BCR-ABL tyrosine kinase. Previously, we identified down-regulation of the negative growth regulator, CCN3, as a result of BCR-ABL kinase activity. CCN3 has a reciprocal relationship of expression with BCR-ABL and consequently reduced CCN3 expression is a prominent feature in both primary human CML cells and cell lines (McCallum *et al.*, Blood 2006; 108(5):1716-23). *Aims.* to investigate the functional consequence of expressing CCN3 in BCR-ABL+ cells. *Methods.* Colony formation assays were performed over a

period of 7 days to investigate CCN3 growth regulation. K562 cells were transfected with vector alone, vector containing CCN3 construct or with treated with Imatinib (1 micromolar) for 24h prior to plating in methyl cellulose. Primary human CML CD34+ cells were isolated from peripheral blood and treated with CCN3 (1 nanomolar) or imatinib (1 micromolar) for 24h before plating into culture. *Results.* K562 cells that had been transfected with vector alone or vector containing full-length CCN3 were compared to cells treated with Imatinib. Increased CCN3 expression significantly reduced colony formation by 65.4%±18.8 when compared to cells transfected with vector alone (p=0.027, n=3). Treatment with Imatinib also reduced colony formation (75%±8.2; p=0.001, n=3) compared to untreated cells. Full-length CCN3 comprises 5 domains. To further characterise the functional component within CCN3, partial length constructs encoding domains 2-5 (NH25), 3-5 (NH35) and 4-5 (NH45) were transfected into K562 cells using nucleofector™ technology. Expression of NH25 and NH35 significantly reduced colony formation capacity by 20% (p=0.001) and 25% (p=0.036) respectively, whilst expression of the NH45 construct did not alter colony formation. We next assessed the clonogenic effects of CCN3 and Imatinib on primary human CD34+ progenitor cells derived from CML peripheral blood samples at diagnosis (n=3). Cells pre-treated with exogenous addition of full-length CCN3 protein for 24h prior to plating in methyl cellulose cultures reduced clonogenic capacity by 25.5±3.9% (p=0.011). Pre- treatment with Imatinib reduced colony formation by 37.9%±19.9 (p=0.010). *Conclusions.* This study demonstrates that CCN3 expression regulates the colony formation capacity of BCR-ABL+ cells. Domains 2 and 3 comprising an insulin-like growth factor binding and Von Willebrand type C domain appear to be significant contributory components in mediating negative growth regulation. Loss of CCN3 expression mediated by BCR-ABL disrupts cell growth regulation confering growth advantage to CML cells.

0531

TRAIL MEDIATED ANTI-LEUKEMIC EFFECTS OF INTERFERON-ALPHA COMBINED WITH G-CSF ON CHRONIC MYELOGENOUS LEUKEMIA DURING IMATINIB THERAPY

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Aims. In the treatment of patients with chronic myelogenous leukemia (CML), a major goal of therapy is the eradication of BCR-ABL-positive leukemic clone. Simultaneous exposure of BCR-ABL-positive leukemia cells to imatinib and interferon-α (IFN) produced synergistic anti-proliferative effects. As G-CSF augments IFN-induced release of soluble TNF-related apoptosis- inducing ligand (sTRAIL) from leukocytes, if G-CSF has a proliferative effect on normal clone but not malignant clone, a combination therapy of imatinib, IFN, and G-CSF is expected to eradicate malignant clone. In this *in vitro* study using bone marrow and peripheral blood mononuclear cells (BMC, PMC) from patients with CML and healthy volunteer donors (HVD), we evaluated whether imatinib in combination with IFN and G-CSF enhanced the anti-leukemic effects via TRAIL/TRAIL receptor (DR4 & DR5) system. *Methods.* Colony assay was performed by a methylcellulose method. Patient's BMC and leukemic cell lines were incubated with imatinib, IFN, G-CSF or recombinant human sTRAIL (rhsTRAIL). Ratio of BCR-ABL- positive cells to -negative cells in pooled colonies was evaluated by RT-PCR with BCR-ABL mRNA. To evaluate indirect cytotoxic effects of IFN-stimulated BMC, PMC, and neutrophils from HVD, their co-culture with K562 was performed using transwell chamber. Apoptosis was detected by Annexin V staining. Concentration of sTRAIL was measured by ELISA. Expression of DR4 and DR5 on leukemic cell lines was examined by FACScan. *Results.* Sequential colony assay of the patients showed that the colony formation decreased paralleled with reduction of Ph clone after imatinib administration, and recovered after achieving a cytogenetic remission. In untreated cases, colonies were effectively suppressed by an addition of imatinib to cultures, while that were more strongly suppressed by IFN than imatinib in patients undergoing imatinib. Although there were no significant differences in BCR-ABL mRNA ratio of pooled colonies between mono-agent treatment and the combinations, IFN with or without G-CSF suppressed BCR-ABL mRNA expression level compared to the control. IFN increased sTRAIL concentration in culture of BMC from patients with CML, also induced sTRAIL from BMC, PMC, or neutrophils obtained from HVD, in a cell number-dependent manner. To clarify the effects of sTRAIL minutely, we examined sTRAIL-induced apoptosis in leukemic cell lines. DR4 and DR5 were highly expressed on K562, KU812, and HL60. rhsTRAIL induced dose- and time-dependent apoptosis in the cell lines, and the effect was completely abolished by anti-TRAIL antibody. Apoptosis of K562 was not induced by a co-culture with IFN, but was induced by IFN-stimulated BMC, PMC, or neutrophils from HVD in the membrane-separated transwell chamber. There were no significant differences in incidence of apoptosis between IFN alone and IFN plus G-CSF treatment. *Conclusions.* We suggest that IFN, adding to its direct effects, induces sTRAIL from BMC, PBC, and neutrophils, which may contribute to reduce leukemic progenitor cells in patients with CML. G-CSF dose not abrogate the cytotoxic effects of IFN, but increases sTRAIL release from blood cells. From these observations, we suggest that G-CSF can enhance anti-leukemic effect of IFN on BCR-ABL-expressing cells via sTRAIL during imatinib therapy.

0532

THE LEVEL OF CIRCULATING T REGULATORY CELLS CORRELATES WITH THE DISEASE BURDEN IN CHRONIC MYELOID LEUKAEMIA

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Chronic myeloid Leukaemia (CML) is characterised by the BCR-ABL gene recombination. We and others have demonstrated that T cells capable of recognising BCR-ABL junctional peptides are present in the circulation of CML patients. We also demonstrated an inverse correlation between the level of these BCR-ABL-specific T cells and the disease burden, as assessed by the BCR-ABL/ABL transcript ratio. However, in spite of the presence of these BCR-ABL-specific T cells, the CML cells persist in the patients. The CD4+ CD25+ naturally-occurring T regulatory cell (Treg) population has immunosuppressive activity involved in limiting immune responses. Studies have also shown that Treg cells can inhibit antitumour immune responses. Thus, we hypothesised that Treg cells may be involved in limiting anti-CML immune responses. In the present study, we carried out an analysis of the amount of Treg cells detected in the peripheral blood of 22 CML patients at multiple time points. All patients were in first chronic phase and currently receiving or having recently received imatinib 400-600 mg daily. The Treg cell numbers were evaluated using flow cytometry for the Treg cell markers CD25 and FOXP3. We correlated these data with BCR-ABL/ABL transcript ratios measured by real-time RT-PCR for each time point. We observed that the Treg population was consistently elevated in patients with high BCR-ABL/ABL transcript ratio (ratio>10%) as compared to patients with low BCR-ABL/ABL transcript ratio (ratio<10%). The median CD4+CD25+ population detected in patients with high BCR-ABL/ABL transcript ratio was 2.29% of all gated lymphocytes whereas this median was 1.29% in patients with low BCR-ABL/ABL transcript ratio (p<0.01 Mann-Whitney two-tailed test). Similarly, the CD4+ FOXP3+ population was consistently elevated in patients with high BCR-ABL/ABL transcript ratio (median of 3.02% for high transcript ratio versus a median of 1.35% for low transcript ratio, p<0.05 Mann-Whitney two-tailed test). We were able to isolate the Treg cell population using CD25-coupled magnetic beads, and are currently investigating the function of these cells in immunity in CML. These data suggest that Treg cells may be involved in limiting anti-CML immunity.

0533

SURVIVIN EXPRESSION REDUCES IMATINIB EFFICACY IN CHRONIC MYELOID LEUKEMIA

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Background and Aims. Imatinib Mesylate (IM) is a semi-specific inhibitor of the BCR-ABL tyrosine kinase and currently represents the treatment of choice for patients diagnosed with Chronic Myeloid Leukemia (CML). Survivin (SVV) is undetectable in normal tissues but highly expressed in most forms of human cancer and functions as both a cell death inhibitor and a mitotic regulator (Altieri, Nat. Rev Cancer 2003;3:46). Previous evidence suggests that SVV is overexpressed in CML cells and that a significant correlation exists between BCR-ABL levels and SVV expression (Conte *et al.* Cancer Lett 2005;225:105). Moreover, our recent findings show that BCR-ABL kinase activity induces SVV expression in both murine and human models of CML (Conte *et al.* submitted). *Patients and Methods.* We investigated the interplay between SVV levels and the clinical response to IM in 20 consecutive patients (pts) with typical BCR-ABL-positive CML (10 males and 10 females; median age 55 yrs, 18 pts in Chronic Phase, 2 pts in Accelerated Phase; 100%

Ph+ cells in the bone marrow). We also evaluated the possible influence of SVV levels on the clinical outcome (time to progression, response to therapy and overall survival) of the pts. All CML pts underwent IM therapy at 400 mg/die and their SVV levels were assessed by quantitative RT-PCR prior to the first IM treatment. Complete hematologic response (CHR), cytogenetic response (Cy-R) and molecular response (MolR) were scored as previously described (Baccarani *et al.* Blood 2006;108:1809). *Results and Conclusions*. All CML pts expressed the SVV transcript (median SVV=727 units). Nineteen out of 20 CML pts (95%) reached a CHR at 3 months; 10/20 pts (50%) obtained a Cy-R (complete + partial) at 12 months; 7/20 pts (35%) achieved a MolR (complete + major) at 12 months. Four pts (20%) displayed a failure to treatment within 12 months. In our analysis, SVV transcripts produced a median time to progression and survival of 31 (p=0.04) and 53 months (p=0.6), respectively. These findings indicate that increased SVV expression reduces IM therapeutic efficacy, in agreement with our previous *in vitro* observation that high SVV levels significantly reduce cell death in CML cells. Thus, our data suggest that strategies aimed at lowering SVV expression may improve the efficacy of IM, therefore representing a possible therapeutic option for both IM-sensitive and IM-resistant CML patients.

0534

DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE ABL KINASE DOMAIN OF CML PATIENTS

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Background. BCR-ABL kinase domain mutations constitute the leading cause of resistance in CML patients treated with imatinib. Detection of BCR-ABL mutations prior to and during the course of imatinib therapy may aid in risk stratification as well as in determining optimal individual therapeutic strategies. More than 50 different point mutations have been described so far. The K247R change has been identified as a rare gene polymorphism occurring also in normal control patients and other cell types. Despite its position near to the P-loop, biochemical and cellular assays of imatinib and dasatinib sensitivity showed no alteration compared to wild-type ABL. *Aims.* Since gene polymorphisms do not automatically necessitate a change in the therapeutic strategy we sought to investigate if other BCR-ABL mutations are rather polymorphisms than mutations associated with resistance to tyrosine kinase inhibitors. *Methods.* In comparison to real BCR-ABL kinase domain mutations, single nucleotide polymorphisms (SNPs) also have to occur on the normal, untranslocated ABL allele. Therefore, we designed an allele-specific PCR for amplification of the normal ABL allele following mutation analysis by denaturing high-performance liquid chromatography (D-HPLC) and subsequent sequencing. A multiplex PCR was performed to ensure that both Ib and Ia ABL splice variants were amplified. Ninety-one imatinib-resistant CML patients (chronic phase, n=88; accelerated phase, n=1; blast crisis, n=2) with 65 different BCR-ABL kinase domain mutations leading to 50 different amino acid changes were investigated in our study. 39 of 65 different mutations were evaluable in two different patients and 26 different mutations in one patient, respectively. *Results.* Amplification of the normal ABL allele succeeded in all cases. The D-HPLC analysis indicated a mutation in the normal ABL allele in eight cases. The nucleotide exchanges were confirmed by direct sequencing in both directions. In all cases the same mutation of the BCR-ABL allele was confirmed in the normal ABL allele (mutated ABL (%)/ mutated BCR-ABL (%)): T240T (50/100), F311V (50/100), T315T (40/100), Y320C (90/30), K247R (100/20; 40/100) and E499E (100/100; 40/100). In three cases the SNP did not lead to an amino acid change (silent mutation). Five patients had a wild-type as well as a mutated ABL allele leading to 50% mutated ABL allele. In these patients the SNP including ABL allele seems to be translocated to BCR-ABL thus containing 100% mutated BCR-ABL. Another patient harbouring the F311V mutation at the BCR-ABL allele did not show the mutation at the normal ABL allele. *Conclusions.* In addition to the previously described polymorphism K247R we uncovered five new polymorphisms, two of these led to amino acid changes. SNPs should be taken into consideration for design of mutation-specific PCRs, particularly the T315T polymorphism for T315X amplifications. Newly identified mutations should be confirmed by amplifying the normal ABL allele to exclude polymorphisms. Clinicians should be aware that polymorphisms do not reflect mutations that automatically demand a change in therapeutic strategy, unless there are other signs of inadequate response to treatment.

0535

MAPK-ACTIVATION IN RESPONSE TO BCR-ABL INHIBITION IS CYTOKINE-DEPENDENT AND CAN BE OVERCOME BY DASATINIB

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Background. Inhibition of BCR-ABL by imatinib mesylate is current standard treatment for patients with chronic myelogenous leukaemia (CML). Despite favourable response rates, imatinib fails to eradicate minimal residual disease in the majority of patients, which is believed to be caused by stem-cell resistance. A proposed mechanism of human CML stem-cell survival is growth-factor- (GF) dependent activation (phosphorylation) of the MAPK Erk1/2 in response to imatinib (Chu *et al.,* Blood 2004). *Aims.* We used IL3-dependent murine haematopoietic cell lines and their IL3-independent BCR-ABL-expressing progenies to study MAPK-signalling in response to imatinib and the high affinity BCR-ABL inhibitor dasatinib. Primary CD34+ enriched human CML cells were used, to validate cell-line results in a clinically relevant cellular context. *Methods.* Murine Baf3 and 32D cells transformed by p210Bcr-Abl were grown in the presence of 5 µM imatinib with and without WEHI-conditioned media as source of IL3. Cytotoxicity was determined by trypan blue viability count. Cells were harvested after 3h, 6h, and 24h and lysed for Western blot analysis. Human CML mononuclear cells derived from 5 patients with first chronic-phase CML prior to treatment were enriched by magnetic separation for CD34+. MAPK-signalling after treatment with BCR-ABL-inhibitors for 16h was studied in serum-free media in the presence of standard GF-mix. *Results.* Baf3p210 and 32Dp210 cells treated with 5 µM imatinib for 24h were rescued by IL3 as determined by trypan blue viability count. Western blot analysis after 3h, 6h, and 24h of treatment demonstrated time-dependent significant activation of Erk1/Erk2 with peak phosphorylation after 6h in the presence of IL3. In the absence of IL3, Erk1/Erk2 phosphorylation was abolished at any time. Simultaneous detection of phosphotyrosine (pTyr) and BCR-ABL demonstrated complete inhibition of BCR-ABL with and without IL3. Similar results were obtained in both cell lines. Exposure of Baf3p210 cells with 60nM dasatinib induced marked MAPK-inhibition independent of IL3 as early as 3h after start of treatment. Treatment of CD34+ enriched CML cells confirmed imatinib-induced MAPK-activation. Exposure of patient (n=5) cells with dasatinib (12 nM, 62 nM) in contrast showed significant (p<0.05) Erk1/2 inhibition consistent with cell-line data. *Conclusions.* GF-dependent MAPK-activation in response to imatinib initially reported in primary CML cells can be simulated in murine haematopoietic cell lines expressing BCR-ABL. This model allows further mechanistic studies and effective screening of alternative BCR-ABL-inhibitors for MAPK-modulation. The multikinase inhibitor dasatinib potently inhibits MAPK in cell lines (± IL3) and primary CML cells and may thus be more effective than imatinib in eradication of CML progenitor cells.

0536

GENETIC POLYMORPHISMS IN THE METHYLENETETRAHYDROFOLATE REDUCTASE GENE ASSOCIATED WITH CHRONIC MYELOID LEUKEMIA

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Background and Aims. Folate metabolism plays an important role in carcinogenesis through DNA methylation and nucleotide synthesis, thus affecting gene expression and DNA instability. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating intracellular folate metabolism. Two common MTHFR single nucleotide polymorphisms (SNPs) - one located in exon 4, C677T, and one located in exon 7, A1298C - have been reported to be associated with reduced enzymatic activity. Perturbation of folate metabolism as a consequence of reduced MTHFR activity has been shown to result in DNA hypomethylation and to promote 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 the present 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 =0.05]. *Conclusions.* On the basis of the present 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.

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0537

IMPACT OF CYTOGENETIC ABERRATIONS IN PHILADELPHIA CHROMOSOME NEGATIVE HEMATOPOIESIS ON EVENT-FREE SURVIVAL OF CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB MESYLATE

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Background. Clonal aberrations (CA) of Philadelphia (Ph) negative metaphases have been described after treatment with interferon α or imatinib (IM) in a minority of patients (pts) with cytogenetic response. Conflicting data suggest selection of pre-existing clones versus induction of aneuploidy by tyrosine kinase inhibitors. The prognostic impact of aberrations in the Ph negative hematopoiesis for the individual pts remains to be determined. *Aims.* To evaluate the incidence and prognostic impact of CA occurring during imatinib therapy, we retrospectively analysed the outcome of our pts with chronic (CP) or accelerated phase (AP) of CML who obtained at least a major cytogenetic response during the first year of IM treatment. *Material and Methods.* between april 2000 and December 2006, 127 pts received IM and 90 obtained a partial cytogenetic response (PCR) or complete cytogenetic response (CCR) during the first year of treatment. Cytogenetic analysis was performed every 3 months during the first year and every 6 months after. CA was defined as abnormalities seen in 2 or more metaphases. Two groups were defined according with the presence or not of CA. Characteristics of each population (age, sex, prior therapies, sokal index, disease phase, response, bcr-abl kinetic) were analysed. During the follow-up, relapse, progression of the disease or death was noted. *Results.* with a median time of follow-up of 41 months, 32 pts developed CA (35%). The median time from the start of IM to appearance of CA was 278 days (range 82-1495 d). In 15 pts these events have been transient and disappeared after a median of 4 months (range, 3-9 months). CA were: -7/del 7 (6 pts); -Y (6 pts), +8 (5 pts), mar (3 pts), der 12p (2 pts), and -1, +6, der 9p, del 10q, der 11p, +15, der 17q, -18, t(3;11), and t(12;14). Characteristic of two groups (CA- vs CA+) were analysed: no significant difference was seen according to age, sex, prior treatment, Sokal index and cytogenetic response. However, the kinetic of bcr-abl transcript decrease was significantly faster in the group of pts without CA. Furthermore, hematologic or cytogenetic relapse and blast crisis occurred in 13/32 pts (41%) and 11/58 pts (19%) in CA^+ and CA^- groups, respectively (p<0.05). The median time of EFS was 55 months for the CA+ group and was not reached in the CA- group. The logrank test showed a significant difference between the two curves (p=0.0335). Mutation analysis showed 8 cases of abl mutation (33%). No significant difference was noted between the 2 groups. Mutations were : P-loop (4 cases); T315I, F317L, E292K and F311I (1 case each). *Conclusions.* Cytogenetic aberration occurring in Ph-negative hematopoiesis during IM therapy defined a group of patient who show an increase risk of IM resistance. CA should be a marker of genetic instability and inadequate control by IM. The present data argues to perform regular cytogenetic analysis even in complete cytogenetic response. In case of CA, abl mutation should be performed and pt requires a more stringent monitoring.

0538

NUCLEAR IMPORT OF NORMAL C-ABL PROTEIN IN RESPONSE TO M-TOR INHIBITOR RAD001 (EVEROLIMUS) DRIVES APOPTOTIC DEATH OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Nuclear accumulation of c-Abl protein in response to genotoxic damage addresses apoptotic cell death. The process, driven by the disruption of its binding to 14-3-3 scaffolding proteins, is independent from c-Abl catalytic state and conditional upon 14-3-3 phosphorylation by the c-jun N-terminal kinase (JNK) (Yoshida *et al.* Nat Cell Biol 7,278,2005). Our work moved from the observation that c-Abl nuclear import in response to gamma irradiation is precluded by the constitutive activation of Abl tyrosine kinase (TK) in p210 Bcr-Abl fusion protein, supporting a role of residual c-Abl protein *loss of function* in the apoptosis-resistant phenotype of Chronic Myeloid Leukemia (CML). In 32D cell clones transducing a temperature-conditional Bcr-Abl mutant, p210 TK inhibition in response to 24 hour exposure to 1 microM Imatinib mesylate (IM) is followed by c-Abl nuclear import and cell commitment to apoptotic death. The two last events are further enhanced by complementary inhibition of the 14-3-3 binding site by R18 peptide, supporting that the negative impact of p210 TK on pro-apoptotic function of residual normal c-Abl arises, at least in part, from the fusion protein effects on 14-3-3sigma. Previous studies proved that the activation of p38 MAP kinase contributes to IM effects in CML (Parmar *et al.*, J Biol Chem 279,25345,2004). Interestingly, p38 MAP kinase is involved in tuberous sclerosis 2 gene protein (TSC2, also known as tuberin) phosphorylation and enhanced binding to 14-3-3 possibly promoting the activation of IM-compensatory signals including m-Tor (Li *et al.*, J Biol Chem 278,13663,2003). Accordingly, in Bcr-Abl-transduced 32D cell clones the enhanced c-Abl nuclear import in response to combined inhibition of p210 TK by IM and m-Tor by RAD001 (everolimus; kindly provided by Novartis Pharma AG) was associated with p38 MAP kinase inactivating dephosphorylation, JNK phosphorylation and 14-3-3 phosphorylation at serine residues critical for client protein binding (including the apoptotic death effectors Bad, Bax and Ask1). C-Abl nuclear shuttling and apoptosis enhancement in response to *in vitro* exposure to IM and RAD001 were confirmed in early ($CD34^+$) myeloid progenitors from CML patients at clinical diagnosis. Our results support the advantage of p210 TK and m-Tor inhibitor association to overcome IM-compensatory pathways that may promote the outcome of a drug-resistant phenotype in CML.

0539

MICRORNAS AS THE TARGET OF DELETIONS ON DER(9) IN CHRONIC MYELOID LEUKEMIA

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Background. 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. Aims. 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 from 260 Kb to 54 Mb and from 230 Kb to 12.9 Mb, respectively. Consultation 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 chromosome 22. Moreover, 4 cases with a complex t(9;22) rearrangement and der(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 derivative was not associated with the miRNAs deletion mapped on chromosome 9. The most recurrent miRNAs deleted on der(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, suggesting a potential role of this miRNA in CpG-island methylation. *Conclusions.* Experimental studies indicate that miRNAs can function as tumor suppressor genes or as oncogenes. In fact, in chronic lymphocytic leukemia associated with del(13)(q14) it has been demonstrated that the miRNAs loss can induce downregulation of the antiapoptotic BCL-2 protein. The novel evidence that deletions on der(9) in CML are associated with miRNAs loss may shed new light on the significance of genomic sequences loss. Further studies are needed since it is known that some microRNAs may have as many as a few thousand targets, so prediction algorithms and strategies allowing large-scale screening of multiple target genes are required.

0540

SEMEN ANALYSIS DURING IMATINIB THERAPY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Imatinib is the main treatment of chronic myeloid leukaemia and induces high rate of cytogenetic response and major molecular response. The 5 years survival from IRIS trial is 87% and the annual risk of relapse for responder patients is about 1% by year. Imatinib is a strong inhibitor of bcr-abl, c-kit and PDGF-R, and could explain sides effects related to the drug. C-kit is involved during spermatogenesis and influenced the spermatogonies maturation. Imatinib induces qualitative and quantitative deteriorations of spermograms in rats. Data about qualitative and quantitative characteristics of spermograms from patients treated with imatinib are lacking and sperm cryopreservation before treatment is recommanded. *Methods.* Semen cryopreservation was performed at diagnosis before treatment to every patient under 50 years of age. After information and fully consenting, patients aged >17 and <50 years old had serial spermograms analysis during treatment with imatinib for chronic myeloid leukaemia to determine any change in sperm volume, sperm number, mobility and morphology of sperm. *Results.* Ten male patients participated to the present study. All patients had no prior treatment before imatinib. All patients received 400 mg of imatinib. The mean age was 35 years (range 24 to 49 years). Spermogram was performed at 6, 12, 18 and 24 months after imatinib start. At the inclusion all patients had a normal sperm with ejaculate volume 3.87±0.25 mL, sperm concentration 92±25.5×10^6/mL, mobility 86±5% and untipycal spermatozoid 23±%. Serial semen analysis showed a reduction of number of spermatozoid: 68±22×10^6/mL at 6 months, 65±25 at 12 months, 43±29 at 18 months and 37±32 at 24 months. No alteration was seen in ejaculate volume, mobility or% of untypical spermatozoid at any time. At 24 months, 1 over 4 patients analysed was oligosperm. Imatinib induces a significant reduction of sperm concentration of: 26%, 29%, 47%, and 60% at 6, 12, 18 and 24 months respectively, without mobility abnormalities and increased of untypical forms. Such deteriorations of sperm characteristics do not induce reduction of fertility. Despites recommendation for contraception, one pregnancy occurred leading to a normal birth. *Conclusions.* these preliminary data show a significant reduction of 35±5% of sperm concentration during imatinib therapy but with limit impact on fertility. However, the duration of imatinib therapy in CML argues to prolong the study with a largest cohort of patients. Lack of sufficient data requires to proposal semen cryopreservation before treatment.

0541

IDENTIFICATION OF BREAKPOINT HOTSPOTS IN PHILADELPHIA NEGATIVE BCR/ABL POSITIVE CML

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Background. Chronic myeloid leukaemia (CML) is a pluripotent stem cell malignancy, characterized by the presence of the Philadelphia chromosome (Ph). The Ph, usually due to t(9;22)(q34;q11) or its variants, results in the formation of the BCR/ABL fusion gene which is a constitutively activated tyrosine kinase. Less than 2% of CML patients appear to be Ph negative by G-banding, but carry a cryptic BCR/ABL fusion that can be located by FISH either at 22q11, 9q34 or in a third chromosome. There are two models to explain the formation of BCR/ABL fusion gene in those patients: direct insertion or two consecutive translocations. In the first model, an insertion either of 9q34 material into BCR region at 22q11 or of 22q11 material into ABL region at 9q34 is postulated. The second model suggests a classical t(9;22)(q34;q11) followed by a second rearrangement between the Ph and the der(9) or a third chromosome, which results in restoring the *normal* chromosome morphology. *Aims.* In order to assess which of the two mechanisms is more plausible, we undertook comprehensive molecular cytogenetics investigations of 4 patients and 1 cell line (CML-T1) with Ph negative BCR/ABL positive CML disease. *Methods.* FISH using a range of DNA probes and high resolution array CGH analysis (Agilent) were used to assess the rearrangements of the region that flanks ABL and BCR breakpoints. *Results.* In all patients the fusion gene was found on chromosome 22. In 3 out of the 4 cases studied, a common fragment from 9q was found inserted into 22q at the telomeric site of BCR breakpoint. The fragment is less than 1 Mbp in length and its proximal boundary coincides with the ABL breakpoint, while the distal boundary falls within a common region of 300 Kbp, flanked by the BAC clones RP11-643E14 and RP11-326N3. In CML-T1 the fusion gene was also found on chromosome 22. CML-T1 cells also showed duplication of the masked Ph and loss of the normal 22. The inserted 9q segment was found to be larger than the one seen in the previous 3 patients and the distal breakpoint falls within RP11-92B21. This BAC includes the 3' end of RXRA gene, which is disrupted as a result of the insertion. Similarly, in the fourth patient the size of the 9q segment was estimated to be nearly 6 Mbp. Surprisingly the breakpoint at 22q remains constant within BCR breakpoint in all cases studied so far, with no material from 22q detected within the structure of the der(9). *Summary.* Our findings show that while the 9q fragment inserted into 22q varies in size, there is no evidence of secondary rearrangements within the region distal to the BCR breakpoint. This leads to the conclusion that the most likely mechanism of formation of the Ph negative BCR/ABL positive rearrangement is an insertion rather than sequential translocations. Ongoing mapping aims to resolve the involvement of the genes housed by the distal breakpoint region, of which RAP-GEF1 is the most plausible candidate.

0542

APOPTOSIS-RELATED GENE EXPRESSION IN T-CELLS FROM IMATINIB TREATED CHRONIC MYELOGENOUS LEUKEMIA PATIENTS

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Background. Imatinib mesylate (Glivec®, Gleevec™) is the first-line treatment for patients with chronic phase CML. Recently several 'off-target' effects have been reported, e.g. immunomodulatory effect, altered serum phosphate levels and effects on cardiomyocytes in animal models. It has been demonstrated that imatinib inhibits T-cell activation and proliferation *in vitro*. However, little is known about the T-cell function in imatinib treated patients. *Aims.* To obtain more information regarding the effect of imatinib on apoptosis pathways in T-cells, an important regulator of the immune response. *Methods.* Eleven imatinib treated (400 mg q.d.) chronic phase CML patients (mean age 57±12 (SD)) and 11 age and sex matched healthy individuals were included in this study. At time of blood sampling, all patients were in complete cytogenetic remission (CCgR) and the median imatinib treatment duration was 48 (range 14-72) months; peripheral blood interphase-FISH for the bcr-abl fusion gene was also *negative* in all patients. Mononuclear cells (PBMCs) were isolated from peripheral blood using Ficoll™ density gradient centrifugation. $CD3^+$ T-cells were separated from PBMCs by immunomagnetic cell sorting (MACS™). Total RNA was extracted from 2-4 millions purified $CD3^+$ T-cells and subject to complementary DNA synthesis. Apoptosis-related gene expression was determined by the Taqman® low density array (LDA) apoptosis panel (Applied Biosystems), which included 94 apoptosis-related genes and two reference genes. On every LDA card, cDNA from one patient and his age and sex matched healthy individual was loaded. The difference in target gene expression (ΔΔCt: patient (Cttarget-Ctreference)- healthy individual (Cttarget- Ctreference)) was calculated for every gene and patient-control pair. If the 95% confidence interval for the mean ΔΔCt did not include zero, the difference in gene expression was considered statistically significant. *Results.* Out of 94 apoptosis-related genes, 16 genes had significantly different expression levels

in CD3+ T-cells obtained from imatinib treated CML patients and healthy individuals. These results are given in the attached Figure 1. *Conclusions.* Most likely the observed difference in gene expression of apoptosis-related genes can be linked to the imatinib treatment, since: (i) all patients were in CCgR and (ii) all had a negative interphase FISH for the bcr-abl fusion gene. Fifteen of 94 studied apoptosis related genes were downregulated in T-cells from imatinib treated patients and only one gene was upregulated. Functional assays are needed to explain how these alterations affect the T-cell function, e.g. proliferation and activation induced cell death (AICD) - an important regulator of the immune response.

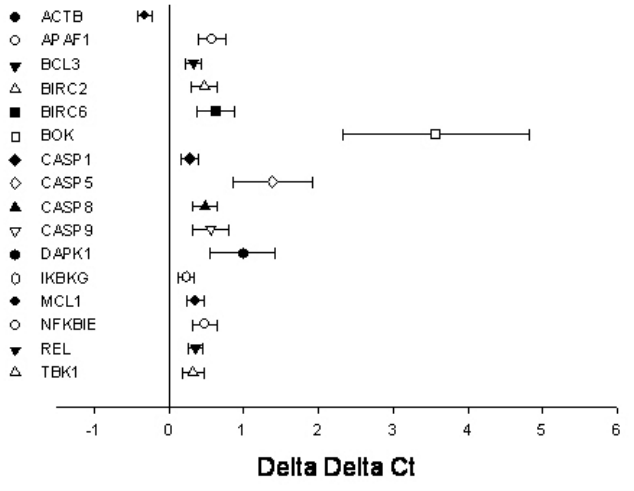

Figure 1.

0543

BCR-ABL KINASE MUTATIONS IN RESPONSE TO DASATINIB

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Background. Dasatinib is a novel Src-Abl kinase inhibitor with efficacy in patients with CML resistant to imatinib. *In vitro* studies show that dasatinib is active against nearly all the known BCR-ABL kinase mutations except T315I. *Aims.* Analysis of the kinetics of BCR-ABL kinase mutations in patients treated with dasatinib. *Methods.* Karyotyping for evaluation of cytogenetic response, RT-PCR for measurement of BCR-ABL transcript levels, direct sequencing and pyrosequencing for detection and quantification of the mutations in serial samples of patients with a suboptimal response to dasatinib. *Results.* 47 imatinib-resistant CML patients received dasatinib. Patients were followed for 3-23 months. 12 patients had mutations in the BCR-ABL kinase domain, prior to treatment with dasatinib. Of these 12, 10 (82%) achieved MCyR with dasatinib usually within three months of starting therapy. 22% later lost their MCyR. Kinetic analysis of the mutant clones revealed 3 patterns of response. Group 1 (n=6): patients initially responded to dasatinib with a 1-3 log reduction in BCR-ABL/ABL ratio, but this was not maintained. A new mutation was noted either in isolation or in addition to pre-existing mutations. Group 2 (n=4): patients achieved a MCyR with dasatinib, which was accompanied by disappearance of the mutant clone (E453V, H396R, F359V). Group 3 (n=2): patients achieved a MCyR with dasatinib. However, the mutant clone persisted with fluctuating levels after more than 18 months follow up. Nearly all the patients with mutations known to be sensitive to dasatinib in cellular studies achieved MCyR with dasatinib. This response was accompanied with decline or disappearance of the mutant clone (group 2), or a fluctuating level of the mutant clone (group3). The only exception was one patient with L248V (group1), in whom L248V remained dominant during the follow-up with a transient MCyR. Group 1 included patients who had an initial response, but subsequently lost this as demonstrated by an increase in the mutant BCR-ABL positive clone. In two patients in this group, a pre-existing mutant clone disappeared along with > 2 log reduction in BCR-ABL/ABL ratio. This response was later lost following the re-appearance of the previously detected mutation along with T315I, Quantitative analysis showed that these mutations co-existed in the same clone. In a further 3 patients in Group 1 there was an initial reduction in the BCR-ABL/ABL ratio but this was lost with the emergence of a new mutation, F317L. The appearance of F317L coincided with disappearance of the pre-existing mutant clones, M244V, M351T and H396R. The F317L induces a 9 to 13.5-fold increase of dasatinib IC_{50} in comparison to wild-type BCR-ABL in cellular assays. These results show the emergence of new mutations under dasatinib and document their different kinetics of mutation.

0544

WHEN IS A DOUBLE PHILADELPHIA NOT A DOUBLE PHILADELPHIA

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Background. Chronic myeloid leukaemia (CML) is a pluripotent haematopoietic stem cell disorder defined by expression of the BCR-ABL fusion gene, a constitutively activated tyrosine kinase. The fusion gene commonly results from formation of the Philadelphia chromosome (Ph) after a t(9;22)(q34;q11) or related variant rearrangement. We studied 48 samples and 12 cell lines from CML patients at various stages of the disease by arrayCGH using a 1 mbase BAC array (Spectral Genomics, Houston, USA), 44k 60 mer oligo arrays (Agilent) and by molecular cytogenetics techniques. Gains in 9q34.1-qter distal to the ABL breakpoint along with gains of 22pter-c-22q11.2 were detected in 1/22 CP, in 3/26 BC samples and in 6 cell lines. This is in keeping with the presence of a second Ph chromosome as confirmed by both G banding, chromosome painting and D-FISH. Contrary to expectations, 5 of these samples (50%) did not display the aCGH profile corresponding to the presence of extra Ph markers instead, various gains were identified from the 9q34.1-9qter segment that (a) failed to cover the whole 9q material of the Ph marker and (b) disagreed with the G banding data. *Aim.* In the absence of BAC array coverage of the ABL breakpoint region, a more precise assessment of the 9q34 imbalances was sought using a high resolution oligo based aCGH analysis of three BC patients and two cell line samples. *Results.* The oligo's aCGH profile confirmed the presence of gains from the 9q34.12 sub-band and delineated a common amplicon with an estimated size of 330 Kbp. Three genes - ABL, LAMC3, NUP214 are located in this amplicon. We constructed a dual colour FISH probe from clones RP11-83J21 (containing ABL from the 5' end to exon 1), RP11-143H20 (covering LAMC3) and RP11-544A12 (partially LAMC3 & NUP214 genes), and applied it to the mitotic figures of bone marrow from 15 CML patient's samples in accelerated phase/blast crisis and with 10 CML cell lines. Extra copies of the region covered by the probe were detected as follows: a) High copy number gains were seen as 'tandem duplications' within similar acrocentric marker chromosomes in 2 cell lines, K562 and KU812; b) Duplication of the ABL-NUP14 region within the Ph chromosome with an apparently normal G-banding morphology in (3/15) patient's samples; similar changes were found in the cell lines MC3, MEG-01, EM-2 and KYO while affecting one of the two copies of the Ph chromosome that were indistinguishable by G-banding; c) The ABL-NUP214 region was found inserted within one of the apparently morphologically *normal* chromosome 22 homologues at the 22q11.2 sub-band in two instances. *Conclusions.* These findings clearly demonstrate that the Philadelphia chromosome is an unstable structure and prone to rearrangements that commonly involve sequences downstream of the ABL breakpoint, specifically the ABL-NUP214 region.

0545

STABILIZED BONE MARROW IS NOT SUPERIOR TO PERIPHERAL BLOOD FOR MOLECULAR ANALYSIS OF CML PATIENTS WITH RESISTANCE TO TYROSINE KINASE INHIBITORS

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Background. Recommendations for treatment surveillance of CML patients on tyrosine kinase inhibitors include systematic molecular monitoring by accurate quantification of BCR-ABL transcripts. Emergence of BCR-ABL kinase domain mutations is considered the major cause of resistance. Inadequate response or loss of response to therapy requires an early and accurate measurement of BCR-ABL transcripts and BCR-ABL kinase mutations indicating the need to change the therapeutic strategy. The sensitivity of the respective assays clearly depends on quality and quantity of RNA derived from peripheral blood (PB) and bone marrow (BM) leukocytes. Bedside RNA preservation systems (e.g. PAXgene) have been developed in order to maintain RNA stability during shipment of the sample from the clinical site to the laboratory. *Aims.* RNA stabilization systems have been tested for PB but not BM cells in previous studies. Therefore, we sought to investigate the applicability of stabilization systems for BM samples and to compare the performance of the system for quantification of BCR-ABL transcripts and detection of BCR-ABL kinase domain mutations in both tissues. *Methods.* Simultaneously stabilized PB and BM samples (PAXgene system, 2.5 mL each) were obtained from n=49 imatinib resistant CML patients in chronic phase to compare RNA yield and purity, quantitative results for housekeeping genes (total ABL and β-glucuronidase, GUS) by RT-PCR, ratios BCR-ABL/ABL and BCR-ABL/GUS, and BCR-ABL mutations analyzed by sensitive denaturing high-performance liquid chromatography (D-HLPC) and by direct sequencing. *Results.* RNA yield was significantly higher in BM (median 9.9 µg RNA/mL BM) than in PB (median 3.8 µg RNA/mL blood, p=0.0013). Using 10 µg RNA for the generation of 40 µL cDNA by reverse transcription, the number of housekeeping gene transcripts indicating sample quality and sensitivity of RT-PCR was comparable between PB and BM (median ABL copies/2 µL cDNA 13,750 vs 26,230, p=0.52; median GUS copies/2 µL cDNA 38,830 vs 59,560, p=0.22, respectively). Further, ratios BCR-ABL/ABL (PB vs BM, median 47% vs 57%, p=0.07) and ratios BCR-ABL/GUS (PB vs BM, median 27% vs 23%, p=0.33) were not significantly different. BCR-ABL kinase domain mutations were detected in 26 of 49 (53%) patients, and in 27 of 49 (55%) patients using PB or BM, respectively. In one patient the D276G mutation was only detectable in PB (20% mutated alleles) but not in BM cells. However, in three patients mutations were only found in BM but not in PB: Mutation H396R (n=1, 20% mutation fraction) and the silent mutation E499E (n=2, mutation fraction 30% and 40%, respectively). In general, the relative fraction of the mutated clone was significantly higher in PB than in BM samples (median 70% vs 50%, p=0.0023). *Summary and conclusions.* Optimum sample quality is a crucial requirement for molecular monitoring of CML patients on therapy. Sensitivities of the assays depend on a sufficient amount of non-degraded RNA in the sample after transit to the laboratory. We conclude that BM is suitable for stabilization with RNA preservation systems but is not superior to PB for quantification of BCR-ABL transcripts and mutation analysis in CML patients.

Chronic myeloid leukemia - Clinical

0546

PLEURAL AND PULMONARY EVENTS IN PATIENTS TREATED WITH DASATINIB FOR CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

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Background. Dasatinib is a novel multi-targeted kinase inhibitor of BCR-ABL and SRC family kinases indicated for patients with chronic myeloid leukemia (CML) or Ph+ acute lymphoblastic leukemia resistant to or intolerant of imatinib. *Aims.* To describe the characteristics and management of pleural and pulmonary events observed in association with dasatinib 70 mg b.i.d therapy. *Methods.* This was a single-center case series of 40 patients with chronic-phase (CP) CML resistant to or intolerant of imatinib, who received treatment with dasatinib in clinical trials. *Results.* Nine of these 40 patients (22.5%) developed pleural effusions and/or pulmonary manifestations. Clinical symptoms included dyspnea, cough, and chest pain; extra-thoracic symptoms (fever, myalgia, arthralgia, or paresthesiae) were present in 3 cases. High-resolution CT scans identified pleural effusions in 6 patients, and lung abnormalities were present in 8. All pleural effusions were exudative and contained lymphocytes. Three patients underwent pleural biopsy: no abnormalities were identified in one patient, while a lymphocytic infiltration and a myelocytic infiltration were observed in the other 2 cases. BAL revealed lymphocytic alveolitis in 4 patients with lung abnormalities and neutrophilic alveolitis in a further case. Treatment with dasatinib was interrupted in all but one case (this patient was administered steroid therapy instead) and 2 patients received antibiotics empirically; diuretics were not routinely given. Resolution of these lung manifestations was evident for all 9 patients. Re-introduction of dasatinib at a reduced 40-mg b.i.d. dose was successfully achieved in 3 of 4 patients without any recurrence; the fourth patient developed a pleural effusion. *Summary and conclusions.* Pleural effusions and pleuro-pulmonary manifestations observed in association with dasatinib 70 mg b.i.d therapy resolve upon treatment interruption. Dose reduction appears to allow resumption of dasatinib therapy.

0547

A COMBINATION OF DAUNORUBICIN, CYTARABINE AND IMATINIB MESYLATE FOR PATIENTS WITH *DE NOVO* PHILADELPHIA POSITIVE ACUTE MYELOBLASTIC LEUKEMIA AND MYELOID BLAST CRISIS CML : RESULTS OF THE AFR01 DOSE ESCALATING STUDY

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Background. Only 15% of patients (pts) with chronic myelogenous leukaemia in myeloid blast crisis (MBC CML) and treated with imatinib mesylate (IM) achieve a complete haematological remission (CHR) and 28% return to chronic phase (CP). For pts in CHR, the median duration of response is only 10 months and overall survival is around 7 months. We conducted a dose escalating study to assess the safety and the efficacy of IM associated with chemotherapy. *Patients and methods*. Pts ≥ 18 years with MBC CML and *de novo* Philadelphia positive AML not previously treated with tyrosine kinase inhibitors (TKI) were eligible. In the first part of the study a fixed dose of IM (600 mg/d) was administered combined with increasing dosages of daunorubicin (cohort 1: no daunorubicin, cohorts 2, 3 : 15 and 30 mg/m^2/day of daunorubicin IV day 1 to day 3 respectively) and cytarabine (Ara-C) 100 mg/m^2/d for 7 days. G-CSF was administered from day 9 until recovery. Responding pts received a second identical course. Hematopoietic stem cell transplantation (HSCT) was then offered to all eligible pts. Intermediate dose Ara-C and IM maintenance was proposed for the others. Pts included in the second part of the study were treated with IM 600 mg/d in combination

with a classical 3+7 chemotherapy (daunorubicin 30 to 45 mg/m^2, Ara-C 100 mg/m^2) (validation cohort). *Results.* 45 pts (38 MBC CML and 7 Ph+ AML) were included[(median age 53 years, (22-74); sex ratio 67%] from 2001 to 2006 with a median follow-up of 3.9 years. 7 pts were treated in cohort 1, 6 in cohort 2, 6+8 in cohort 3 and 18 in the validation cohort. Haematological responses were observed in 31 pts (68.9%) including 26 patients in CR (57.8%) and 5 patients in CP (chronic phase). The rate of CR and CP was significantly higher in pts treated in cohorts 3 and in the validation cohort (21 CR, 2 CP, 8 failures) compared to cohorts 1 and 2 (3 CR, 3 CP, 7 failures) (p=0,013) indicating a benefit of combining standart chemotherapy and IM. After induction, a complete cytogenetic response was observed in 13 pts (42%) including 4 of them with a major molecular response. 5 pts (11%) died early during induction due to disease progression (n=3), septicaemia (n=1) and following splenectomy for haematoma (n=1). All responding pts had a neutrophil recovery before day 45 (median 22 days). The median duration of grade 3-4 thrombocytopenia was 25.5 days. For pts with MBC CML (n=38), response rate (CP and CR) was 63.1%. However, median survival and DFS were 9.8 and 14.4 months respectively. Only pts who received allogeneic stem cell transplantation (n=11) experienced a prolonged DFS (not reached versus 10.9 months, p=0,01) and a trend in survival (65.9% at 3 year). For pts with Ph+ AML (n=7), the CHR rate is 100% and median survival and DFS were not reached. At 3 year, estimated survival and DFS were 85.7%. 4 out of 7 Ph+ AML received allogeneic HSCT. Their survival was +4.2, +6.5, +19.3 and+23.1 months compared to +43.6, +17.9 and 4 months for non allografted patients. *Conclusion.* IM combined with the classical 3+7 induction protocol produce 63.1% of haematological remission in pts with MBC CML without significant toxicity but only pts who received allogeneic HSCT experienced prolonged DFS and survival. All our 7 pts with Ph+ AML obtained a CR and long survivors were observed either after allogeneic HSCT or maintenance with IM and chemotherapy.

0548

ALTERED BONE AND MINERAL METABOLISM IN CHRONIC PHASE CML PATIENTS TREATED WITH IMATINIB

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Background. Imatinib mesylate (Glivec®, Gleevec™) is the drug of choice for most patients with chronic phase CML. It was recently suggested that hypophosphatemia develops in imatinib treated patients as a consequence of suppression of bone turnover and renal phosphate wasting. *Aims.* To gain more information regarding the imatinib-associated hypophosphatemia we analyzed bone and mineral metabolism in imatinib treated CML patients and age and sex matched healthy individuals. *Material.* Eighteen imatinib treated CML patients (11 males/7 females; mean age 60±11 (SD) years) and 19 helthy volunteers (11 males/8 females; mean age 58±12 (SD) years) were included. All CML patients were treated in first chronic phase, targeting an imatinib dose of 400 mg q.d. At time of study, all patients were in complete cytogenetic remission and the mean imatinib treatment duration was 48±21 (SD) months.

Table 1.

	CML (n=18)	Controls (n=19)	P-value
S-Potassium (mmol/L)	4.1±0.3	4.2±0.2	<0.05
S-ionized Calcium (mmol/L)	1.21±0.02	1.24±0.03	<0.001
S-Phosphate (mmol/L)	0.89±0.17	1.06±0.16	<0.01
S-Magnesium (mmol/L	0.79±0.05	0.84±0.05	<0.01
S-Creatinine (µmol/L)	93±25	79±10	<0.05
tU-Calcium (mmol/24 hours)	3.19±1.79	5.25±1.86	<0.01
tU-Phosphate (mmol/24 hours)	28±11	31±8	ns
S-Parathyroid hormone (ng/L)	46±19	35±13	0.056
S-25-OH-vitamin D (ng/L)	24.6±6.8	28.1±7.6	ns
S-1,25 $(OH)_2$ vitamin D (ng/L)	42.5±10.4	49.2±9.2	<0.05
S-Osteocalcin (µg/L)	7.2±2.7	10.1±3.7	0.01

Blood was collected in the morning, between 8-10 am, after a light breakfast. All serum and plasma samples were stored frozen at -80°C until analysis. Twenty-four hours urine collections, for determination of Calcium and Phosphate excretion, were also obtained from all patients and controls. *Results.* The results from the biochemical evaluation are given the attached table. Results from bone density measurements (DEXA and pQCT) are pending. *Conclusions.* The imatinib treated patients had lower levels of 1,25-(OH)2 vitamin D, lower ionized Calcium, lower S-Phosphate, lower S-Osteocalcin and increased S-Parathyroid hormone; the changes were small but still statistically significant. These alterations in mineral metabolism resemble those seen in hereditary, vitamin D-dependent rickets type 1, i.e. a dysfunctional renal 1-OHase enzyme. It is still unknown if the altered mineral metabolism has any clinically relevant effect on the bone mineralization. Results from our DEXA and pQCT measurements will be presented.

0549

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

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Background. Imatinib 400 mg (SD) is the established first line treatment of chronic myeloid leukemia (CML) in chronic phase. The efficacy of imatinib in early chronic phase has been demonstrated by phase 2 and 3 controlled trials like the IRIS study (Druker BJ et al NEJM 355:23, 2006). Large multicentric studies aimed to evaluate the impact of imatinib 400 mg/daily outside strictly monitored frameworks are not yet available. AIMS The GIMEMA (Gruppo Italiano Malattie Ematologiche dellAdulto) CML Working Party opened in January, 2004, an observational study (serial n. CML/023) to investigate the efficacy of imatinib SD in newly diagnosed CML patients in chronic phase. Since response to treatment is risk related and reporting on the cumulative response rate irrespective of risk is confusing, we report the results obtained in low (Sokal) risk patients. This allows a more suitable comparison with other published series. METHODS Clinical and anagraphical data were collected through a web-based system. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR, Bcr-Abl/Abl x 100 - Taqman) were centralized in Bologna. RESULTS Overall, between Jan 2004 and Jan 2006, 55 italian centers enrolled 217 low Sokal risk newly diagnosed CML pts . Median age was 50 yrs (range 18-84), 136 (63%) males and 81 (37%) females. 217 patients were evaluable for response at 3 months, 181 at 6 months and 118 at 12 months. Median observation time is 12 months. At 6 months, 86% of evaluable cases obtained a complete cytogenetic response (100% Ph-neg, CCgR). A major molecular response (MMolR) defined as a Bcr-Abl/Abl x 100 ratio < 0.05%, was shown in 54% of CCgR pts. At 12 months, the CCgR rate was 88% and the MMolR rate in CCgR pts was 60%. With this short observation period, only 4 pts (1,8%) progressed to accelerated/blastic phase.CONCLUSIONS 201 low Sokal risk pts were enrolled in the IRIS trial: at 12 months CCgR and MMolR (reduction of Bcr-Abl/Bcr ratio level > 3 logs) rates were 76% and 66%, respectively (T. Hughes *et al.,* NEJM 349:15, 2003). Our multicentric study confirms and reinforce the results of the IRIS trial in the same Sokal risk category, showing that imatinib is highly effective and manageable outside of academical structures as well as in strictly monitored trials. ACKNOWLEDGMENTS COFIN 2003, FIRB 2001, AIRC, Fondazione del Monte di Bologna e Ravenna, European Leukemia Net, BolognaAIL. *Background.* Imatinib 400 mg (SD) is the established first line treatment of chronic myeloid leukemia (CML) in chronic phase. The efficacy of imatinib in early chronic phase has been demonstrated by phase 2 and 3 controlled trials like the IRIS study (Druker BJ et al NEJM 355:23, 2006). Large multicentric studies aimed to evaluate the impact of imatinib 400 mg/daily outside strictly monitored frameworks are not yet available. AIMS The GIMEMA (Gruppo Italiano Malattie Ematologiche dellAdulto) CML Working Party opened in January, 2004, an observational study (serial n. CML/023) to investigate the efficacy of imatinib SD in newly diagnosed CML patients in chronic phase. Since response to treatment is risk related and reporting on the cumulative response rate irrespective of risk is confusing, we report the results

obtained in low (Sokal) risk patients. This allows a more suitable comparison with other published series. METHODS Clinical and anagraphical data were collected through a web-based system. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR, Bcr-Abl/Abl x 100 - Taqman) were centralized in Bologna. RESULTS Overall, between Jan 2004 and Jan 2006, 55 italian centers enrolled 217 low Sokal risk newly diagnosed CML pts . Median age was 50 yrs (range 18-84), 136 (63%) males and 81 (37%) females. 217 patients were evaluable for response at 3 months, 181 at 6 months and 118 at 12 months. Median observation time is 12 months. At 6 months, 86% of evaluable cases obtained a complete cytogenetic response (100% Ph-neg, CCgR). A major molecular response (MMolR) defined as a Bcr-Abl/Abl x 100 ratio < 0.05%, was shown in 54% of CCgR pts. At 12 months, the CCgR rate was 88% and the MMolR rate in CCgR pts was 60%. With this short observation period, only 4 pts (1,8%) progressed to accelerated/blastic phase.CONCLUSIONS 201 low Sokal risk pts were enrolled in the IRIS trial: at 12 months CCgR and MMolR (reduction of Bcr-Abl/Bcr ratio level > 3 logs) rates were 76% and 66%, respectively (T. Hughes *et al.,* NEJM 349:15, 2003). Our multicentric study confirms and reinforce the results of the IRIS trial in the same Sokal risk category, showing that imatinib is highly effective and manageable outside of academical structures as well as in strictly monitored trials. ACKNOWLEDGMENTS COFIN 2003, FIRB 2001, AIRC, Fondazione del Monte di Bologna e Ravenna, European Leukemia Net, BolognaAIL.

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IMATINIB HIGH DOSE (800 MG) IN INTERMEDIATE SOKAL RISK PATIENTS CML 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 (MMolR) 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-istitutional prospective study (serial 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 MMolR (Bcr-Abl/Abl x 100 ratio < 0.05%). At 12 mos, the CCgR rate was 88% and the MMolR 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 103:2873). ACKNOWLEDGMENTS COFIN 2003, FIRB 2001, AIRC, CNR, Fondazione del Monte di Bologna e Ravenna, European LeukemiaNet, AIL.

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MULTIPLE ANALYSES AFFIRM THE INDEPENDENT ADVERSE INFLUENCE OF ABL/BCR BREAKPOINT SPANNING DELETIONS ON SURIVAL IN CHRONIC MYELOID LEUKEMIA

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Background. Deletions at or encompassing the ABL/BCR junction on the derivative chromosome 9 [(der(9)] are seen in 10-15% of patients with chronic myeloid leukemia (CML). However whether deletions are genuinely associated with an adverse prognosis on survival remains controversial. We investigated the prognostic significance of der(9) deletions in 339 patients enrolled in three randomized German CML trials and allocated to receive interferon-α (IFN). Initial univariate analysis showed no significant survival difference between 59 deleted and 280 non-deleted patients. Only the 21 patients with breakpoint-spanning deletions had a significantly poorer survival compared to patients without deletions. *Aims.* We sought to investigate whether der(9) deletion status (no deletion vs. one-sided deletions vs. deletions spanning the ABL/BCR breakpoint) provided independent prognostic information with regard to survival when additional factors (age, spleen enlargement, white blood cell count hemoglobin, and the proportions of blasts, eosinophils, and basophils in peripheral blood) were also considered. *Methods.* Deletions were defined using a novel DNA-based deletion screen based on multiplex ligation-dependent probe amplification. Associations between the candidate variables and survival were assessed with multiple Cox regression. In order to attribute survival probabilities to IFN treatment, survival times were censored at the start of imatinib therapy or the date of an allogeneic stem cell transplantation for patients still in chronic phase. *Results.* Firstly, we examined the prognostic value of deletion status in a common Cox model with the Hasford score (Hasford et al., JNCI 1998; 90:850-858) which was developed and validated to differentiate three prognostic groups with regard to survival after start of IFN treatment. Of 338 patients with deletion status and score available, 131 had died. Patients without deletions (n=279, 83%) had a median survival of 6.8 years. Whilst the 21 cases (6%) with deletions at the whole breakpoint showed a median survival of 6.1 years, the 38 patients (11%) with one-sided deletions (whether ABL or BCR was concerned made no difference in survival) had a six-year survival probability of 0.74. Of the 12 patients observed beyond that time, nobody died. The Hasford low-risk group contained 132 patients (39%, median survival: 8.6 years), intermediate-risk group 166 (49%, median survival: 6.8 years), and the high-risk group 40 patients (12%, median survival: 5.6 years). For both Hasford score and deletions status, the logrank test indicated a statistically significant discrimination of survival probabilities. As prognostic factors in a common model, deletion status and Hasford score kept their statistical significance (p=0.007 and p=0.011). In a second approach, all candidate variables were entered into the model. Backward elimination yielded a final model with three independent variables: deletion status (p=0.007), age (p=0.018), and spleen enlargement (p<0.001). In both models, compared to baseline category *no deletion*, one-sided deletions were associated with a significant beneficial effect and deletions at the whole breakpoint with significantly worse survival. *Conclusions.* Our results indicate that deletion status at diagnosis provides independent, statistically significant prognostic information. In particular, the unfavorable influence of ABL/BCR breakpoint spanning deletions on survival was affirmed, in conjunction with the Hasford score or with age and spleen size.

0552

GRADE 3/4 ADVERSE EVENTS IN IMATINIB RESISTANT/INTOLERANT CHRONIC PHASE CML (CML-CP) PATIENTS TREATED WITH NILOTINIB AND DASATINIB

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Background. Toxicities of new treatments for CML may impact ability to maintain the treatment regimen and increase the care and resources required to manage patients. *Aims.* The objective of this study was to compare the incidence rates of Grade 3/4 AEs reported for nilotinib and dasatinib, the second generation tyrosine kinase inhibitors (TKIs), in imatinib resistant/intolerant CML-CP pts in preparation for an economic analysis. *Methods.* A systematic search was conducted to identify published articles, registration documents, or abstracts of clinical trials in CML-CP for nilotinib 400 mg BID and dasatinib 70 mg BID. The base case incidence rates were selected from the most rigorous sources, specifically data from the dasatinib product label and the 120-day update of the nilotinib phase II trial. The AEs selected for the comparison were based on frequency (>5%) and/or high likelihood of incurring incremental costs for medical treatment/monitoring. The full ranges for the incidence of AEs (if available) were summarized from publication to provide ranges for sensitivity analyses in future economic analyses. The nilotinib median duration of exposure was 8.1 months while the median treatment duration of dasatinib was 5.6 months. *Results.* The Grade 3/4 hematologic AEs are the most common AEs (table below) and are approximately twice as high for dasatinib compared to nilotinib. Common Grade 3/4 biochemistry abnormalities include hyperglycemia in

11% and lipase elevations in 15% of nilotinib pts (1 patient developed Grade 1/2 pancreatitis; no Grade 3/4 pancreatitis), while dasatinib abnormalities are hypophosphatemia in 11% and hypocalcemia in 22%. Rates of Grade 3/4 non-hematologic AEs such as pleural effusion, GI bleeding, arrhythmia, and pneumonia/infection are >2-7 times higher for dasatinib than nilotinib. *Summary and Conclusions.* The toxicity profiles of the second generation TKIs for imatinib resistant/intolerant CML-CP appear to be different and favor nilotinib with its reduced rates of Grade 3/4 AEs even with longer median duration of drug exposure compared to dasatinib. Further analysis is required to understand the cost impact to health care payers of the differences in safety profiles among the new TKIs.

Table 1.

	Base AE Occurrence Rate % (Range)	
Grade 3/4 Adverse Event	**Nilotinib**	**Dasatinib**
Anemia	7.9 (5.3-9.9)	18.0 (9.0-20.0)
Neutropenia	28.3 (13.1-33.1)	49.0 (36.4-58.0)
Thrombocytopenia	28.5 (19.9-32.9)	48.0 (35.0-54.0)

0553

WITHIN THE P-LOOP GROUP, THE Y253H AND E255K/V ABL KINASE DOMAIN MUTATIONS ARE OF PARTICULAR SEVERE PROGNOSIS IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS RESISTANT TO IMATINIB MESYLATE

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Despite its remarkable efficacy in chronic myelogenous leukemia (CML) resistance to imatinib mesylate (IM) may arise, and the onset of BCR-ABL point mutations remains the main cause identified, being responsible for resistance. Among these mutations, some located in the ATP binding loop (P-loop: residues 248-255) or at residue 315 seem particularly critical by disturbing IM binding or by altering the flexibility of the ABL kinase domain. The poor clinical prognosis of these mutations is generally accepted but still debatable. Among the P-loop group, biochemistry and cellular assays suggest that there is some heterogeneity in impairing the response to IM and mutations located at residues 253 and 255 are responsible for particularly high IC_{50} values *in vitro*. In a vast retrospective study from 5 different French centers, we analysed and compared the features and clinical outcomes of 75 CML patients treated with IM and presenting either clinical, cytological, cytogenetical resistance or molecular progression. Progression has been defined as a loss of any previous response, progression to a more advanced phase and a ≥2-fold increase in BCR-ABL/ABL ratio confirmed on 2 assessments 3 months apart. Fourty-six patients (32M/14F, median age 52) harboured 49 P-loop mutations [Group 1 (G1)] and 29 patients (19M/10F, median age 52) harboured 37 mutations with at least a T315I mutation (± other) [Group 2 (G2)], as detected by direct sequencing. At mutation discovery 24 patients (52%) were in chronic phase (CP), 12 (26%) in accelerated phase (AP), 10 (22%) in blast crisis (BC) in G1 and 12 (41%), 4 (14%), 13 (45%) in G2 respectively. The median duration of IM was 16 months (1.3-65.6) for G1 and 19 (0-57.6) for G2 (p=ns). In patients who had received IFN prior to IM, duration of IFN was similar between the 2 groups (5 months (0-89) for G1 vs 9 months (0-131) for G2, p=ns). The median interval between diagnosis and day 1 of IM was 16 months (0-61.5) for G1 and 13 months (0-49.5) (p=ns) for G2. Univariate analysis for gender, age, sokal, prior treatment with IFN, initial dose of IM, intervals between diagnosis-IM start, diagnosis-mutation detection, IM-mutation detection and major cytogenetic response with IM, did not show any significant impact of these variables. Multivariate analysis demonstrated a significantly worse PFS since IM start for E255K/V+Y253H mutants vs other P-loop mutants (p=0.016, HR:2.96, 95%CI: 1.22-7.16). IFN treatment prior to IM was associated with better survival rates (p=0.00013, HR:0.12, 95%CI: 0.04-0.35). Progression free survival (PFS) Kaplan Meier curves for all phases, since IM start showed a significant better survival for other P-loop mutations than Y253H, E255K/V and T315I groups (p=0.03) (See Figure 1). In conclusion, our results confirm that P-loop and T315I mutations are associated with poor prognosis, but they show that Y253H and E255K mutations are of particularly severe prognosis within the P-loop group as suggested by *in vitro* data. Those mutations would require early therapeutic intervention in order to improve survival

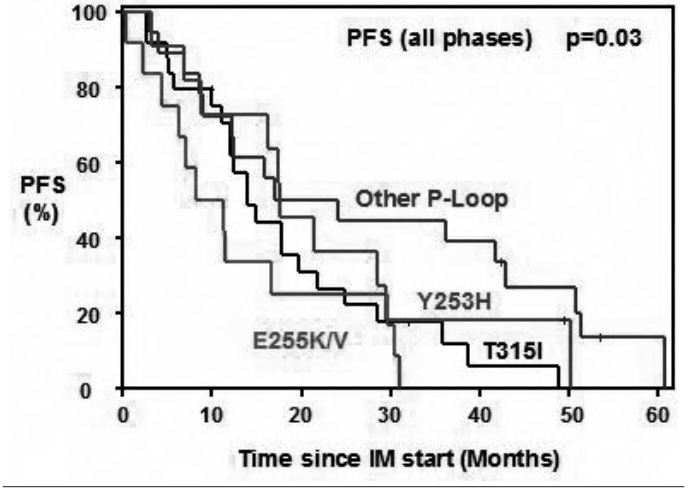

Figure 1. PFS, CML all phases according to mutation status.

0554

NILOTINIB THERAPY AFTER DASATINIB FAILURE IN PATIENTS WITH IMATINIB-RESISTANT OR-INTOLERANT CHRONIC PHASE AND ACCELERATED PHASE PHILADELPHIA-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA

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Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor which is 30-fold more potent *in vitro* than imatinib and active against 32/33 imatinib-resistant cell lines with Bcr-Abl mutations. *Aims.* These results are from an ongoing phase II study evaluating the efficacy and safety, as defined by hematologic and cytogenetic response rates (HR/CyR), of nilotinib administered at a daily dose of 400 mg BID to patients (pts) with Philadelphia-positive (Ph^+) chronic myelogenous leukemia (CML) in chronic phase (CP), accelerated phase (AP), and blast crisis (BC) who were resistant or intolerant to imatinib and dasatinib. *Methods.* All pts signed an informed consent and received nilotinib for an unapproved indication. *Results.* Data from 42 pts are available. Included were 16 CP, 9 AP, and 17 BC; 13 myeloid; 4 lymphoid) pts were included. Median age 58 (19 -78) years; median time since first diagnosis of CML was 18 (0.1-166) mos. Of the 42 pts, 10 (24%) had extramedullary involvement. Median exposure was 81 (4-420) days with

median dose intensity being 800 (216-1195) mg/day. 13 (31%) pts have ongoing treatments and 29 (69%) pts discontinued treatment (16 disease progression, 6 adverse event, 5 other reasons, and 2 deaths). The primary endpoint for CP pts was CyR, while the primary endpoint for AP pts was HR. 13/16 CP pts had no baseline complete hematologic response (CHR). 4/13 (31%) pts had major cytogenetic response (MCyR); 2 (15%) complete and 2 (15%) partial cytogenetic response: 1 minor, 2 minimal, 3 none, 1 not assessable, and 2 disease progression. 5/13 had CHR. Of the 9 AP pts, 2 (22%) returned to CP, 6 (67%) were not available and there was 1 (11%) death. Of the 17 BC pts, 3 (18%) had CHR 1 (6%) returned to chronic phase, 5 (29%) had stable disease, 4 (24%) were not available, and 4 (24%) had progressive disease. Overall, thrombocytopenia (33%), pyrexia (29%), anemia (24%), and neutropenia (24%) were the most common adverse events including all grades. The most common grade 3/4 adverse events were thrombocytopenia (26%), neutropenia (24%), and anemia (7%). Any hematologic and/or cytogenetic responses were observed in 4/8 CP and 3/7 AP patients with baseline mutations vs. 6/7 CP and 2/3 AP patients without baseline mutations. *Summary and Conclusions.* Nilotinib has clinical activity in CML-CP, -AP, and -BC pts who were resistant or intolerant to imatinib and have also previously failed dasatinib therapy. In these heavily pre-treated pts, nilotinib administration has acceptable safety and tolerability similar to that previously profiled. Updated information will be presented at the meeting.

0555

A PHASE II STUDY OF NILOTINIB, A NOVEL TYROSINE KINASE INHIBITOR ADMINISTERED TO IMATINIB-RESISTANT AND -INTOLERANT PATIENTS WITH PHILADELPHIA-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE

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Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor that *in vitro* is 30-fold more potent than imatinib and active against 32/33 imatinib-resistant BCR-ABL mutations. *Aims.* This open-label study was designed to evaluate the safety and efficacy of nilotinib, as defined by hematologic/cytogenetic response rates (HR/CyR), administered at a daily dose of 400 mg BID to patients (pts) with imatinib-resistant or -intolerant Philadelphia-positive (Ph^+) chronic myelogenous leukemia (CML) in chronic phase (CP). *Methods.* Nilotinib could be escalated to 600 mg BID for pts who did not adequately respond to treatment. All pts signed an informed consent and received nilotinib for an unapproved indication. *Results.* 318 pts were enrolled including 223 (70.1%) with imatinib resistance and 95 (29.9%) with imatinib intolerance. Median age was 58 (21-85) years and the median time since CML diagnosis was 57.3 mos. 50.6% were men. Treatment with nilotinib is ongoing for 221 (69.5%) pts. A total of 97 (30.5%) pts have discontinued treatment. The median duration of nilotinib exposure was 245 (1-502) days. The median average dose intensity for all pts was 796.6 (151-1111.5) mg/day. The nilotinib dose was escalated for 41 (12.9%) pts. Efficacy data are available for 280 pts with ″6 months of follow-up. Major CyR (MCyR) was observed in 145 (51.8%) pts, of which 96 (34.3%) were complete and 49 (17.5%) partial. 10 (3.6%) pts had disease progression. The median time to MCyR was 2.8 (0.9-11.1) months. 95 (33.9%) had a CHR at baseline and 185 (66.1%) did not. 137 (74.1%) of the 185 pts without baseline CHR achieved CHR. The median time to CHR was 1 (0.9-8.3) mo. Of 101 pts with baseline mutation analysis performed, 45 (44.6%) had BCR-ABL mutations. Of the 318 pts included in the safety analysis, overall the most frequent grade 3/4 adverse events included thrombocytopenia (n=70, 22%), neutropenia (n=49, 15.4%), anemia (n=22, 6.9%), and elevated serum lipase (n=22, 6.9%). 3 (0.9) pts experienced QTcF >500 msec. Overall, there were 4 deaths (1 myocardial infarction and ventricular rupture, 1 coronary artery disease and sudden death, 2 sepsis). *Summary and conclusions.* Nilotinib has demonstrated significant clinical activity as defined by 51.8% MCyR rate and 74.1% CHR rate, and an acceptable safety and tolerability profile in pts with imatinib-resistant or -intolerant CML-CP.

0556

NILOTINIB MONOTHERAPY IN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT PH+ CHRONIC MYELOGENOUS LEUKEMIA IN BLAST CRISIS OR RELAPSED/ REFRACTORY PH^+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor of BCR-ABL that *in vitro* is 30-fold more potent than imatinib. It is active against 32/33 imatinib-resistant BCR-ABL mutations. *Aims.* This open-label phase II study was designed to evaluate the safety and efficacy of nilotinib at a dose of 400 mg BID in adult patients (pts) with imatinib-resistant or -intolerant CML-BC or in pts with relapsed/refractory Ph+ALL. The primary endpoints were investigator assessments of best hematologic response (HR) and complete remission, respectively. *Methods.* Daily doses of nilotinib could be escalated to 600 mg BID for pts who did not adequately respond to treatment, and in the absence of safety concerns. All pts signed an informed consent and received nilotinib for an unapproved indication. *Results.* Safety and efficacy data are reported for 120 BC (87 myeloid, 27 lymphoid, 6 unknown) and 41 Ph^+ ALL pts (37 active disease, 4 residual disease; 38 relapsed, 3 refractory). 60% of pts had >35% Ph^+ metaphases for BC and 31% for Ph^+ ALL. Median ages were 54 yrs for BC and 46 yrs for Ph^+ALL pts. Chromosomal abnormalities other than Ph^+ were noted in 64 (53%) BC and 12 (29%) Ph^+ ALL pts. Extramedullary involvement was present in 44 (37%) BC and 3 (7%) Ph^+ ALL pts. Median treatment duration was 53 (1-441) and 72 (3-363) days for BC and Ph^+ ALL, respectively. Median dose intensity was 800 mg/day for both pt groups. Treatment is ongoing for 21 (18%) BC and 4 (10%) Ph^+ ALL pts. Most discontinuations were due to disease progression (61 [51%] in BC; 26 [63%] in Ph^+ ALL). Hematologic responses (HR) were observed in 42 (35%) BC pts. Of the 120 BC pts, complete HR was reported in 25 (21%) pts, marrow responses (no evidence of leukemia) in 7 (6%) pts, and return to chronic phase in 10 (8%) pts. Complete remission was reported in 10 (24%) Ph^+ ALL pts, of which 1 pt had minimal residual disease. The most common grade 3/4 AEs were thrombocytopenia (41%), neutropenia (28%), pneumonia (11%), and anemia (27%) in BC and thrombocytopenia (24%) in Ph^+ ALL pts. During the study period, death occurred in 9 (8%) BC and 3 (7%) Ph^+ ALL pts. No Ph^+ ALL pt developed CNS disease while on therapy. *Summary and conclusions.* Nilotinib monotherapy has significant clinical activity and is well tolerated in pts with imatinib-resistant or -intolerant BC and pts with relapsed/refractory Ph+ ALL. Nilotinib represents an important new treatment option for these pts, in which there remains a high unmet medical need. Given the safety and efficacy of nilotinib monotherapy, the combination of nilotinib with systemic chemotherapy warrants investigation in Ph^+ CML-BC or ALL. Updated data will be presented at the meeting.

0557

A PHASE II STUDY OF NILOTINIB, A NOVEL TYROSINE KINASE INHIBITOR ADMINISTERED TO IMATINIB-RESISTANT OR -INTOLERANT PATIENTS WITH PHILADELPHIA-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA IN ACCELERATED PHASE

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Background. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor of BCR-ABL that *in vitro* is 30-fold more potent than imatinib. It is active against 32/33 imatinib-resistant BCR-ABL mutations. *Aims.* This open-label study was designed to evaluate the safety and efficacy of nilotinib, as defined by hematologic/cytogenetic response (HR/CyR) rates, at a dose of 400 mg BID in patients (pts) with Philadelphia-positive (Ph^+) imatinib-resistant or -intolerant chronic myelogenous leukemia (CML) in accelerated phase (AP). *Methods.* Nilotinib could be escalated to 600 mg BID for pts who did not adequately respond to treatment. All

pts signed an informed consent and received nilotinib for an unapproved indication. *Results.* Data are available on the 120 enrolled pts, of whom 97 (80.8%) pts were resistant and 22 (18.3%) were intolerant to imatinib. 67 (55.8%) were male. The median age was 58 (22-79) years and the median time since first diagnosis of CML was 71.3 (2.2-298.2) mos. The median duration of nilotinib exposure was 137.5 (2-503) days and median average dose intensity for all pts was 796.6 (145.4-1149) mg/day. Nilotinib dose was escalated for 26 (21.7%) pts. Treatment is ongoing for 64 (53.3%) patients, and 56 (46.7%) have discontinued (25 [20.8%] for disease progression, 14 [11.7%] for adverse events). Baseline mutation data are available for 46 of the 64 pts. Of the 96 pts who had ≥6 months of follow-up, confirmed hematologic response (HR) occurred in 43 (44.8%) patients. 18 (18.8%) pts had complete HR, 9 (9.4%) had marrow responses (no evidence of leukemia), and 16 (16.7%) returned to chronic phase. Time to confirmed HR was 1 (0.8-3) month. Major CyR occurred in 29 (30.2%) pts, of whom 15 (15.6%) had complete CyR and 14 (14.6%) had partial CyR. 12 (12.5%) pts had minor CyR, and 20 (20.8%) had minimal CyR. The rate of MCyR for resistant and intolerant AP patients was 30.4% and 29.4%, respectively. Of the 120 patients included in the safety analysis, overall the most frequent grade 3/4 adverse events included thrombocytopenia (n=41, 34.2%), neutropenia (n=24, 20%), anemia (n=16, 13.3%), and elevated serum lipase (n=11, 9.2%). No pts experienced QTcF intervals >500 msec. Overall, there were 9 deaths including 4 for disease progression, 2 due to other malignancies, 1 related to progressive disease complicated by a cerebral hemorrhage, 1 cardiac failure, and 1 due to sepsis. *Summary and Conclusions.* These data demonstrated that nilotinib is clinically active and has an acceptable safety and tolerability profile when administered to pts with imatinib-resistant or -intolerant Ph^+ CML-AP.

0558

REDUCED INTENSITY CONDITIONING OF ALLO-SCT IN CML PATIENTS IS SUPERIOR OVER MYELOABLATIVE CONDITIONING AND RESULTS WITH 60% OF LONG TERM SURVIVAL

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Background. In the era of Imatinib a number of allo SCT in CML patients has been considerably reduced. However, in some patients we come to the decision of allo SCT while toxicity or progression to more advanced stages of the disease become clinically apparent. There are rather rare events but still this problem may be relevant up to 20% of patients especially during the first three years of treatment. An optional proposal to proceed with allo SCT should have a rational basis behind. *Methods.* In this study we present the data of allo SCT performed in one center in two cohorts composed of patients receiving myeloablative (cohort 1: Busulfan 16 mg/kg b.w., Cyclophosphamide 120 mg/kg b.w. + anti-thymocyte globulin - ATG 20 mg/kg b.w. in alternative donors transplantation) and non myeloablative (cohort 2: Bu 8 mg/kg b.w., Flu 120 mg/kg, ATG usually 10 mg/kg b.w.) preparative regimens. Patients characteristics in cohort 1 and cohort 2 are as follow: n=40 (n=58), 1st CP: 31 (49), > 1st CP: 2 (9), ACCP or BC: 7 (0), sib/alternative donors: 30/10 (23/35), marrow/PBPC: 35/5 (14/44), years of transplant: 1989-2000 (1999-2006). The results of the transplant procedure when compared cohort 1 vs cohort 2 differed with respect to the incidence of transplant related toxicity ≥ III grade (10/40 vs 1/58, p<0,001), but not with respect to aGvHD (19/40 vs 18/58, p=0.098), extensive cGvHD (11/40 vs 15/58, p=0.857) and relapses incidence (5/40 vs 9/58, p=0,675). Overall survival of RIC patients (cohort 2) was significantly better than myeloablative patients (cohort 1) (Log-rank test, p=0.029). From an univariate analysis it became apparent that the results of the procedure was affected by: time from diagnosis to transplantation (p<0.025), years of transplant (p<0.007), stage of the disease (p<0.031) and intensity of conditioning (p<0.030). There are several bias behind the latter comparisons: myeloablative and RIC transplantations were performed in two different time periods what may affect the outcome due to the improvement in supportive care, on the other hand cohort 2 patients received more frequently VUD then sibling transplantation. Therefore, a multivariate analysis was performed which documented that only time from the diagnosis to allo SCT (p<0.012) and intensity of conditioning (p<0.011) independently affected the outcome. In conclusion patients transplanted under RIC with a low dose of ATG enjoy in 60% long term survival affected by 25% incidence of extensive cGvHD independent whether they received sibling or MUD transplant providing high resolution typing.

0559

IMATINIB 400 MG IN LATE CHRONIC PHASE CML PATIENTS: LONG TERM EVALUATION OF RESPONSES AND SURVIVAL OF A PHASE II STUDY OF THE GIMEMA CML WP

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Background. Imatinib is the drug of choice for the treatment of Ph+ CML, where a complete cytogenetic response (CCgR) is achieved in more than 80% of early chronic phase (ECP) patients. In patients who start imatinib in late chronic phase (LCP) the cytogenetic response rate is lower (40-50%) and the long term effect on survival is not yet determined. *Aims.* The GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) CML Working Party is conducting a phase II, multi-istitutional prospective study (CML/002) to investigate the long-term effects of imatinib in LCP patients, treated with imatinib 400 mg daily after failure of Interferon-γ. 295 patients were enrolled; median age was 52 yrs (range: 19-82). The median duration of chronic phase prior to imatinib treatment was 38 months (range: 1-202). We monitored the hematologic, cytogenetic and molecular response to imatinib in this cohort, with focus on the rate of major molecular response and on progression-free survival and overall survival of complete cytogenetic responders. The median observation time is 62 months (range, 6-72). *Methods.* Patients were monitored for cytogenetic and molecular response every 6 months. Cytogenetics was performed with conventional methods. Molecular response was assessed in Bologna on peripheral blood samples by quantitative PCR (RQ-PCR) using TaqMan methodology and expressed as a ratio of BCR-ABL to ABL%. Major molecular response (MMR) was defined as BCR-ABL/ABL% below 0.05, whereas a complete molecular response (CMR) was defined as undetectable BCR-ABL transcript levels (below 0,001%) by RQ-PCR confirmed by nested PCR. *Results.* 158 patients (53%) achieved a CCgR at a median time of 6 months from start of imatinib and 124 of them (78%) are still in continuous CCgR at 60 months. 34 patients (22%) lost the CCgR: 23 (67%) within 24 months from the date of its first achievement and 11 at a later timepoint (5 between 25 and 36 months, 6 after 37 months). For patients who obtained a CCgR, the 5-year progression free survival is 92% and overall survival is 91,5% (versus 60% e 73,5% for those without a CCgR, p=0.00001). 115/124 patients with stable CCgR were evaluable for molecular response. Considering all timepoints, the median number of evaluable samples was 100 (range: 73-115). The frequency of MMR increased during follow-up (38%, 46%, 53% at 6, 12 and 24 months) up to 70% at 36 and 60 months. During the course of treatment, 44 patients (38%) had a stable MMR; 27 (23%) fluctuated between MMR and undetectable levels of BCR-ABL (BCR-ABL/ABL% below 0,001, nested PCR positive) and one patient maintained a stable RT-PCR negativity. 24 patients (21%) never achieved a MMR, although in stable CCgR. Four patients (3%), achieved a CMR, which was unstable during follow-up. *Conclusions.* For complete cytogenetic responders progression-free and overall survival are likely to be as good as for ECP patients, suggesting that the achievement of CCgR represents a main prognostic factor for CML patients. Moreover, even in late chronic phase CML patients, imatinib determines a high and early frequency of MMR, which increases over time.

0560

A RETROSPECTIVE COMPARISON OF THE SAFETY PROFILES OF HIGH-DOSE IMATINIB AND DASATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. Patients with chronic myeloid leukemia (CML) treated with kinase inhibitors may experience undesirable side effects. *Aims.* This study aims to compare the safety profiles of high-dose (HD) imatinib and dasatinib in chronic phase (CP) CML patients who have been previously treated with standard dose 400 mg daily imatinib using data

from pivotal clinical trials. *Methods.* Adverse event (AE) data were extracted for patients who had at least two occurrences of daily imatinib dose escalated from 400 mg to 600 or 800 mg from the 5-year IRIS study in newly diagnosed CP-CML patients (N=158) and, for those receiving dasatinib 70 mg twice daily for imatinib-resistant or intolerant CP-CML, from the FDA's publicly-available Sprycel Drug Approval Package (N=186 to 214). AEs were selected based on their frequency, clinical significance and availability. Conservative approaches to systematically favor dasatinib were employed throughout the analysis. Incidence rates were reported as number of patients with the AE per 100 patient-years of observation, and incidence rate ratios were used to compare both drugs using Poisson distribution. The imatinib group's observation period was constructed based on time periods of imatinib treatment beginning from the date of dose escalation to > 400 mg, and ending at the earliest of the last imatinib treatment date or the AE end date, thus assuming AE occurrence even during treatment gaps. For the dasatinib group, in the absence of specific patient-level data, it was conservatively assumed that all AEs occurred only at the latest treatment end date (279th day) for the group, artificially increasing the denominator for the incidence rate calculation which in turn underestimated the AE incidence for the dasatinib group. All AEs (drug-related or not) were considered for imatinib, whereas only those judged by the investigator as drug-related were included for dasatinib. Sensitivity analyses based on the subset of HD imatinib patients receiving ≥ 800 mg or the HD imatinib patients receiving interferon-alfa plus cytarabine before crossing over to standard imatinib dose were also conducted. *Results.* At baseline, relative to HD imatinib, the dasatinib group was older (mean age; 56.5 vs. 49.5 years, $p<0.05$), had more males (53.8% vs 33.5%, $p<0.05$), and had comparable ECOG performance scores (mean; 0.280 vs 0.284, $p=0.96$). The median daily dose during observation was 667.1 mg in the HD imatinib group, and 114 mg in the dasatinib group. The dasatinib group presented statistically significantly ($p<0.05$) higher incidences for all studied AEs than the HD imatinib group (Table 1). Sensitivity analyses confirmed the statistically significantly different AE incidences between the two groups. *Summary and conclusions.* CP-CML patients receiving dasatinib may be at greater risk for a variety of clinically important AEs than those treated with HD imatinib following treatment with standard dose imatinib, as suggested by the significantly higher incidence rates observed for dasatinib despite an overwhelmingly conservative analytical approach in favor of dasatinib and despite a lower median daily dose of dasatinib than prescribed (140 mg). Further analysis addressing the limitations stemming from lack of randomization is warranted.

Table 1. Comparison of AE incidence rates between HD Imatinib and Dasatinib patients.

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IMATINIB 400 MG AND PEGYLATED RECOMBINANT INTERFERON-ALPHA IN EARLY CHRONIC PHASE CML: HIGH FREQUENCY OF COMPLETE CYTOGENETIC AND MAJOR MOLECULAR RESPONSES IN THE LONG TERM

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Background. Imatinib 400 mg is the established first line treatment of chronic myeloid leukemia (CML) in chronic phase. *Aims.* Between July and December 2001, 76 patients were enrolled in a phase II study of the Italian Cooperative Study Group on CML (serial n. CML/011) to investigate the efficacy of imatinib 400 mg daily and a variable pegylated IFN-α dose (50, 100 and 150 microg/wk) in early chronic phase (ECP) CML patients. *Methods.* Patients were monitored for conventional cytogenetic and molecular response every 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 (MMR) was defined as BCR-ABL/ABL% less than 0.05. *Results.* 76 ECP CML patients were monitored, with a median observation time of 54 months (range: 12-65). Median age at diagnosis was 47 yrs (range 18-68). According to Sokal scale, 45% were low, 31% intermediate and 24% high risk. 75 patients discontinued IFN-α after a median time of 10 months (range: 1-49) because of side effects (96%) or unsatisfactory therapeutic effect (4%). Imatinib have been discontinued in 10 (12%) patients at a median time of 21 months (range: 4-56) because of: unsatisfactory therapeutic effect (4), submission to allogeneic SCT while in CCgR (1), CCgR loss (3), progression to AP (1), consent withdrawal (1). The mean daily imatinib dose was 348 mg. 66 (87%) patients obtained a CCgR (specifically 72%, 96% and 88% of high, intermediate and high risk patients, respectively). 56 patients (78%) are still in continuous CCgR at 60 months. 59 patients (90%) achieved the CCgR within 12 months of therapy, 2 patients between 13 and 24 months and 5 (6,5%) after 25 months. 3 patients (2 low and 1 intermediate Sokal risk) lost the CCgR, after 9, 31 and 24 months from its first achievement, respectively. 6 patients underwent allogeneic SCT. 3 patients died: 1 after allogeneic SCT and 2 from a cause unrelated to CML. The 5-year overall progression free survival (PFS) is 82% and overall survival (OS) is 97%. Analyzing patients by Sokal risk, no difference in PFS and OS resulted statistically significant among the 3 groups. 60/63 patients with stable CCgR were evaluable for molecular response. Considering all timepoints, the median number of evaluable samples was 56 (range: 55-60). The frequency of MMR was very high at 6 months (60%) and increased over time (72%, 80%, 86%, 89% and 80% at 12, 24, 36, 48 and 60 months). During follow-up, 27 patients (45%) had a stable MMR; 25 (42%) fluctuated between MMR and undetectable levels of BCR-ABL (BCR-ABL/ABL% below 0,001, nested PCR positive) and one patient maintained a stable RT-PCR negativity. Only 1 patients (1,5%) never achieved a MMR. *Conclusions.* Our study shows that imatinib is highly effective and manageable in ECP CML patients, reinforcing the results of the IRIS trial.

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0562

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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Background. Imatinib is the drug of choice for the treatment of Ph+ CML, with very high cytogenetic response rates both in patients treated in early (ECP) and in late (LCP) chronic phase. As the rates of cytogenetic remission improves, monitoring minimal residual disease by PCR has become more important. *Aims.* We evaluated the pattern and the clinical significance of molecular response to imatinib by comparing patients treated in ECP and in LCP. We analyzed the results of quantitative PCR (RQ-PCR) in 187 patients with CML who achieved a stable CCgR with imatinib 400 mg daily (124 treated after failure of IFN-α and 63 previously untreated). The patients on study have been enrolled in two different phase II, multi-istitutional prospective study of the Italian Cooperative Study Group (serial no.: CML/002 and CML/011). Median observation time for both groups is 60 months (range, 6-72). *Methods.* Patients were monitored for conventional cytogenetic and molecular response every 6 months. Molecular response was centralized in Bologna, assessed on peripheral blood samples by quantitative PCR (RQ-PCR) using TaqMan methodology and expressed as a ratio of BCR-ABL to ABL%. Major molecular response (MMR) was defined as BCR-ABL/ABL% below 0.05. Complete molecular response (CMR) was defined as undetectable BCR-ABL transcript levels (below 0,001%) by RQ-PCR confirmed by nested PCR. *Results.* 295 patients were treated with imatinib after failure of Interferon-α (administered for a median time of 38 months, range: 1-202). 158 patients achieved a CCgR: 124 (78%) are still in continuous CCgR and 115/124 are evaluable for molecular response at 60 months (median number of evaluable samples: 100, range: 73-115). 76 ECP CML patients were treated with imatinib and a variable pegylated IFN-α dose (50, 100 and 150 microg/wk), which has been discontinued by 75/76 patients after a median time of 10 months (range: 1-49). 66/76 patients obtained a CCgR (specifically 72%, 96% and 88% of high, intermediate and low risk patients). 63 (83%) patients are still in continuous CCgR and 60/63 are evaluable for molecular response at 60 months (median number of evaluable samples: 56, range: 55-60). The frequency of MMR increased during follow in both early and late CP patients, but the rapidity of achievement and the quality of the molecular response was higher in ECP patients. Specifically, the proportion of patients treated in ECP achieving a MMR was 60%, 72%, 80%, 86%, 89% and 80% at 6, 12, 24, 36, 48 and 60 months (versus 38%, 46%, 53%, 70%, 68%, 68% of LCP patients at the same time-points). Even the rate of RQ-PCR negativity was higher in ECP (22%) than LCP patients (7%) at 5 years. During follow-up, MMR was stable in 88% and in 78% of early and late CP patients, respectively. *Conclusions.* Our long-term results shows that Imatinib induces a very high rate, which increases over time, of MMR both in early and late CP patients. Notably, ECP patients, maybe even thanks to the association of IFN-α in the first year of treatment, obtained earlier, higher and more durable molecular responses.

0563

HIGH SENSITIVITY TESTING IDENTIFIES ADDITIONAL LOW LEVEL BCR-ABL KINASE DOMAIN MUTATIONS IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA AND RESISTANCE TO IMATINIB

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Background and Aims. Mutations of the BCR-ABL kinase domain (KD) are the most frequent cause of acquired resistance to imatinib (IM) in patients with chronic myeloid leukaemia (CML), most likely due to selection of mutant clones on IM treatment. As new BCR-ABL inhibitors become available, precise quantification of low level mutation will be required to monitor response. Here we report our results for 5 key mutations (G250E, Y252H, E255K, T315I, F359V) in both IM resistant and newly-diagnosed CML patients using a sensitive and quantitative Ligation-PCR (L-PCR) assay in comparison to direct sequencing. *Patients and Methods.* Forty three CML patients on IM (24 male, 19 female) with a median age of 60 (range 20 to 75) years in blast crisis (n=11), accelerated phase (n=20) or chronic phase (n=12) and IM failure were analysed using both approaches. Sequencing of the ABL KD was performed using forward and reverse primers of the ABL exons 4 to 7, while the L-PCR analysis focused on the G250E, Y253H, E255K, F359V and T315I mutations. Briefly, pairs of probes specific for either wild type (wt) or mutant BCR-ABL were added to the RT-PCR amplified BCR-ABL KD, and then ligated under conditions optimized for specificity. Ligated probe pairs were then amplified in a quantitative PCR using universal primers. Values were expressed as% BCR-ABLmut/BCR-ABLwt based on ct values. In our hands, this assay can detect 0.05-0.1% mutant allele in a BCR-ABLwt *Background.* Results were scored positive only if two independent runs showed amplification exceeding the lowest controls. *Results.* Patients were treated with a median IM dose of 600 (range 500-800) mg for a median of 15.5 (range 1 to 75) months. Dose reductions due to toxicity were necessary in 18 (42%) patients. Overall, one (n=16) or two (n=2) mutations of the BCR-ABL KD were detected by direct sequencing (42%). In contrast, L-PCR for G250E, Y253H, E255K, F359V T315I detected 40 (one n=16, two n=7, three n=2, four n=1) mutations in 26 (60%) patients with a median of 0.25% (range 0,07-100) BCR-ABLmut in BCR-ABLwt. Direct sequencing identified mutations in only 9 (21%) of the 26 patients (p=0.002). On median follow-up of 7 (range 1-53) month in 11 patients, one progression from 0.1 to 23% BCR-ABLE255K in BCR-ABLwt within 30 days was observed; all other mutated clones remained stable at low levels. No mutations were detected in 14 newly diagnosed CML patients either by direct sequencing or L-PCR. *Conclusions.* (1) L-PCR detects low levels of mutant clones in a high proportion of patients on IM who remain negative by direct sequencing. (2) The failure of L-PCR to detect mutations in newly diagnosed patients is consistent with selection on IM therapy. (3) Limited follow-up data suggest that some mutant clones may remain stable at low level over prolonged periods of time. Their prognostic significance remains to be determined.

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RATIONAL USE OF THE REAL-TIME QUANTITATIVE PCR PROTOCOL TO MEASURE TELOMERE LENGTH IN BLOOD SPECIMENS

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Background and Aims. It has been presumed that the measurement of telomere length is troublesome and time-consuming, despite the fact that telomere attrition is closely linked to bone marrow failure syndrome as well as leukemia and lymphoma. We present here an automated telomere measurement system that combines magnetic filtration and real-time PCR. *Methods.* Genomic DNA was extracted from 100 µL of whole blood by an automated magnetic bead system, then quantitative PCR was done in a closed tube using a carefully designed pair of oligonucleotide primers as reported by Cawthon *et al.* (Nucleic Acid Res 2002, 30: e47). In this assay, the quantity of telomere repeat in each experiment sample was measured as the level of an arbitrarily chosen reference sample, then the telomere signal was normalized to the signal from a single copy gene to generate a relative ratio (T/S). *Results.* In 107 peripheral blood specimens obtained from healthy volunteers (aged 8 to 84) and 6 leukemia cell lines, the T/S ratio was significantly associated with the mean telomere restriction fragment (TRF) measured by traditional Southern blot analysis [y=TRF, x=T/S, y=6.55x±2.39, R2=0.564]. The relative telomere ratio in blood specimens obtained from healthy volunteers was 1.06±0.21 (mean ± S.D). In 87 peripheral blood specimens obtained from various hematologic diseases, the relative telomere ratio was as follows: chronic myeloproliferative disorders (n=34), T/S=0.93±0.41, acute leukemia (n=31), T/S=0.77±0.75, myelodysplastic syndrome (n=23), T/S=0.39±0.34. Of note is that the T/S ratio in myelodisplastic syndrome was more closely linked to the length of the G-tail rather than TRF, which included the subtelomeric region. *Conclusions.* Our results suggest automated telomere measurement is suitable for high throughput of samples. The resolution of this assay should be adequate for many genetic and epigenetic studies. This assay will facilitate investigations of the biology of telomeres and their roles in hemopoetic stem cell biology as well as bone marrow failure syndrome.

0565

COOPERATING MUTATIONS OF RECEPTOR TYROSINE KINASE/RAS PATHWAY IN ADULT CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA: DIFFERENT PATTERNS BETWEEN AML1-ETO AND CBFB-MYH11

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Background. Two-hit model of leukemogenesis has been proposed for AML; class I mutations that drive proliferation and survival, and class II mutations that block differentiation. *Aims.* We sought (1) to determine the cooperating mutations of class I including receptor tyrosine kinase (RTK)/Ras signaling pathway in adult AML patients with core-binding factor (CBF) translocations which are class II mutations, and (2) to determine the difference in the patterns of cooperating mutations between AML with AML1-ETO and AML with CBFβ-MYH11. *Patients and Methods.* By RT-PCR analysis, 123 adult patients were identified to have CBF AML, 93 with AML1-ETO and 30 with CBFβ-MYH11. Bone marrow samples at diagnosis were analyzed for FLT3-ITD, FLT3-TKD, N-ras, K-ras, c-KIT and c-FMS mutations. Mutational analysis was performed by DNA-PCR followed by GeneScan analysis for FLT3/ITD, and by PCR-RFLP followed by direct sequencing for FLT3-TKD, by DNA/cDNA PCR followed by direct sequencing of all PCR products for N-ras, K-ras, c-KIT and c-FMS. *Results.* Forty-six of 93 patients (49.5%) with AML1-ETO had RTK/Ras mutations compared with 21 of 30 patients (70%) with CBFβ-MYH11 (p=0.059). The frequencies of RTK/Ras mutations in 93 AML1-ETO AML were 5.4% (n=5) for FLT3/ITD, 7.5% (n=7) for FLT3/TKD, 1.1% (n=1) for N-ras, 3.2% (n=3) for K-ras, 33.3% (n=31) for c-KIT, and 1.1% (n=1) for c-FMS mutation (A273V). The frequencies of RTK/Ras mutations in 30 CBFβ-MYH11 AML were 3.3% (n=1) for FLT3/ITD, 26.7% (n=8) for FLT3/TKD, 20% (n=6) for N-ras, 20% (n=6) for c-KIT, and none for K-ras and c-FMS mutations. All RTK/Ras mutations were mutually exclusive except two, one of them had both N-ras and K-ras mutations, the other harbored FLT3/TKD and c-KIT mutations. Patients with CBFβ-MYH11 had a significantly higher frequency of FLT3/TKD and N-ras mutations than patients with AML1-ETO (p=0.010 for FLT3/TKD, and p=0.001 for N-ras). Taken together, c-KIT mutations accounted for 30% in CBF AML, and there was no difference in the frequency between AML1-ETO and CBFβ-MYH11 groups. Of the 31 patients with AML1-ETO and c-KIT mutations, 23 had mutations located at kinase domain (exon 17), 3 in exon 8, 1 in exon 9, and 4 in exon 11. Of the 6 patients with CBFβ-MYH11 and c-KIT mutations, two each had mutations in exons 8, 11 and 17. Patients with AML1-ETO were more frequently associated with c-KIT mutations in exon 17 as compared with patients with CBFβ-MYH11 (p=0.073). *Conclusions.* Our results showed that occurrence of cooperating mutations of RTK/Ras pathway are common in adult patients with CBF AML. The patterns of mutations were different between AML1-ETO and CBFβ-MYH11 groups.

0566

COOPERATING MUTATIONS IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background. There have been rare reports on the cooperating mutations of class I mutations which drive proliferation of acute myeloid leukemia (AML) blasts and class II mutations which block differentiation of blasts in childhood AML. *Aims.* We sought to systematically study the cooperating mutations in a large series of childhood AML. *Patients and Methods.* By cytogenetics and RT-PCR analysis,166 children with newly diagnosed AML were divided into t(8;21) (N=31), inv(16) (N=14), MLL rearrangement (N=21), t(15;17) (N=14), Down syndrome (N=6), unfavorable group (N=15) which included -7 or 7q-, complex karyotype and t(6;9), and intermediate group for all other patients (N=65). Bone marrow samples at diagnosis were examined for mutations of FLT3-ITD, FLT3-TKD, N-Ras, K-Ras, c-KIT, c-FMS, CEBPα, and NPM1. Mutational analysis was performed by PCR followed by GeneScan analysis for FLT3-ITD, by PCR-RFLP followed by direct sequencing for FLT3-TKD, and by PCR followed by direct sequencing for other genes. *Results.* c-KIT mutations were more prevalent in t(8;21) than others (p<0.0001), and in inv(16) than others (p<0.0001). The frequencies of c-KIT mutations between t(8;21) and inv(16) were not statistically different (p=1.0). FLT3-ITD was more frequently present in the intermediate group (22/65) than others (p<0.0001). In 28 patients with FLT3-ITD mutations, 4 had allelic ratio of mutant to wild type >2.

Table 1. Gene mutations in childhood AML.

The frequencies of FLT3-TKD between the intermediate group and others were not statistically different (p=1.0). N-Ras mutations were more prevalent in patients with inv(16) than others (3/14 vs 2/61, p=0.042), and in the intermediate group than others (9/65 vs 2/61, p=0.055). The frequency of K-Ras mutations in patients with MLL rearrangement was not statistically different from those of others (3/21 vs 3/75, p=0.117) or the intermediate group (6/65) (p=0.682). The frequency of CEBPα mutations in the intermediate group (8/53) was higher than others (2/94) (p=0.005). Similarly, the frequency of NPM1 mutations in the intermediate group (6/57) was higher than others (0/94) (p=0.002). In patients with recurrent chromosomal translocations, two

cooperating mutations were only found in 2 patients: FLT3-TKD + K-Ras in one with t(11;19) and c-KIT + N-Ras in another with inv(16) , all others with two cooperating mutations occurred in the intermediate group: FLT3-ITD + NPM1 in 5, FLT3-ITD + CEBPα in 4, FLT3-TKD + K-Ras, FLT3-TKD + CEBPα, and N-Ras + K-Ras in one each. *Conclusions.* In childhood AML, the core-binding factor leukemias are more frequently associated with c-KIT mutations. AML with inv(16) has more N-Ras mutations. Patients with intermediate cytogenetic risk group have more FLT3-ITD, CEBPα, and NPM1 mutations.

0567

DETECTION OF PATIENT-SPECIFIC BCR-ABL GENOMIC DNA IN CML PATIENTS WITH NO DETECTABLE BCR-ABL BY QUANTITATIVE REVERSE TRANSCRIPTASE PCR

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Background. With prolonged imatinib therapy for CML an increasing proportion of patients have persistently undetectable BCR-ABL using real-time reverse transcriptase quantitative PCR (RQ-PCR), referred to as *complete molecular remission* (CMR). However, most patients in CMR have residual disease, and imatinib withdrawal may result in early relapse. More sensitive molecular methods are required to identify patients at higher risk of relapse and to quantify residual disease so that the efficacy of therapy given after attaining CMR can be assessed. *Aims.* To determine if a patient-specific DNA PCR assay can detect residual disease below the level of CMR as measured by RQ-PCR. *Methods.* The patient-specific DNA PCR assay was developed after determining the genomic BCR-ABL breakpoint in each patient. DNA was extracted from cells frozen at presentation. The BCR-ABL breakpoint was identified using long template PCR with a forward primer in BCR and reverse primer in ABL. The sequence flanking the breakpoint was amplified and characterised. A sensitive DNA PCR assay with patient-specific primers and probe was then used for detection of BCR-ABL in follow-up samples. First, the breakpoint region was amplified using TaqGold DNA polymerase, then the breakpoint amplicon was detected in real-time PCR using nested primers and a TaqMan probe spanning the breakpoint. Each PCR was performed in two independent experiments. There were two negative controls in each assay; no template, and DNA from another CML patient. DNA PCR results were compared with prior RQ-PCR *Results.* RNA was reverse transcribed with random hexamer primers and Superscript II. BCR-ABL cDNA was quantified using RQ-PCR with TaqMan probes on the ABI Prism 7000. *Results.* We report on two DNA PCR assays developed for the first patients enrolled in the Australasian Leukaemia and Lymphoma Group study of imatinib withdrawal in patients with CMR for ≥2 years. To assess specificity each DNA assay was tested on 5 other CML patients. No non-specific amplification was detected. The limit of detection for serial dilutions of the breakpoint amplicon from presentation samples was similar for both patients suggesting comparable sensitivity. Both patients had received imatinib treatment for ≥3 years with no detectable BCR-ABL by RQ-PCR for 2 years prior to cessation of imatinib (baseline). Patient #1 had molecular relapse by RQ-PCR 3 months after imatinib cessation. Retrospective analysis by DNA PCR detected BCR-ABL in this patient's samples collected immediately prior to imatinib cessation and 6 months earlier. In contrast, for patient #2 the available DNA samples at baseline and 14 months earlier were negative by DNA PCR. This patient remains in CMR 6+ months after imatinib cessation. *Conclusions.* A major limitation of sensitive RQ-PCR is the potential for BCR-ABL contamination from other patient samples. Patient-specific DNA detection should overcome this problem. This DNA PCR assay for BCR-ABL is more sensitive than RQ-PCR in the two patients analysed to date. In these cases DNA PCR results correlated with remission/ relapse status after cessation of imatinib therapy. We aim to develop a quantitative DNA PCR assay which might enable more precise enumeration of residual CML cells.

0568

SENSITIVE DETECTION OF C-KIT POINT MUTATIONS BY D-HPLC AND QUANTITATIVE PCR IN PERIPHERAL BLOOD AND BONE MARROW SAMPLES FROM PATIENTS WITH SYSTEMIC MASTOCYTOSIS

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The majority of patients (pts) with systemic mastocytosis (SM) is characterized by the presence of the transforming mutation D816V of the c-kit gene, resulting in a factor-independent activation of the receptor tyrosine kinase KIT. The mutation is regarded the causative event for the pathogenesis and a potential target for therapeutic intervention with novel tyrosine kinase inhibitors like dasatinib, nilotinib (AMN107) and midostaurin (PKC412). However, the sensitivity of screening procedures for mutations are compromised by a minor proportion of malignant cells in the bone marrow (BM) sample. We therefore established and compared sensitive strategies for the detection of c-kit mutations in BM and peripheral blood (PB) samples on mRNA level: (i) D-HPLC (denaturing-high performance liquid chromatography) combined with direct sequencing in case of a positive D-HPLC signal and (ii) a LightCyclerTM based quantitative PCR assay for the D816V mutation amplifying the c-kit wildtype (wt) and the mutated allelen. Levels of the D816V mutation are expressed as ratios D816V C-KIT/wt C-KIT. The different techniques have been established using serial dilutions of c-kit D816V positive HMC-1 cells in a background of NB4 cells harboring wildtype c-kit and pts mRNA in control mRNA. D-HPLC and the quantitative PCR were optimized for the detection of the D816V mutation down to 0.1-0.5% fraction of the mutated clone (cell line and RNA dilution). No false positive results were observed in 20 different control samples obtained from healthy donors PB samples. In comparison, the detection limit for D816V mutations by conventional sequencing was 10 to 15%. These techniques were applied to BM (n=122) and PB (n=65) samples from 102 pts (55 m, 47 f) meeting the WHO criteria for SM. Median age was 51 yrs (range, 23-81). At diagnosis, D-HPLC was positive in 89% of the BM samples (110/123) and the quantitative PCR in 89% of the BM samples (59/66) whereas conventional sequencing revealed the D816V mutation in 73% of the BM samples (90/123). Two patients showed the D816H and one patient the D816L mutation. The analysis of PB samples revealed D-HPLC positivity in 48% of the samples (31/65) with a consecutive detection of the D816V mutation by direct sequencing alone in 40% of pts (26/65). D816V mutation was detected by quantitative PCR in 58% of pts (14/24). The mutant proportion of the D816V mutation analysed in the positive samples by quantitative PCR in BM samples was in median 22% (range, 0.5-67%) and in PB samples 22% (range, 2.8-91%). In conclusion, i. D-HPLC is a reliable and sensitive method for the screening of c-kit mutations in SM which is superior to conventional direct sequencing. ii. The quantitative PCR assay for the most common mutation D816V offers easy handling but overlooks rare mutations and is suitable for the surveillance of pts with SM during therapy with novel tyrosine kinase inhibitors. iii. Employing these sensitive techniques, the D816V mutation could be detected in BM as well as in PB samples, but the reliability for a positive result of the mutation was higher in BM.

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A NOVEL DENATURING-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (D-HPLC)-BASED METHOD FOR KIT MUTATION SCREENING OF PATIENTS WITH SYSTEMIC MASTOCYTOSIS

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Background. Systemic mastocytosis (SM) is a clonal disorder characterized by anormal growth and accumulation of mast cells (MC) in various tissues. Kit is a tyrosine kinase transmembrane receptor on the surface of MC. The presence of an enzimatic site (ES) mutation (e.g., D816V) renders Kit resistant to inhibition by imatinib, whereas Kit with juxtamembrane (JM) mutations (e.g., V560G) remains sensitive to imatinib. Sensitive methods are required to assure appropriate therapeutic management of SM patients as the proportion of malignant cells in the available tissues which carry the mutation is small. *Aims*. Our aims were:

to set up and optimize 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. *Methods.* Kit mutation analysis was performed on 77 bone marrow and/or peripheral blood samples obtained from 51 patients. For each sample a PCR product of 287 bp, spanning codons 763-858 corresponding to the catalytic loop and to the activation loop, was 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 enzime HinfI to detect a GAC-to-GTC nucleotide change at codon 816, leading to a Asp-to-Val amino acid substitution (D816V). For each sample scored as wild-type by D-HPLC and by RFLP analysis, a PCR product of 350 bp, spanning codons 510-626 corresponding to the transmembrane domain and to the juxtamembrane domain, was screened by D-HPLC combined with direct sequencing. *Results.* By RFLP analysis 34/51 pts were positive for the D816V. By D-HPLC analysis, an abnormal eluition 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 in all the 34 RFLP-positive cases and showed that in two of these cases a I798I polymorphism was also present. The two pts scored positive by D-HPLC but negative by RFLP were found to have the I798I polymorphism. The 15 pts who did not harbour ES type mutations were further investigated by D-HPLC analysis of a RT-PCR product spanning the transmembrane and juxtamembrane domains. D-HPLC showed an abnormal elution profile in 5 pts. By direct sequencing one patient showed the K546K mutation and 4 pts showed the M541L mutation in the TM domain. *Conclusions.* 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 TM domain, is still unclear.

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0570

PREPARING FOR JAK2 V617F TARGETED THERAPY: DEVELOPMENT OF A HIGHLY SENSITIVE AND SIMPLE REAL-TIME RT-PCR METHOD FOR JAK2 V617F TRANSCRIPT QUANTIFICATION

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Background. The identification of the JAK2 V617F mutation, occurring in almost all cases of polycythemia vera (PV) as well as in approximately 60% of cases of essential thrombocythemia (ET), has strongly simplified the diagnosis and classification of these diseases. Recently, it has been suggested that the JAK2 V617F burden may have prognostic significance. In addition, new promising JAK2 V617F targeted drugs are under development. *Aims.* We developed a novel real-time RT-PCR method for quantification of JAK2 V617F transcripts that would allow determination of JAK2 V617F burden at diagnosis as well as the evaluation of the response to newly developed therapeutic agents. *Methods.* RT-PCR reactions were performed on a Rotor-Gene 3000 (Westburg) in single tubes with 1 set of primers and two differently labelled, allele-specific TaqMan probes, directed to respectively wild-type (WT) and mutant (Mut) JAK2 sequences (1 mismatch). Probes were adapted by Locked Nucleic Acid (LNA) modification for increased hybridization specificity and enhanced allelic discrimination. Standard curves were constructed with JAK2 V617F WT and Mut plasmids. Results are expressed as percentage of JAK2 V617F of total JAK2. Whole peripheral blood or bone marrow samples of a total of 54 JAK2 V617F positive cases, including 23 untreated and 7 conventionally treated PV cases and 19 untreated and 5 conventionally treated ET cases, were analysed. In addition, also 30 peripheral blood samples of normal individuals were analysed. *Results.* Reaction efficiencies of this single tube assay for JAK2 Mut and JAK2 WT were equal (97%). Quantities down to 10 copies of JAK2 Mut plasmid amongst WT cDNA and patient JAK2 V617F cDNA diluted down to 0,09% into WT cDNA could be reliably detected. Low intra- and inter-assay variabilities ensure good reproducibility of the assay. None of the negative control samples showed any increase of the fluorescent signal derived from the Mut probe, demonstrating the high specificity of the assay and no requirement for defining a cut-off value. For PV patient samples, the assay showed mean JAK2 V617F quantities of 82% for untreated cases versus 56% for treated cases. Untreated ET cases showed a significantly lower mean JAK2 V617F% compared to untreated PV cases (55% versus 82%). *Conclusions.* We have developed a robust and simple method for quantification of JAK2 V617F transcripts that is more sensitive than all previously described methods. It provides the potential to evaluate the prognostic significance of the JAK2 V617F burden at diagnosis as well as the response to JAK2 V617F targeted therapy that will become available in the near future.

0571

THE VALUE OF CLOSE MONITORING OF WT1 EXPRESSION LEVEL DURING THERAPY AND POST-THERAPY FOLLOW-UP OF ACUTE MYELOID LEUKEMIA: AN INDEPENDENT PROGNOSTIC FACTOR AND A VALUABLE PREDICTOR OF RELAPSE

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Background. The Wilms' tumour gene 1 (WT1) is expressed during normal fetal development, but only to a low extent in adult tissues. However, since it is highly expressed in virtually all AML patients, it has been suggested as a tool for minimal residual disease (MRD) detection and for individualized therapy decisions.[1-2] *Aims.* a) To determine whether high residual WT1 expression in first complete remission (CR1), established by morphology and immunophenotyping, is an adverse prognostic factor, and b) to evaluate the value of WT1 expression levels in peripheral blood (PB) and bone marrow (BM) for prediction of disease relapse. *Methods.* WT1 levels were quantified using real-time quantitative RT-PCR by normalization to the control genes β2M and ABL, and in follow-up samples expressed as a fraction of the BM diagnostic level. (described in detail in (1)) Normal BM and PB WT1 levels were defined as described in.[1] 185 patients (160 adults, 25 children) treated at the Departments of Haematology and Paediatrics, Aarhus University Hospital, were analyzed at diagnosis. A cohort of 89 patients (73 adults, 16 children) were selected for follow-up based on high WT1 expression and lack of fusions transcripts. These patients were sampled at every visit during therapy and follow-up (median number of WT1 determinations per patient: 11, range 2-38). Prognostic difference between groups was determined using the Cox Proportional Hazards statistical model including age, sex, cytogenetics, *de novo* secondary leukemia and FLT3-ITD. When comparing the predictive value of rising WT1 expression levels in PB and BM Wilcoxon's ranksum test was employed. *Results.* When we analyzed the WT1 expression of patients achieving CR1 we found that the disease free survival (DFS) in the group with BM WT1 expression above normal levels at CR1 was significantly shorter than in the BM WT1-normal group (Hazard ratio (HR) = 6,88 (95% Confdence interval (CI) 2,07-22,9), p=0,002). Similarly, the DFS was significantly shorter in the PB WT1 high group vs. the PB WT1 normal group (HR=8,60, CI 1,76-41,9, p=0,008). Of even greater importance, we were able to address relapse kinetics in 29/32 relapses observed in the 89 patient cohort. We were able to detect WT1 above normal levels in 100% of the BM samples that available 3 months before relapse. (Range 1-8, median 4 months). 33% of PB samples were positive 3 months before relapse (Range 0-8, median 1 month). (p=0,0086) *Conclusions.* CR1 WT1 expression levels in both BM and PB are independent prognostic factors in AML. Relapse was seen significantly earlier in BM than PB, but WT1 levels above normal can still be seen in PB in 33% of patients 3 months prior to relapse.

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0572

A MOLECULAR CYTOGENETICS STUDY OF THE ROLE OF CHROMOSOME 9P IN CML CELL LINES AND CMPD PATIENT SAMPLES

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Chronic Myeloproiferative Disorders (CMPDs) are a spectrum of haematological malignancies of which the molecular pathogenesis remains unknown. Chronic Myeloid Leukaemia (CML) being the only disorder with a recognisable pathogenomic abnormality, the Ph chromosome. Recent published data has supported the role of tyrosine kinases

in the disease pathogenesis, in particular the role of a cytoplasmic tyrosine kinase JAK2. It has been shown that >80% of Polycythaemia Vera (PV) patients and 30% of Essential Thrombocythaemia (ET) patients demonstrate a clonal and recurrent mutation V617F in the JH2 pseudokinase domain of JAK2 located on 9p24.1. Array CGH investigations of cell lines, established from lymphoid and myeloid blast phase samples revealed a complex pattern of imbalances affecting the short arm of chromosome 9: on the background of 2-3-fold amplification, a loss of the 9p24.1 region, which harbours the JAK 2 along with a number of other kinases, was repeatedly observed. The aim of this investigation was to clarify the aCGH observations using BAC DNA probes from the 9p24.2 and 9p23 region. FISH mapping was carried out to map the JAK2 gene and the flanking sequences. Results so far indicate the presence of both imbalances (amplifications & deletions) and rearrangements involving the JAK2 region in 5 cell lines. This work is now being extended to include BAC clones telomeric and centromeric to JAK2. An on going FISH study of CMPD patients (CML, PV, ET and MF) with normal and abnormal karyotypes has revealed 9p24 imbalances in 18% (5 out of 28). The pathegenomic significance of aberrations found in the region surrounding JAK2 has yet to be defined and our study demonstrates the importance of molecular cytogenetics in exploring the importance of 9p in the CMPD disease model.

0573

ANALYSIS OF 482 PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) FOR THE IDENTIFICATION OF MUTATIONS IN THE PROMOTER REGION AND THE CODING SEQUENCE OF THE CEBPA GENE BY CAPILLARY ELECTROPHORESIS

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Background. The CEBPA gene encodes a transcription factor, CCAAT/enhancer binding protein (C/EBP)α. The wildtype (WT) protein is essential for the lineage specific differentiation of myelocytic haematopoietic precursors into mature neutrophils. Approximately 9% of all AML patients harbour one or more mutations in this gene, while the frequency in the cytogenetically negative group of patients is reported to comprise up to 15%. Until recently, the mutational status of the gene has been determined using cloning and sequencing. *Aims.* We designed a fragment analysis using capillary electrophoresis that enabled us to discriminate as little as a single base pair insertion or deletion accounting for about 90% of all CEBPA mutations. *Methods.* Diagnostic bone marrow (BM), or when not available peripheral blood (PB), from 444 adults and 38 children diagnosed with AML at a single centre from 1980 to date and from whom bio banked material was available were assessed for mutations employing fragment analysis. Mononuclear cells were isolated, cryopreserved, and stored until use at -80°. Genomic DNA was extracted from approximately 1 million mononuclear cells and eluted in a volume of 100 µL using MagNa-Pure LC Robot (Roche Diagnostics, Basel CH). Four overlapping and tailed primer pairs were designed to cover the entire coding sequence and the region upstream the intronless gene using Oligo (Primer Analysis Software, version 6.83, Molecular Biology Insights, CO, USA) and purchased from Applied Biosystems (Foster City, CA, USA). PCR was performed for each primer pair and subsequently the PCR products for each patient were pooled and transferred to capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems). The T-Cell line 8402 was included as a wildtype control. The results were analysed using GeneMapper software version 3.7 (Applied Biosystems). Samples harbouring insertions or deletions were identified and sequenced directly on both forward and reverse strand. *Results.* We analyzed 482 patients hereof 38 children. In total, we identified 45 abnormal PCR fragments in the CEBPA gene in 37/444 (8,3%) and 1/38 (2,6%) adult and childhood AML patients respectively. Using the method described above we were able to distinguish between N- and C- terminal mutations and reached the same mutational frequency as described in the literature. *Conclusions.* We conclude that fragment analysis by capillary electrophoresis can be used as an easy and high throughput diagnostic procedure for the mutational status in the CEBPA gene.

0574

GENE POLYMORPHISMS OF METHYLENTETRAHYDROFOLATREDUCTASE IN MALIGNANT DISEASE

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Background. Methylentetrahydrofolatreductase (MTHFR) plays a role in the human folate metabolic pathway. The MTHFR 677 C>T polymorphism is associated with a reduced enzyme activity, which leads to a reduced probability of DNA double-strand breakage. A number of studies focused on the polymorphism's influence as a risk factor for different malignancies, however, data are conflicting. *Aims.* It was the aim of our study to analyse the prevalence of mutations in patients with malignant disease and healthy controls. *Methods.* Between October 2003 and July 2006, 427 patients with solid malignant tumours (225 female/202 male; mean age=62y) of the breast, lung, stomach, colon and prostate and 80 patients with haematological malignancy (32 female/48 male; mean age=56y) including Hodgkin's lymphoma, non-Hodgkin's lymphoma and multiple myeloma were enrolled. Seventy-four healthy controls were matched for age and sex (36 female/38 male, mean age=61y). MTHFR genotype was analysed with MS-PCR. *Results.* Genotype distribution was in Hardy-Weinberg equilibrium and 41% CC, 45% CT and 13% TT in patients with solid tumours, 38% CC, 54% CT and 9% TT in those with haematological malignancies and 31% CC, 51% CT and 18% TT in healthy controls (p-values for pair-wise comparisons not significant). TT genotype was not associated with a significantly reduced risk for malignancy in univariate analyses (solid tumours: OR=0.6 [95% CI=0.3-1.2], p=0.14, haematological malignancies: OR=0.4 [0.1-1.2], p=0.10, respectively). In a subgroup analysis for colorectal carcinoma (n=97, 46% CC, 44% CT and 9% TT) MTHFR 677 TT was associated with a significantly lower risk in univariate (OR= 0.35 [0.13-0.95], p=0.039) and bivariate analyses corrected for age (OR=0.37 [0.137-0.997], p=0.049). Summary/*Conclusions.* MTHFR 677 C>T polymorphism seems to modulate the risk for selected malignancies, but it does not seem to influence the risk for developing cancer in general.

0575

A HIGHLY SENSITIVE METHOD FOR DETECTION OF HAEMOPOIETIC CHIMERISM IN ALLOGENEIC HAEMOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Background. The ability to detect donor cell engraftment in reduced intensity conditioning allogeneic hematopoietic stem cell transplant recipients with high sensitivity would enable early detection of patients at high risk of relapse. *Methods.* Sixteen specific biallelic polymorphic markers were selected to identify informative markers for analysis of chimerism in transplant donor/recipient pairs. Four informative markers were then used in SYBR green Q-PCR to quantify post-transplantation chimerism in serial samples from these patients. The percentage of donor cells was calculated using a standard curve, constructed using artificially mixed donor/recipient chimeric DNA in 8 serial dilutions (0.01-100%). A linear correlation with r higher than 0.98 and a sensitivity of 0.1% proved reproducible. DNA from 9 donor/recipient pairs was retrospectively screened for informative markers and 38 post-transplant samples were monitored for chimerism using the SYBR green Q-PCR method. *Results.* We were able to find at least 4 different informative markers for 7 donor/recipient pairs, 2 markers for 1 donor/recipient pair and 1 marker for 1 donor/recipient pair. These results were compared to the existing method using PowerePlex 16 microsatellite STR analysis kit (Promega). The detection limit of the Q-PCR method was 0.1%, which is significantly higher than that achieved with STR methodology (5%). This high sensitivity enabled us to predict relapses up to 4 months earlier than the STR method. *Conclusions.* this assay is highly sensitive and provides an accurate quantitative assessment of mixed chimerism that can be useful in evaluating patients' response to transplantation and distinguishing those at high risk of relapse at an early stage to enable implementation of additional treatments. Detailed analysis of data from large cohort of transplant patients will be presented.

0576

WESTERN BLOT IDENTIFICATION OF NPM1 LEUKEMIC MUTANTS IN CYTOLOGICAL AML SAMPLES: A POWERFUL DIAGNOSTIC TECHNIQUE

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Nucleophosmin (NPM1) mutations occur in 50-60% of adult acute myeloid leukemia (AML) patients with normal karyotype and are predictors of favourable prognosis. For these reasons, analysis of NPM1 mutations has recently become a major new step in the diagnosis and prognostic stratification of AML patients. About 40 different molecular NPM1 mutation variants have been so far identified, with about 90% represented by mutation A (about 80%) and B (about 10%), other NPM1 mutations being extremely rare. Mutations of the NPM1 gene can be reliably identified by molecular biology techniques or by immunohistochemistry through detection of aberrant cytoplasmic NPM positivity (NPMc+). Mutational analysis is carried out only in specialized laboratories; immunohistochemistry overcome this problem but it is only applicable to paraffin sections from bone marrow biopsies. Hereby, we describe a highly sensitive and specific Western blot 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 NPM mutant protein (type A). Western blot analysis of a selected number of AML samples proved that these antibodies reacted specifically with the NPM mutant but not with the wild-type NPM protein. These findings prompted us to use this method to analyze systematically cytological leukemic samples and to compare blindly the results obtained by Western Blot with those derived from immunohistochemical studies and, when available, from NPM1 mutational analysis. A total of 114 AML patients classified by immunohistochemistry into $NPMc^+$ (cytoplasmic-positive; n=57) and NPMc- (cytoplasmic-negative; n=57) were enclosed in the study. Western Blot analysis was performed retrospectively in 83 AML cases and prospectively in 32 cases. We investigated a total of 174 cytological preparations (82 from $NPMc^+$ and 92 from NPMc- AMLs) of various types, including frozen dry cell pellets of Ficoll-isolated leukemic cells; 1 to 2 drops of fresh whole bone marrow or peripheral blood; or even cells obtained scraping the surface of leukemic cytospins or smears. Western Blot analysis was performed according to standard procedures. Seventy-two out of 82 (88%) $NPMc^+$ AML samples resulted positive at Western Blot analysis. Results obtained in cytological material of different types were comparable, indicating the high flexibility of the method. However, retrospective Western Blot analysis of badly preserved leukemic smears sometimes gave negative *Results.* In addition to NPM mutant A, the specific anti-NPM mutant antibodies recognized NPM mutant proteins of type B, D, E, and L. For patients studied prospectively, Western Blot analysis predicted NPM1 mutation in all (16 out of 16) $NPMc^+$ AML patients investigated. Importantly, no false-positive results were registered. In conclusion, Western Blot analysis represents a new highly sensitive, specific and low cost assay for detecting the most common NPM1 mutations in AML cytological samples processed in different ways. This also represents the first example of employment of Western Blot for identification of a specific genetic lesion in AML, that should turn out to be very useful not only for leukemia diagnosis but also for research purposes.

0577

HLA AND IMMUNOGENIC MARKERS IN PATIENTS WITH LGL-CD3+ PHENOTYPE

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Background. T - Large granular lymphocyte lymphocytosis (LGL) represents a clonal expansion of both $CD3^+$ cells and natural killer (NK) cells. The most common phenotype typical for this condition is T α/β $CD3^+$, $CD8^+$, $CD57^+$. Autoimmune diseases (arthritis, erithroblastopenia) are frequently associated with LGL, although, these conditions frequently have a benign course. Recent studies support an idea that a subtype of LGL, TCR α-β+/$CD4^+$ T-large granular lymphocyte (LGL) lymphocytosis, represents a clonal expansion with a CDR3 sequence homology in TCR and a common HLA DRB1*0701 genotype, and has an antigen-driven mechanism underlying its origin (Garrido et al. Blood. 2007. February. PMID: 17303697). Considering that HLA DRB1*0701 is a frequent allele in the Caucasian population, a possible defect in the immune regulation could be relevant in the development of this clonal expansion. OBJECTIVES: To analyse the possible implications of functional polymorphisms in IFN- γ, CTLA - 4, IL10, IL4, TNF-α, FAS, FASL, and MICA genes in association with autoimmune diseases and persistent viral infections predisposing development of LGL $CD4^+$, $CD8^+$. *Methods.* In 41 cases with LGL - T- $CD3^+$, $CD8^+$ and in 40 cases with LGL- $CD4^{++}$, CD8+ we performed a single nucleotide polymorphism (SNPs) genotyping using a Taqman 5´ allelic discrimination assay. PCR reactions were carried out in a total reaction volume of 5 microlitres with the following amplification protocol: denaturation at 92°C for 15 seconds, followed by an annealing and extension at 58ªC for 1 min. Post-PCR, the genotype of each sample was assigned automatically by measuring the allele -specific fluorescence on the ABI FAST 7500 Sequence Detection Systems using the SDS 1.3.1 software for allelic discrimination (Applied Biosystems, Foster City, CA, USA). The results were compared with 170 DNA samples from a healthy population obtained from a blood bank. *Results.* the frequencies of the studied allelic polymorphisms in the LGL $CD4^+$, $CD8^+$ population were similar to that found in control group. *Conclusions.* The results obtained suggest that the analyzed polymorphisms do not seem to play a major role in the LGLs susceptibility.

GvHD - GvL - graft rejection

0578

LUNG TRANSPLANTATION AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background and Aim. Pulmonary complications cause significant morbidity and mortality after haematopoietic allogeneic stem cell transplant (HSCT). We describe our experience with bilateral lung transplantation as a therapeutic option in HSCT patients with end-stage lung disease. *Methods.* Cases were identified via search of database information from St Vincent's Hospital and personal contact with lung transplant units in Australia. Information from medical records of identified cases was analysed. Pathology specimens of the end stage lung were reviewed. *Results.* Five cases have been identified. All received HLA matched allogeneic transplants from related donors (four sibling, one maternal) for haematological disease (three CML, one AML, one aplastic anaemia). Conditioning was with Cy/Bu in four patients and Cy/TBI in one. Graft versus Host Disease (GVHD) prophylaxis was with CsA/MTX. Acute GVHD (grades II-III) occurred in two patients. Chronic GVHD other than lung occurred in three. Pathology review confirmed the diagnosis of bronchiolitis obliterans (BO) in three patients, interstitial fibrosis in a fourth, and a mixed process in the fifth. Time from HSCT to lung transplant ranged from 23 to 125 months. Four of five patients are alive after lung transplant with no evidence of relapse of haematological malignancy (range +8 to+74 months). One patient died three years after lung transplant from Post-Transplant Lymphoproliferative Disorder. The remaining patients maintain normal pulmonary function. Morbidity in the living patients includes gastro-oesopharangeal reflux disease, osteoporosis, hypertension, mild renal impairment, and opportunistic infections (CMV, aspergillus colonization, MAC reactivation, herpes zoster and simplex). Marrow function is normal and performance status ECOG 0-1 in the surviving patients. *Conclusions.* Lung transplantation is a viable therapeutic option for patients with prior HSCT and end-stage lung disease. Interesting questions requiring further research include those related to causative processes, HLA matching, stem cell dose, prevention and therapy of end stage lung disease. Ongoing immunosuppression does not appear to affect recipient bone marrow function or contribute to relapse.

0579

THE GRAFT CONTENT OF DONOR T-CELLS EXPRESSING γ/δ TCR+ AND CD4+FOXP3+ PREDICTS THE RISK OF ACUTE GRAFT VERSUS HOST DISEASE AFTER TRANSPLANTATION OF ALLOGENEIC PERIPHERAL BLOOD STEM CELLS FROM UNRELATED DONORS

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Purpose. Recently, high numbers of regulatory T-cells within the stem cell graft were described to be associated with less graft versus host disease (GVHD) after related peripheral blood stem cell transplantation (PBSCT). Studies in mice also suggest a distinct role of γ/δ TCR+ T-cells in mediating GVHD. Therefore, the aim of this study was to define the yet unknown role of regulatory and gamma/delta TCR+ T-cells in human PBSCT from unrelated donors. *Experimental design.* The frequency of both T-cell subsets within the graft was analyzed in 63 patients receiving unrelated allogeneic PBSCT. The respective amounts were quantified by flowcytometry and PCR and further correlated with clinical outcome. *Results.* The grafts contained a median of 11.2×10^6/kg $CD4^+foxp3^+$ and 9.8×10^6/kg γ/delta TCR^+ T-cells, respectively. Patients receiving more $CD4^+foxp3^+$ cells had a lower cumulative incidence of acute GVHD II-IV (44% vs. 65%, p=0.03). Interestingly, in patients who received higher concentrations of donor γ/delta TCR^+ T-cells acute GVHD II-IV was more frequent (66% vs. 40%, p=0.02). In multivariate analysis only the graft concentration of γ/δ TCR^+ T-cells (p=0.002) and a positive CMV-status of the recipient (p=0.03) were significantly associated with the occurrence of acute GVHD II-IV. *Conclusions.* Graft composition of T-cell subsets seems to affect the outcome of patients receiving allogeneic PBSCT from unrelated donors. Therefore, selective manipulation or add-back of particular subsets might be a promising strategy to reduce the incidence of GVHD.

0580

TREATMENT OF STEROID REFRACTORY GRAFT VS. HOST DISEASE BY INTRA-ARTERIAL INFUSION

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Introduction. Graft versus host disease (GVHD) still is the major draw-back of allogeneic stem cell transplantation (SCT). In its resistant form, it carries high morbidity and mortality. The mainstay of GVHD therapy is preventive and once it is developed the first line therapy is systematically high dose steroids, and cyclosporine or tacrolimus. It has been shown by us and other groups, that intra-arterial targeted steroid therapy may be useful even in steroid refractory GVHD. *Methods.* A total 35 patients with 37 cases of steroid resistant/dependent gastrointestinal (GI) (n=16), hepatic (n=15) or combined (n=6) GVHD, were included and given intra-arterial treatment, for one or more of the following arteries - the hepatic, gastro-duodenal, superior mesenteric, inferior mesenteric, internal iliacs. We defined GI partial response and complete response as the day that symptoms decrease or resolved, respectively. Hepatic partial response and complete response were defined as the day that bilirubin level began to decrease or decreased below 30% of initial level, respectively. *Results.* The procedure was safe with no major complications. We found that Intra-arterial catheter guided steroid therapy was associated with partial and complete remission among patients with steroid resistant or dependent GI or Hepatic GVHD. Hepatic partial response was observed in 14 (66.6%) patients among whom 7 (33.3%) reached complete response. GI partial response was observed in 19 (86.4%) patients among whom 12 (54.4%) reached complete response. An early administration of the local therapy, female gender, myeloid basic disease, and a non-active status of the basic disease at the day of transplantation were found related for predicting a better response for the intra-arterial treatment. *Conclusions.* Intra-arterial catheter guided steroid therapy is safe and effective in steroid resistant or dependent GVHD. A further research is warranted characterizing the patients benefit most.

0581

TREATMENT OF CHRONIC EXTENSIVE GRAFT VERSUS HOST DISEASE WITH ALEFACEPT

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Introduction. Alefacept (AMEVIVE®) is an immunosuppressive dimeric fusion protein that consists of the extracellular CD2-binding portion of the human leukocyte function antigen-3 (LFA-3) linked to the Fc (hinge, CH2 and CH3 domains) portion of human IgG1. We have recently shown its effect in acute, steroid resistant/dependent GVHD. In this study, we describe the effect of alefacept treatment on chronic extensive graft versus host disease (cGVHD). *Patients and Methods.* A total of 12 patients (13 cGVHD episodes) were included in this study, 7 males and 5 females with median age 27 years (range 3-60 years).

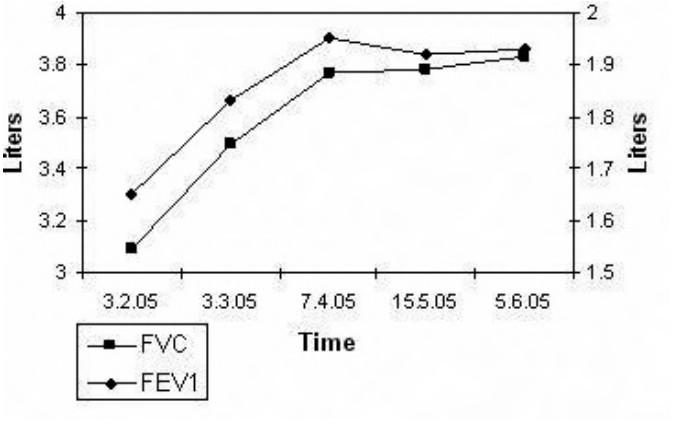

Figure 1. Pulmonary function test.

Basic disease was AML (n=5), ALL (n=3), NHL (n=1), hemophagocytosis (n=1) and malignant melanoma (n=1). Seven patients were transplanted from HLA-A, B, C and high resolution DR fully matched family members (siblings=6, father=1); 2 patients received stem cell graft from fully matched unrelated donor (MUD) non-reactive in mixed lymphocyte culture; 2 from mismatched family members and 1 from a matched unrelated cord blood unit. All patients had an extensive cGVHD.Alefacept dose for children was 15 mg given intramuscularly (IM) once weekly. The dose for adults was 30 mg IM once weekly. *Results.* a median of 9 (range 1-25 injections were given to the patients. Eight out of 12 patients (9/13 episodes) showed response. The median time to initial response was 2.25 weeks (range 1-8). The response was either marked (n=3), moderate (n=2) or minimal (n=4). One patient with pulmonary GVHD had a consistent improvement (Figure 1). In 2 responding patients, the response was only temporary. Treatment complications included infection (n=3), pericarditis (n=1) and squamous cell carcinoma of the lip (n=1). All these events may be related to other drugs given simultaneously. Currently, 7/12 patients are alive all with stable or improved cGVHD. Five patients died due to GVHD progression. Conclusions. Alefacept is effective for the treatment of cGVHD, dose and treatment's time intervals should be explored.

0582

EPIGENETIC REGULATION OF ADHESION IN HEMATOPOIETIC AND LEUKEMIC STEM CELLS

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The $\alpha 4 \beta 1$ integrin very late activation antigen-4 (VLA-4) plays a key role in the adhesion of both hematopoietic progenitor cells and leukemic blast cells to bone marrow stromal cells expressing the vascular cell adhesion molecule-1 (VCAM-1). VLA-4 is an a4 (CD49d)/b1 (CD29) heterodimer and its expression on leukemic cells has been associated with bone-marrow minimal residual disease (MRD). Conversely, the absence of VLA-4 reduces bone marrow retention of both hematopoietic progenitor and leukemic blast cells. In this study, we have demonstrated a downregulation of VLA-4/CD49d on various AML cells lines, on primary cells from AML patients and on hematopoietic stem cells and peripheral blood mononuclear cells from healthy donors upon treatment with the histone deacetylase inhibitors suberoylanilide hydroxamic acid (SAHA) and valproic acid (VPA). This was also functionally associated with decreased adhesion to mesenchymal stromal cells. These findings suggest that HDAC-inhibitor treatment might impair homing of both normal and leukemic progenitors to the bone marrow. On the other hand it might also facilitate the mobilization of hematopoietic progenitors.

0583

CLINICAL BENEFIT OF TREATMENT WITH SYNTHETIC PGI-2 IN PATIENTS SUFFERING FROM RESISTANT SCLERODERMIC CHRONIC GVHD AFTER ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION

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Background. Scleroderma is a late manifestation of cGVHD, presenting as progressive tightness of the skin and hampering patients' quality of life. In recent times Iloprost, a synthetic PGI-2, has been proving effective in the therapy of idiopathic systemic sclerosis, which has a similar pathogenesis to sclerodermic cGVHD. *Patients and Methods.* From 2001 to 2006 we observed 9 pts. affected by diffuse or limited sclerodermic cGVHD. The patients (8 affected by acute leukemia, 1 by NHL) were transplanted with classical conditioning regimen (FTBI 1200 cGy plus Cytoxan 120 mg/Kg) from their sibling donors. The source of stem cells was bone marrow in 3/9 cases and peripheral blood in 6/9 cases. Chronic GVHD was observed at a median of 20 months from HSCT (range 11-36), presenting as diffuse skin changes (tightness and thickening of the face, neck, hands, thorax and abdomen), cutaneous dyschromia, digital pitting scars and functional joint impairment. In two patients these alterations were linked with itching and pain. In all cases cGVHD severely affected quality of life. It was limited to the skin in 8/9 cases, and involved internal organs in the other one (liver and lungs); in two cases diffuse scleroderma was associated with severe discomfort in tendons or muscles. In seven cases it presented as *de novo* cGVHD. All pts had already been treated with various regimens including CSA, azathioprine, mofetil mycophenilate, steroids, repeated cycles of PUVA with little or no effect on the skin. Iloprost, 50 microgram a day over 8 hours IV continuous infusion for 5 days every month, was started after a median of 8 months from the appearance of cGVHD (range 1-64). *Results.* The drug was well-tolerated with little or no severe side effects: the most frequent side effect was mild hypotension. No other major side effects were observed. In 8/9 cases we saw a significant clinical benefit after a median of 5 cycles (range 3-12). Further improvement was observed over the subsequent courses. Iloprost courses were given in Outpatient Department for at least 8-10 courses every month until a satisfactory clinical response that was obtained after a median of 5 courses. In all cases the patients received a maintenance therapy with 3-4 treatments a year. At present, one patient has died because of relapse of acute leukaemia, 7 out of the 8 remaining pts are alive and well, with satisfactory improvement of quality of life. One patient, poor responder, is still on Iloprost, with stable severe skin Scleroderma after 14 cycles. *Conclusions.* In our study Iloprost seems to be one of the most effective drugs in reverting sclerodermic diffuse cGVHD and in reducing the extension of the skin lesions. Skin tenderness and disappearance of pain were reached after less than 6 months from the beginning of symptoms. The quality of life has improved dramatically in all but one case.

0584

ANALYSIS OF BONE MARROW-RESIDING CD4+CD25+FOXP3+ T REGULATORY CELLS IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction. Peripheral tolerance is largely maintained by immunosuppressive regulatory T cells (Treg), such as $CD4^+CD25^+$ T cells co-expressing transcription factor forkhead box P3 (FOXP3) and it has been suggested that Treg contribute to the prevention of graft-versus-host disease (GVHD) following allogeneic stem cell transplantation (alloSCT). Unfortunately, Treg also represent a main obstacle of an effective anti-tumor T cell response and depletion of $CD4^+CD25^+FOXP3^+$ Treg seems to enhance anti-tumor immunity. It is unclear, however, whether a reduced number or function of Treg might play a role in the induction of graft-versus-myeloma (GVM) effects in multiple myeloma (MM) patients post alloSCT and very little is known about Treg residing in the bone marrow (BM) of these patients. *Aims and Methods.* We performed the first systematic analysis of Treg numbers and function in the BM and in the peripheral blood (PB) of MM patients treated with alloSCT (N=40), newly diagnosed MM patients (N=17), and healthy BM donors (N=20) using flow cytometry and functional assays. Mechanisms which might serve as potential mediators of the immunosuppressive function of BM Treg were investigated using real-time PCR. *Results.* Following alloSCT, donor-derived $CD4^+CD25^+FOXP3^+$ Treg expanded faster than conventional $CD4^+$ T cells, leading to an accumulation of Treg in the BM of transplanted patients. Since patients post alloSCT are devoid of a relevant thymic function, Treg reconstitution was most likely based on peripheral expansion. This idea was supported by the fact that reconstituted BM $CD4^+CD25^+FOXP3^+$ Treg of MM patients post alloSCT consisted preferably of CD45RA-CCR7- memory T cells. BM-residing Treg of newly diagnosed and MM patients post alloSCT showed a strong inhibitory function and transforming growth factor (TGF)-β1 seemed to represent an important mediator of Treg function in the BM of MM patients post alloSCT and might also be involved in the expansion of BM-residing $CD4^+CD25^+FOXP3^+$ Treg. *Conclusions.* Our study demonstrates for the first time that BM-residing Treg expand outside the thymus and accumulate in the BM of MM patients post alloSCT. These Treg, which are donor-derived and lead to an efficient replenishment of Treg in the periphery, might be necessary for the prevention of GVHD. However, BM Treg might also contribute to the failure of an effective GVM effect in these patients.

0585

IS THERE A DIFFERENCE IN OUTCOME AND INCIDENCE OF ACUTE/CHRONIC GVHD IN PEDIATRIC PATIENTS UNDERGOING UNMANIPULATED MUD-PBSCT VS MUD-BMT? SINGLE LARGE CENTER EXPERIENCE

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There is an unresolved question whether unmanipulated matched

unrelated donor (MUD) PBSCT leads to a worse overall outcome and higher incidence of severe GvHD in both adults or children in comparison with well established MUD-BMT. It is well known that PBSC grafts contain ca 1 log more mature T cells, which may result in higher rate of severe often fatal acute/chronic GvHD after MUD-PBSCT. The aim of our study was to compare the results of MUD-PBSCT vs MUD-BMT in a large pediatric patient population. We analysed the records of 157 pediatric pts (ALL n=57, AML n=25, CML n=28, MDS/JMML n=14, NHL/HD n=6 and nonmalignant disorders n=27), who underwent MUD-BMT (39 pts) or MUD-PBSCT (118 pts) between 1999 and 2006 in our institution. Probability of overall survival for all pts after MUD-PBSCT was 0.53 and after MUD-BMT was 0.45, whereas pDFS after MUD-PBSCT was 0.53 and after MUD-BMT - almost significantly worse 0.39 (p=0.07). Median follow up of surviving 90 pts (57%) was 2 years and of dead 67 pts was 4 months. Probability of DFS for pts after MUD-BMT was for ALL 0.4, for AML 0.17 and for CML 0.67, respectively, whereas it was much higher for pts after MUD-PBSCT for AML 0.69 (p=0.01 log-rank) and not lower for pts with ALL 0.38 and CML 0.56, respectively. Furthermore, there was no difference between the incidence of both acute GvHD grade III-IV between the recipients of MUD-PBSCT (19.5%) or MUD-BMT (20.5%) and extensive chronic GvHD (19.6%for MUD-PBSCT recipients and 15.4% for MUD-BMT recipients), respectively. In conclusion the results of MUD-PBSCT in a large prospective pediatric cohort of pts are similar if not better that the results of MUD-BMT. There seems to be no increased risk of severe GvHD after MUD-PBSCT. MUD-PBSCT seems to offer a better disease control in patients with malignancies, especially with AML due to more pronounced T-cell mediated GvL effect.

0586

STUDIES IN C3 DEFICIENT MICE REVEAL BENEFICIAL ROLE OF COMPLEMENT IN AMELIORATING CONSEQUENCES OF GRAFT-VERSUS-HOST DISEASE AFTER HEMATOPOIETIC ALLOTRANSPLANTATION

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Graft-versus-host disease (GvHD) is the main complication after allogeneic hematopoietic stem cell transplantation. Immunosuppressive regimen used in its prevention and treatment is only partially effective making research focusing on new possibilities for control of GvHD necessary. While activation of complement during GvHD has been observed there are no published data concerning its significance for GvHD pathophysiology. The aim of the study was to evaluate the role of complement in pathogenesis of GvHD by employment of C3 knock-out animals. $C3^{+/+}$ (n=90) and $C3^{-/-}$ (n=74) mice from the C57/BL6 background were transplanted with 5×10^6 BM cells and 4×10^6 or 10×10^6 splenocytes isolated from allogeneic H2 and sex mismatched C3H mice or from C57/BL6 syngeneic animals(21 $C3^{+/+}$, 22 $C3^{-/-}$) one day after ablative TBI. Mice were weighted and physically examined by looking for changes in skin, posture, physical activity and chimerism in transplanted animals was confirmed by detection of sry gene with real-time PCR. Peripheral blood morphology assessment and fluorocytometric analysis of peripheral blood lymphocytes were performed for some animals on days +17, +31 and +45. Histopathological examination was performed in randomly chosen animals on day +8 and +16 and furthermore on most of mice which died during the experiment. Evident physical (weight loss, skin desquamation, hunching) and histopathological (involvement of liver, skin, spleen and gut) symptoms of GvHD were observed in all mice transplanted with allogeneic cells but not in mice transplanted with syngeneic cells. Analysis of peripheral blood of allotransplanted animals revealed severe lymphopenia affecting B and T cells, strongly increased expression of CD69 activation antigen on $CD4^+$ and $CD8^+$ cells and lower values of RBC, Hb, Hct and MCV as compared to syngeneic controls. The most important difference between $C3^{-/-}$ and $C3^{+/+}$ allogeneic graft recipient populations was significantly worse survival of C3 deficient animals (median: 50 vs. 63, mean: 112 vs. 158 days, hazard rate=1.62, p=0.028; Figure 1). Characteristic pattern of weight loss was observed consisting of a fall to ca 86% of initial body weight on day +7 followed by return to the initial weight on days +14 to +17 and another decrease in weight leading either to the death of the animal or to a long time plateau. The average weight of $C3^{-/-}$ animals was significantly lower on day +10 as compared to $C3^{+/+}$ mice (90,1% vs. 93,7%, p=0.002). Interestingly, the body weight on day +10 was strongly correlated with overall survival (p=0.001). Blood examinations revealed higher WBC count in C3-/- knock-out mice on day +45 (19 vs. 11×10^3/uL, p=0.024). Skin changes tended to be less severe in wild type animals but observed differences were not statistically significant. This study showed for the first time the beneficial role of the complement cascade in ameliorating consequences of GvHD in mice. The positive influence of the cascade on the overall survival is particularly interesting because of its potential clinical implications. In the light of our data, assessment of the role of complement in the course of GvHD in transplanted patients could be interesting in the future.

Figure 1. Kaplan-Meier survival curves for populations of $C3^{-/-}$ (ko) and $C3^{+/+}$ (wt) mice transplanted with allogeneic (allo) and syngeneic (syn) cells.

0587

A COMPARISON OF THE CYTOTOXIC EFFECTS OF 4 COMMERCIALLY AVAILABLE PREPARATIONS OF ANTI-T CELL GLOBULINS IN VARIOUS HEMATOLOGICAL MALIGNANCIES

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Background. Polyclonal anti-T-cell globulins (antithymocyte globulins), ATGs are widely used in allogeneic stem cell transplantation mainly due to their anti-T cell activity. ATGs however contain a wide range of antibodies targeting antigens expressed on various hematopoetic cells. Lymphoglobulin® and Atgam® are produced by immunizing horses with human thymocytes. Thymoglobulin® is produced by immunizing rabbits with human thymocytes, while ATG-Fresenius® is produced by immunizing rabbits with the jurkat cell line. We have previously reported potent ant-myeloma activity of ATG-Fresenius®. Similar data were later reported for Thymoglobulin®. Various ATG preparations have been shown to induce apoptosis in malignant B cell lines. Future conditioning regimens may therefore take advantage of the direct effects of ATGs against hematological malignancies. *Aims and Methods.* We sought to compare the cytotoxic activity of 4 commercially available ATG preparations in myeloma cell lines and myeloma patient samples, B-NHL cell lines and CLL patient samples, myeloid leukemia cell lines as well as primary T cell samples. Viability was assessed by staining with 7AAD and subsequent flow cytometry. *Results.* In all, Atgam® showed significantly lower anti-T cell activity compared to the other 3 ATG preparations. Atgam® also showed significantly lower anti-leukemia activity compared to the other 3 ATG preparations. The anti-CLL effect of Thymoglobulin® was twice as strong as that of ATG-Fresenius®. Thymoglobulin®, ATG-Fresenius® and Lymphoglobulin® had similar anti-myeloma activity. Thymoglobulin®, ATG-Fresenius® and Lymphoglobulin® showed potent complement-mediated cytotoxicity against myeloid leukemia cell lines but no significant complement independent cytotoxicity could be observed against myeloid leukemia cell lines. *Conclusions.* Our data show that Thymoglobulin®, ATG-Fresenius® and Lymphoglobulin® have potent cytotoxic effects against myeloma, CLL and other B cell lymphomas, but limited activity against myeloid leukemia. These findings would be helpful in choosing the ATG preparation and dose when targeting hematological malignancies.

0588

EXTRACORPOREAL PHOTOPHORESIS FOR TREATMENT OF ACUTE GVHD IN PAEDIATRIC PATIENTS

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Background. Extracorporeal photophoresis (ECP) represent a possible and efficacious treatment in management of acute or chronic Graft versus Host Disease (aGvHD/cGvHD) resistant to first line treatment consisted in steroid therapy given to low (2 mg/Kg/day) or high dose (5mg/Kg/day). *Methods.* In our department ECP was used to treat drug resistant acute and chronic GvHD, to reduce the early and late steroid toxicity and to increase the immunological reconstitution until to decrease the incidence of opportunistic infections. ECP (1,5 TBV utilizing COBE Spectra Auto PBSC6.1) was administered according to a schedule consisted in 2 cycles/week for the first month, 2 cycles/every 15 days for the second month and 2 cycles/month for the following 4 months. *Results.* During the period between 2000-04, we enrolled 14 children (median age 9.3 yrs) affected by aGvHD after an allogeneic haematopoietic stem cell transplantation. ECP was administered to cure aGvHD (global grade 1 = 8 pts, grade 2 = 3 pts; grade 4=1 pt) treated with steroid therapy (2 mg/Kg/day in 8pts, 5 mg/Kg/day in 6pts) or to maintained a CR obtained with steroid (2 pts). Steroids were associated to other immunosuppressive therapies in 3 pts (anti CD25 in 2 pts and anti-TNF in 1). We evaluated the response to ECP at the end of first, second and six months of treatment. After the 1st month of ECP, 7/14 pts improved, 4/14 pts not responded, while 3/14 pts worsened. At the end of 2nd month of ECP, 12/14 pts raised CR of aGvHD (3 pts received other immunosuppressive therapies), 1 pt showed a grade 1, and 1 a grade 2 of aGvHD. The cGvHD evaluated at the end of ECP schedule (after 6 months) was absent in 11/14 pts, it was limited in 1/14 pts and extensive in 2/14 pts. After 6 months, 6 pts received ECP for relapse of cGvHD (4/6) or for a consolidation of CR obtained after a first treatment (1/6) or to reduce the immunosuppressive therapy (remaining 1). Five pts died :2 for cGvHD, 2 for sepsis and 1 for relapse of underlying disease. 6 pts without a relapse of cGvHD are alive and they are in CR, 2 of pts who not relapsed of cGvHD died for haematological relapse. *Conclusions.* We confirm that ECP represent an efficacious treatment of aGvHD also in paediatric patients. No events adverse were observed in our populations. In our experience the major response was obtained after the first 2 months of therapy, when ECP was administered more frequently, in particular the patients who obtained a CR during this first period rarely developed a cGvHD. Patients who relapsed after a complete treatment of ECP and who were treated with a new ECP treatment not obtained a improved of cGvHD (2 of them died for cGvHD). We suggested that probably ECP cycles should be increase in the first months of treatment until to obtained a better and prolonged response.

0589

INFLUENCE OF NON-HLA GENETIC POLYMORPHISMS ON THE INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AND MORTALITY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Hematopoietic stem cell transplantation (HSCT) may be associated with a variety of complications such as graft-versus-host disease (GVHD) and major non infectious transplant-related complications. Although HLA compatibility has a central role in selecting donors and determining transplant outcome, the sequencing of human genome has revealed numerous non HLA single nucleotide polymorphisms (SNPs), whose significance in allogeneic HSCT could be relevant. Due to these polymorphisms, patients may be predisposed to releasing high levels of certain cytokines pre and post trasplant during the *cytokine storm* and therefore may be more predisposed and/or protected from GVHD and mortality. In our study we examined the association of SNPs at position 677 and 1298 in the MTHFR gene, at -308 in the TNF α gene and -252 in the TNF β gene, at -1082 and -592 in the IL10 gene, at -238 in the IL10 receptor gene and at 908, 702, 1007 in the NOD2/CARD15 gene, on incidence of GVHD (grade II-IV) and mortality in a group of 42 patients submitted to allogeneic HSCT in our Institute from 2004 and in their donors respectively. Variant genotypes were determined using PCR/restriction fragment length polymorphisms, except for variant 1007 and 702 determined using allele-specific PCR. Table 1 presents patients' characteristics. Kaplan Meyer incidence curves, with differences compared by the Log-Rank test, were used to assess the association between polymorphisms and clinical covariates (age, disease status, regimen conditioning, gender compatibility, HLA mismatch) and GVHD development and mortality. The level of significance was performed to $p<0.05$. Our results showed that global incidence of GVHD (grade III-IV) was 21.4%. In univariate analysis we found an association statistically significant between development of GVHD and TNF β homozygous variant recipient (p: 0.002) and TNF α homozygous variant donor ($p=0.002$). We did not observe significant correlation of genotypic variants and clinical characteristics with acute GVHD. Regarding mortality we found a higher incidence in patients with TNF α homozygous variant ($p=0.004$); in addition we observed that patients with advanced status disease had a lower survival ($p=0.001$) . These data suggest that the determination of functional genotypes in relevant cytokine pathways may have clinical utility for risk assessment, counseling and treatment planning before transplantation.

Table 1. Patients' characteristics (n=42).

0590

PERSISTENCE OF RECIPIENT LANGERHANS CELLS FOLLOWING ALLOGENEIC HAEMOPOIETIC STEM CELL TRANSPLANTS WITH REDUCED INTENSITY CONDITIONING

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Langerhans cells (LCs) are members of the dendritic cell family found predominantly within the suprabasal area of the epidermis where they form the first immunological barrier against the external environment. They are thought to originate from bone marrow precursors although studies in mice suggest they have the ability to renew *in situ* and are only replaced by precursors from the bone marrow after inflammatory changes. Murine models have also shown that recipient LCs are required for development of acute graft versus host disease (GVHD) following allogeneic haemopoietic stem cell transplantation (allo-HSCT). Engraftment of LCs following human allo-HSCT has previously been assessed using migration from isolated epidermal sheets, which may be inaccurate because of differential migration capacity of donor and recipient cells. We therefore studied LC engraftment using fluorescence immunophenotyping and simultaneous *in situ* hybridisation for X/Y chromosomes in sex mismatched transplants. Skin biopsies were performed at days 28, 56 and 100, 6 months and 1 year post transplant on 9 patients receiving alemtuzumab based RIC allo-HSCT regimens. Ten micron cryosections were taken, LCs labelled with anti-CD1a and X/Y chromosomes detected with the CEP X/Y probe kit. Slides were examined on a Zeiss LSM510 Meta confocal microscope and Z-stack images were collected to cover a volume of 100 microns cubed. Chimerism of purified $CD15^+$ and $CD3^+$ peripheral blood cells was determined in parallel. Results are tabulated below and show that compared to $CD15^+$ myeloid blood cells, donor LC engraftment is markedly delayed (day 28, 56 and 100 paired t-tests give $p<0.0001$, $p=0.0006$ and $p=0.0207$ respectively). Of the 9 patients studied, 3 developed biopsy proven

GVHD and in all cases, recipient LCs were still present. These results show that LC engraftment after RIC-allo-HSCT is markedly delayed with persistence of recipient cells at up to 1 year.

Table 1.

Cell Type	Day 28	Day 56	Day 100	6 Months	1 Year
LC	21.4 (n=9)	72.85 (n=8)	90.5 (n=8)	93.85 (n=7)	96.6 (n=3)
$CD3^+$ PB	98 (n=9)	85 (n=7)	73 (n=7)	82 (n=5)	100 (n=3)
$CD15^+$ PB	100 (n=9)	100 (n=7)	100 (n=7)	100 (n=5)	100 (n=2)

Median % donor chimerism at specific time points Post allo-HSCT

0591

DECREASED CHRONIC GVHD RATE AFTER REDUCED INTENSITY CONDITIONING WITH LOW DOSE ATG IN ALLOGENEIC HEMATOPOIETIC SCT

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Background. Reduced intensity conditioning (RIC) regimens have been established to diminish toxicity in patients not eligible for standard myeloablative allogeneic hematopoietic stem cell transplantation (SCT). RIC is associated with higher rates of morbidity and mortality from graft versus host disease (GvHD). Anti-thymocyte globulin (ATG), in addition to its dominant action as a T cell depleting agent, has been implicated to beneficially influence GvHD by depleting host antigen presenting cells. *Aims.* We studied the effect of low dose rabbit ATG (4×5 mg/kg) on the morbidity and mortality due to GvHD. *Methods.* Forty-eight patients (23AML, 2ALL, 4CML, 7CLL, 3NHL, 9MM), not eligible for myeloablative SCT, either by age (median 52 years, range 21-72) or co-morbidities, underwent RIC-SCT. Seven patients had PD, 26 were in PR, 3 in CR2 and 12 in CR1. Eighteen patients (38%) had matched unrelated, 26 (54%) matched related and 4 (8%) mismatched unrelated donors. As a conditioning regimen they received fludarabine 3-5×30 mg/m^2 (with additional cyclophosphamide 3×300 mg/m^2 for NHL or melphalan 100 mg/m^2 for Myeloma) and TBI 2-4 Gy. GvHD prophylaxis consisted of cyclosporine A (2x1.5mg/kg starting at day +1) and mycophenolate mofetil (2x15 mg/kg starting 6h after SCT). In particular patients with an unrelated donor (n=21) received rabbit ATG (5 mg/kg at days -4 to -1; Fresenius, Germany). *Results.* After a median follow up of 708 days 28 of 48 patients (58%) were alive. The 1 year overall survival as well as the transplant related mortality after 1 year was not different between the study groups. The incidence of acute GvHD grade II-IV was 38% with ATG compared to 33% without ATG (p=ns). The occurrence of overall chronic GvHD was 52% in controls and 14% in the ATG arm (p=0.03). In addition the groups also differed in chronic GvHD grading (p=0.04) with extensive chronic GvHD of 7% versus 19%. GvHD related deaths occurred in one ATG treated patient and two controls. There was no statistical significant difference in developing a positive CMV-PCR (38% vs 31%) between the groups within one year. *Conclusions.* RIC including low dose (4×5 mg/kg) rabbit ATG resulted in a considerably low incidence of chronic GvHD. Prospective randomized studies are warranted to corroborate these *Results.*

0592

CHIMERISM OF DENDRITIC AND REGULATORY T-CELLS AFTER CHEMOTHERAPY BASED CONDITIONING IN GVHD MOUSE MODEL

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Background. Hematopoietic stem-cell transplantation (HSCT) remains an important curative therapy for many malignant and non-malignant diseases. However, transplantation related complications such as veno-occlusive disease and graft-versus-host disease (GVHD) are still the major causes of mortality and morbidity following allogeneic HSCT. Dendritic cells (DCs) as the most potent antigen-presenting cells (APCs) play a central role in the development of acute and chronic graft-versus-host disease. Regulatory T (Treg) cells and host APCs have been implicated in GVHD, but their relative contributions remain unclear. *Aims.* To evaluate the time course and pattern of chimera of DCs and Treg cells after allogeneic HSCT in a mice model of GVHD. *Methods.* Female BALB/c were transplanted with male C57BL/6 mice in the allogeneic settings (group I and II) while female BALB/c were transplanted using female BALB/c in the syngeneic setting (group III and IV). Group I and III were treated with busulfan (Bu) 20 mg/kg/ day for four days while group II and IV received 25 mg/kg/day for four days. Cyclophosphamide (Cy) was given as 100 mg/kg/day for two days in all groups. The conditioning started at day -7 days and both Bu and Cy were administrated IP. GVHD was studied by histological analysis of skin, intestine and liver. The chimerism and engraftment were surveyed by FACS analysis in spleen. The plasma levels of cytokine were measured by Multiplex Antibody Bead kits. *Results.* Symptoms of acute GVHD started at day 5 post transplantation mainly in group II. Full donor chimerism achieved faster (day+7) and was durable in group II compared to group I. Percent of DCs in spleen increased at day 7 in group I and II compared to the syngeneic transplanted mice (group III and IV). The percent of DCs in group II was higher than group I at day+7. The number of DCs normalized from day14 in both groups. Full donor chimerism was persistent in group II compared to group I. T regulatory cell population in both allogeneic transplanted groups was higher at day +7 compared to syngeneic settings. Treg population in group I decreased by the time and followed total chimerism pattern whereas in group II it decreased even less than normal level in spite of full donor chimerism, which probably due to the development of GVHD. *Conclusions.* Short term engraftment of both DCs and T regulatory cells may not predict the level of chimerism or the development of GVHD. Moreover, the allogeneic transplantation settings have more pronounced effect on DCs and T regulatory cells chimerism compared to syngeneic settings, which may play an important role in initiation of GVHD.

0593

EXPANSION OF NATURAL KILLER CELLS WITH LYTIC ACTIVITY AGAINST ACUTE MYELOID LEUKEMIA BLASTS UNDER GOOD MANUFACTURING PRACTICE CONDITIONS. A POSSIBLE NEW STRATEGY FOR ALLOGRAFTED PATIENTS?

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Background. Natural killer (NK) cells are known to play a key role in the context of haploidentical KYR mismatch stem cell transplantion (SCT), that is when a donor-versus-recipient NK cell alloreactivity is present. We recently demonstrated the possibility of expanding and activating NK cells both from normal donors and from leukemic patients; moreover, we verified the capacity of this population of expanded effectors to recognize and kill allogeneic and autologous primary myeloid and lymphoid leukemia blasts. This population of effector cells has been also demonstrated capable of controlling the leukemic expansion in experimental models. These observations open the way to possible future clinical trials for the management of minimal residual disease (MRD), also in the setting of an HLA-compatible or haplotype-mismatch allogeneic SCT, regardless of the KYR epytope matching. *Aims.* In order to translate these pre-clinical data into a potential clinical trial, the 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. *Methods.* We investigated the peripheral blood of 17 adult donors. Ficoll density gradient separated peripheral blood lymphocytes (PBL) underwent NK cell enrichment by negative depletion after adherence to plastic and were co-cultured with allogeneic or autologous irradiated mononuclear feeder cells in addition to different concentrations of IL-2, IL-15 and phytohemagglutinin-M (PHA). Expanded NK cells were then engaged in cytotoxic tests against fresh primary acute myeloid leukemia (AML) blasts, by means of the annexin-V flow cytometry technique. *Results.* Enriched NK cells co-cultured for 14 days at 37 °C with irradiated autologous lymphocytes (lymphocyte : NK cell ratio =2.5:1) plus IL-2 500 U/mL and IL-15 50 ng/mL (n=5) presented an expansion capacity (mean fold increase 37.2±14.9) comparable to the expansion obtained in the presence of an allogeneic feeder and PHA. $CD3^-/CD56^+$ NK cells represented 97.1%±2.0 of this expanded population, the remaining cells being $CD3^+$. Preliminary data also show that by adding to the culture medium IL-2 500 U/mL + IL-15 50 ng/mL during the last 24 hours of the expansion period, NK cells

acquire a cytolytic capacity against leukemia cell lines and against allogeneic primary AML blasts. *Summary and conclusions.* These results confirm that NK cells can be significantly expanded under GMP conditions and indicate that from 100 mL of donor peripheral blood it is possible to obtain 1-5×10^8 NK cells, which means the opportunity of infusing into a recipient of 70 kg 1.5-7×10^8 NK cells/kg of body weight. Taken together, these findings indicate a possible new strategy for the expansion of cytolytic effectors that may be considered for the management of AML patients with evidence of disease persistence or recurrence after an allogeneic SCT. Clinical protocols appear feasible, particularly considering that the infusion of NK cells should induce very limited toxicity and no or very low risk of graft-versus-host disease, thus avoiding the potential complications associated to donor T-lymphocyte infusions.

0594

PLASMA CHANGES OF ENDOTHELIAL INJURY MARKERS IN PATIENTS TREATED WITH DIFFERENT CONDITIONING REGIMENS FOLLOWED BY HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The endothelial injury caused by conditioning regimen is thought to play a central role in the non-infectious complications in patients undergoing haematopoietic stem cell transplantation. The aim of the study was to evaluate the plasma changes of endothelial injury markers in patients treated with different type of preparative regimens and to investigate whether these changes are associated with toxicity of conditioning schedule. *Patients and Methods.* Plasma levels of von Willebrand factor antigen (vWF Ag), trombomodulin (TM) and vascular endothelial growth factor (VEGF) were measured by immunoassay tests in 21 patients (pts), median age 30 (19-67) years, transplanted with allogeneic (18 pts) or autologous (3 pts) haematopoietic stem cells after BuCy2 (busulfan 16 mg/kg, cyclophosphamide 120 mg/kg) - 5 pts, TBI/Cy (total body irradiation 12 Gy, cyclophosphamide 120 mg/kg)- 5 pts, Treo/Cy (treosulfan 14 mg/m^2, cyclophosphamide 120 mg/kg)- 6 pts or fludarabine-based reduced-intensity regimen- 4 pts for AML (10 pts), ALL (5 pts), CML (4 pts) and AA (1 patient). For the statistical analysis patients were divided into the subgroups according to the type of conditioning schedule: 1.BuCy2 2.TBI/Cy 3.myeloablative regimens (BuCy2, TBI/CY) and 4.reduced-toxicity regimens (fludarabine-based regimen, Treo/CY). Endothelial injury markers were measured before conditioning regimen (day -10), and on the day of stem cells infusion, 48 hours after finishing preparative schedule, but before stem cells infusion (day 0). *Results.* After conditioning regimen vWF Ag concentration increased significantly on the day 0 in comparison to the day -10 ($p<0.05$) in the whole study group. wWF Ag concentration did not differ significantly between subgroups on the day 0. In the subgroup treated with TBI based regimen (5 pts) VEGF level increased on the day 0 in comparison to the day -10 ($p<0.05$). Plasma level of TM did not change significantly either in the whole study group or in the subgroups. *Conclusions.* vWF Ag is a sensitive endothelial injury marker, but not specific enough to determine toxicity of different conditioning regimens. Further studies with a larger group of patients are necessary to evaluate the role of VEGF as a marker of chemotherapy induced endothelial injury and a predictor of non- infectious complications.

0595

ACTIVATING KILLER IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) COMPATIBILITY IS ASSOCIATED WITH IMPROVED SURVIVAL AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANATION

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Killer immunoglobulin-like receptors (KIRs) regulate function of NK cells and a subset of T cells. KIR genotype is highly polymorphic and includes 14 genes for activating and inhibitory receptors. Two groups of haplotypes may be distinguished: haplotype A including KIR2DS4 as the only activating KIR and haplotype B with varying number of activating KIRs. The goal of this prospective study was to evaluate prognostic value of donor and recipient KIR genotype in a setting of allogeneic hematopoietic cell transplantation (alloHCT). Presence of particular genes in donors and recipients as well as incompatibility of the whole genotype and in particular loci were analyzed. Mismatches in graft-vs.-host (GVH) direction were considered if gene was present in the recipient and absent in the donor. Incompatibility in HVG direction was recognized in opposite situation. Seventy consecutive patients with hematological malignancies, aged 32.5 (18-58)y, given transplant from HLA-matched related (MRD) (n=28) or matched unrelated donor (MUD) (n=42) were included. The conditioning regimen was myeloablative and based on chemotherapy alone (76%) or TBI (24%). GVHD prophylaxis consisted of cyclosporin, methotrexate, and, in case of MUD-HCT, pretransplant ATG. KIR genotype mismatch was established in 68% of MRD-HCT and 90% of MUD-HCT. At two years the OS rate was 100% for KIR-matched and 70% for KIR-mismatched transplants (p=0.04). The difference resulted mainly from incompatibility regarding activating KIRs (100% vs. 67%, $p=0.01$). In a multivariate analysis including other potential risk factors, increasing number of activating KIR mismatches in HVG direction (analyzed as continues variable) was associated with decreased DFS (RR=3.45; $p=0.008$), increased NRM (RR=1.43, $p=0.02$), and the incidence of grade II-IV acute GVHD (RR=1.35; $p=0.03$). Presence of at least one activating KIR incompatibility in HVG direction was associated with higher peripheral blood $CD8^+/CD4^+$ ratio up to 100 days after alloHCT. Number of mismatched loci correlated positively with this ratio on days +28, +56 and +100. Increasing number of activating KIR disparities in GVH direction resulted in higher risk of relapse (RR=4.43; $p=0.046$) and CMV infection (RR=1.59; $p=0.02$). *Conclusions.* Disparity regarding activating KIR genotype is an independent adverse prognostic factor for patients undergoing HLA-matched alloHCT. Activating KIR mismatch seems to enhance donor-recipient alloreactivity and the effect depends on the mismatch direction. Results of this study may contribute to better donor selection and optimization of the alloHCT procedure.

Health economics

0596

COMPLIANCE WITH HOME TREATMENT USING ENZYME REPLACEMENT THERAPY FOR TYPE 1 GAUCHER DISEASE A UK CENTRES PERSPECTIVE

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Background. Gaucher disease is an inherited metabolic disorder in which accumulation of glucocerebroside in the reticulo-endothelial system results in pathology within the bone marrow, liver, spleen and skeleton. In neuronopathic forms (types 2 and 3) there is CNS involvement. Treatment with enzyme replacement therapy (ERT) is currently long-term, possibly life-long. *Objective*. Management of Gaucher disease involves the meeting of therapeutic goals.[1] These are: the establishment and maintenance of specific haematological parameters, reduction in hepato-splenomegaly, avoidance of bone crises and improvement in quality of life. Currently the annual drug cost of treating a 70-kg adult at the recommended dose of 60 iu/kg 2-weekly would be £325,000. UK practice has been to individualise dose according to clinical status, once disease has been debulked - making the average cost approximately £86,000 per patient.[2] Hollak *et al*.[3] have previously demonstrated the efficacy of dose titration on goals and disease biomarkers. In the UK home treatment with ERT for Gaucher was established within 3 years of the available therapy being licensed and has become the standard. More than 50% of patients become self-infusers with support from treating centres and a designated home nursing team. *Methods.* In view of this independence we examined compliance amongst our co-hort of type 1 Gaucher patients receiving treatment with Cerezyme™. We used a patient-led assessment tool and 3 to 6- monthly monitoring with an approved bio-marker (chitotriosidase). We also compared data to a similar study on patients having intravenous immunotherapy in the home. *Results.* Of 35 patients 21 (60%) stated that they had never missed a dose of their treatment. 14 (40%) had missed a dose at some time and the main reason given for this were vacations lasting more than 2 weeks. Patients were asked to score themselves on a scale of 1 to 10 (1 = non-compliant, 10 = totally compliant, receiving every dose of treatment at the recommended 2- week intervals). 32 (91%) of patients scored themselves between 8 and 9 for compliance. 18 patients (51%) had never experienced any problems with home infusions. Where problems did occur - 11 (35%) had found some difficulties with cannulation when they were still learning. 19 (54%) found that home infusion meant that they felt more in control of their treatment and condition. *Summary.* In the UK the treatment of type 1 Gaucher disease with ERT has shown major clinical benefits to patients. Providing delivery of care in the home has been beneficial to patients in terms of quality of life and maintenance of independence. Compliance with unsupervised treatment has not been shown to be major issue - having positive implications for disease management in the long-term.

References

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0597

CAN WRITTEN ADVICE PROVIDE A SAFE AND ACCEPTABLE ALTERNATIVE TO A NEW PATIENT ASSESSMENT FOR SELECTED REFERRALS TO HAEMATOLOGY

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Background. Traditionally we have seen all patients referred with haematological problems. An alternative approach for selected patients, in which the possibility of significant pathology is low, is to provide written advice to the referrer instead of seeing the patient in person. If this approach is safe and acceptable to patients and referring doctors, it may allow resources to be used more effectively for those other patients who require most attention. *Aims.* We wished to discover what happens to patients referred and given written advice but not seen and compare this with other patients who were referred and seen in person. *Methods.* After obtaining approval from our IRB we reviewed all referrals to the department between 11/03 and 06/06. Those referrals which were managed with written advice (WA) were examined for content of advice and subsequent course. The diagnoses of these patients were compared with those referrals subsequently seen non-urgently as new patients (NUNP). We asked those who referred patients and received written advice, and recent patients who were managed in this way, for their opinions on usefulness and acceptability of this advice. *Results.* We gave immediate written advice to the referring doctors of 714 (37%) of 1907 referrals. All the remaining referrals were seen according to clinical need, such that 438 (37%) were seen non-urgently after a wait of 4 weeks or more (median wait 56 days). All referrals were followed up for a minimum of 8 months. Diagnoses in WA and NUNP were similar with no acute pathology in either group. There was a higher proportion of MGUS, haemochromatosis and haemostasis problems amongst WA and more myeloproliferative and lymphoproliferative disease amongst NUNP. 4% of WA v 9% of NUNP have died. 13% of WA were subsequently re-referred and this led to 52 (7%) attending in person after a median interval of 4 months from first referral. 21 of these remain current patients of whom 13 have required treatment. There were 5 patients who received a diagnosis which had not been predicted at the time of the written advice letter, and all had been re-referred promptly. 223 (74% of all referring doctors) gave their opinion on this process by questionnaire. Most were unsurprised by receiving written advice for their patient(s). 90-98% of respondents believed the process was rapid, helpful, comprehensive and effective and that they could easily re-refer patients if necessary. 90% of additional comments, when made, were favourable. 31 (28% of all patients referred in 2006 whose doctors received WA) gave their opinion, most being aware of their referral and pleased not to be seen in the clinic. Most of additional comments, when made, were favourable. *Summary and Conclusions.* Providing written advice as opposed to arranging a new patient visit does not disadvantage selected patients or miss important pathology. 662 (35% of all referrals) were not seen at all in the course of our review which allowed some reallocation of resources to other patients. Skilled referrers, appropriately supported by written advice overwhelmingly approved of the process.

0598

COST UTILITY ANALYSIS OF DEFERASIROX VERSUS DEFEROXAMINE (DESFERAL) FOR PATIENTS REQUIRING IRON CHELATION THERAPY IN THE UNITED KINGDOM

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Background. Patients suffering from β-thalassemia (β-thal), sickle cell disease (SCD), and myelodysplastic syndrome (MDS) may require life-long blood transfusions and are at risk of iron overload. If they are not treated with iron chelation therapy (ICT), they can suffer serious organ damage and reduced life expectancy. Desferal, infused subcutaneously for 8 to 12 hours per day, 5 to 7 times per week, has been the gold standard of ICT. Exjade is a once daily oral iron chelator, which has been approved in Europe and the US for the treatment of transfusional iron overload. *Aims.* To estimate the incremental cost per quality adjusted life year (QALY) of using Exjade instead of Desferal in patients with β-thal, SCD, or MDS who require iron chelation, from a UK NHS perspective. *Methods.* A cost utility analysis was performed to compare Exjade with Desferal. The results from Study 107, a pivotal, open-label, randomised, controlled study comparing the efficacy of Exjade to Desferal in 586 adults and children with β-thal, demonstrate non-inferiority of Exjade compared to Desferal in patients who received ≥20 mg/kg/day Exjade. The cost-utility analysis is therefore based on a comparison of the health related quality of life associated with the two forms of administration ie oral therapy versus slow subcutaneous infusion, assuming equivalent clinical efficacy. Drug costs were informed by observed use in the equivalence arms of the 107 trial (the ≥20 mg/kg Exjade, and ≥35 mg/kg Desferal arms)with a mean patient weight of 42kg. The annual equipment cost for the administration of Desferal was informed by a primary study of 29 patients (11 β-thal; 14 SCD; 4 MDS; 31% male; mean age 30.6±20.1 years) from four UK treatment centers on the basis of chart reviews and interviews. The model also includes additional costs associated with Exjade treatment for creatinine monitoring and adverse events. A utility study involving 120 UK general population respondents used the time trade-off approach to estimate utility values for clinically

informed vignettes describing health states involving subcutaneous and oral administration of ICT. The mean utility values were 0.66 and 0.84 for sub-cutaneous and oral ICT, respectively. The 95% CI for the utility difference was 0.147-0.212. Utility decrements were applied to account for the minor adverse events associated with Exjade. The model was run over a 1-year period and unit costs from 2004/2005 GBP were applied. *Results.* In the reference case analysis, Exjade dominates Desferal with lower aggregate costs and improved quality of life,as shown in the results Table 1. A range of one- and multi-way sensitivity analyses are also presented. *Conclusions.* Exjade fills a current unmet need for iron chelation via a simple and convenient mode of administration which significantly reduces the patient burden and improves quality of life. The reference case cost-effectiveness results show that Exjade provides more QALYs and costs less than Desferal; in other words, Exjade *dominates* DFO.

Table 1.

Base case	Desferal	Exjade	Difference
Drug costs	£6463	£12580	£6117
Admin costs	£7552	£0	-£7552
Monitor/AE costs	£6	£25	£19
Total costs	£14021	£12605	-£1416
Utility value	0.644	0.808	0.164
Incremental cost per QALY (ICQ)			Exjade dominates

Sensitivity analysis	Δ costs	Δ QALYs	ICQ
Mean weight increased to 62kg	£2061	0.164	£12566
50% patients use pump, 50% balloons	£1315	0.164	£8017
Weight 62kg and 50% pump use	£4321	0.164	£26348
Weight 62kg, 50% pump use, and utility gain reduced by 25%	£4321	0.119	£36311

0599

THE PRICE FOR SURVIVAL ARE ALL PATIENTS EQUAL?

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Background. In the UK hospitals are currently reimbursed for SCT according to prices negotiated on a regional level and agreed in advance of the next financial year based on the hospital's historical and predicted future activity. By 2008 this arrangement will change to a fixed national tariff, *Payment by results*, based on a national average cost of a SCT. It is currently unclear how this figure will be validated. *Aims.* We undertook a comprehensive costing exercise in our institution. We looked at variability in transplant costs and compared our results to our current charges. METHOD We selected ten transplants from the three main transplant modalities: autologous PBSC (ASCT), HLA-matched sibling, and matched unrelated donor transplants. In each group we selected patients with shortest and longest inpatient stays. The other eight transplants were selected at random to include a selection of disease groups, disease status, patient age, and conditioning regimens. The cost for each patient transplanted was calculated from the decision to imminently transplant, to 6 and 12 months for ASCT and allografts respectively. We reviewed clinical activity from the casenotes and medication charts and derived investigations from our computerised results system. Using these data and costs for overheads and utilities, we produced an accurate cost for each patient. In the case of unrelated allogeneic transplants, donor search and stem cell procurement were also included. *Results.* Both myeloablative and reduced intensity conditioning regimens were included. In all three transplant types, cost varied considerably. The average price for an ASCT was 50,575.12 Euros (range 28,932.54-127,083.15 Euros), for HLA matched sibling SCT, 105,577.39 (range 72,818.26 - 265,038.33 Euros) and for matched unrelated SCT, 154,953.04 Euros (range 56,401.78-265,038.33 Euros). These costs are remarkably similar to those currently reimbursed with the exception of ASCT (36,000). A wide range in costs was seen for all aspects of the SCT procedure including donor search and cell procurement (range 13,807.50 Euros - 35,328.45 Euros), pharmacy costs (range 2,881.5 Euros - 108,580.5 Euros), and post-transplant follow up. SCT are inherently heterogeneous and complications are often unpredictable so the high and wide ranges of costs were not unexpected. Current actual repayments underestimate the costs of ASCT in particular. The earliest suggestion for the national tariff, underestimates the cost of all procedures necessitating further accurate negotiations with our purchasers. Our experience of the Payment by Results consultation process is that centres are calculating cost in a variety of ways, with differing *Results.* We believe that our costs are accurate although we did not take into account capital asset depreciation. Failure to include all costs will falsely underestimate costs and the fixed tariff will result in an imbalance between expenditure and gain. As a result, trusts may have to select patients for SCT in order to adhere to a fixed price and novel transplants will no longer be an option. Whilst we admit that the present situation is not ideal, the speed of introduction of new regulations and the lack of standardisation is likely to have a detrimental effect on the UK transplant experience.

0600

COST-EFFECTIVENESS OF CHOP-LIKE CHEMOTHERAPY PLUS RITUXIMAB VERSUS CHOP-LIKE CHEMOTHERAPY ALONE IN YOUNG PATIENTS WITH GOOD-PROGNOSIS DIFFUSE LARGE-B-CELL LYMPHOMA

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Background. There has been little research into the treatment costs associated with conventional haematologic drug regimens. Published economic evaluations focused on the impact of Stem Cell Transplantation in this therapeutic area. *Aims.* To estimate the cost-effectiveness of CHOP-like chemotherapy plus rituximab therapy versus CHOP-like chemotherapy alone therapy, in young patients with good-prognosis diffuse large-B-cell lymphoma, based on data from a large international study (MabThera International Trial MInT Group). *Methods.* The present work used a 3 years model in which two cohorts of patients received CHOP-like chemotherapy plus rituximab or CHOP-like chemotherapy alone. On the basis of efficacy data derived form the above mentioned study, the model simulated a complete or non-complete response to the initial treatment at 5 months and at 3 years. In case of lack of efficacy the model assumed a rescue-therapy (debulking phase plus autologous transplant) would be administered to the non-respondent patients. The analysis was conducted from the perspective of the Italian National Health Service. The model provided estimates of overall survival (LYs - Life Years) and direct medical costs (pharmacological treatment, hospitalization, management of rescue therapy, etc.). Overall survival data were calculated based on the results of the international study (MInT Study) and direct medical costs were based on italian treatment patterns and reported in 2006 Euro. Benefits and costs were discounted at 3%. One-way sensitivity analysis on key clinical parameters was performed. *Results.* The overall survival (per patient) with CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone was respectively 19.69 LYs and 17.78 LYs (giving 1,91 LYs Gained). The expected cost (per patient) was € 23,617.57 with CHOP-like chemotherapy plus rituximab and € 25,370.08 with CHOP-like chemotherapy alone. Therefore it was not necessary to calculate the Incremental Cost Effectiveness Ratio (ICER) of CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone, since the former was dominant. The study results were sensitive to a 5-months complete response and to a 3-years complete response. Namely, sensitivity analysis simulated the worst case for CHOP-like chemotherapy plus rituximab with reference to such parameters. For a 5-months complete response an ICER was calculated of € 1,273.43 for CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone. For a 3-years complete response an ICER was calculated of € 661.43 for CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone. *Conclusions.* This economic evaluation suggests that CHOP-like chemotherapy plus rituximab is a dominant strategy versus CHOP-like chemotherapy alone for treatment of young patients with good-prognosis diffuse large-B-cell lymphoma. The sensitivity analysis showed CHOP-like chemotherapy plus rituximab was a cost-effective approach even in the worst case assumption.

0601

EUROPEAN LEUKEMIANET - INTEGRATION OF THE LEADING NATIONAL LEUKEMIA NETWORKS (CML, AML, ALL, CLL, MDS, CMPD) AND THEIR INTERDISCIPLINARY PARTNER GROUPS IN EUROPE

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Leukemias serve as a model for a variety of diseases and possess exemplary relevance for basic research and patient care. Leukemia research and therapy have achieved high standards and even a leading position in several European countries. A true European world leadership, however, had not been accomplished due to national fragmentation of leukemia trial groups, diagnostic approaches and research activities and the lack of central information and communication structures. To overcome national fragmentation the European LeukemiaNet (ELN) was started in 2002. It is funded within the 6th EU-Framework Program since 2004. The ELN now integrates 92 leading national leukemia trial groups (CML, AML, ALL, CLL, MDS and CMPD), 86 interdisciplinary partner groups (diagnostics, treatment research, registry, guidelines) and industry partners across Europe to form a cooperative network for advancements in leukemia-related research and health care. The trial groups and their partners which represent several thousand participating centers and ten thousands of study patients treated within the trial groups, form the backbone of the network. The network with its integration and interdisciplinary cooperation brings together 133 participating institutions and approximately 1000 researchers from 24 countries. The network consists of 16 thematically distinct workpackages. Of these, six deal with the various disease entities and represent subnetworks on their own. Seven workpackages represent interdisciplinary platforms, which provide the support and research expertise required for high quality networking and excellence. Three central service workpackages provide central communication, information and management services for the whole network and support integration. Scientific highlights of accomplished work include: 1. Establishment of information, communication and management structures. Communication is mostly accomplished via the information center (ELIC) and by the network management center (NMC) through annual symposia, regular network- and WP-meetings, an ELN website, and biannual newsletters. A European Leukemia Trial Registry (ELTR) was developed in accordance with the guidelines of the International Committee of Medical Journal Editors and the WHO. ELTR will be connected to the WHO Meta-Registry, as soon as the WHO has defined definitive interfaces for data-transfer. 2. European registries have been started for CML, ALL, ET and MDS. 3. Clinical trials on an European level are ongoing. 4. Quality control rounds and consensus recommendations in diagnostics on a European level were achieved e.g. for molecular monitoring in CML, cytogenetic analysis in CLL and morphological diagnosis of leukemias. 5. Several guidelines and management recommendations were completed and in part published, e.g. management recommendations for CML, recommendations for harmonizing methodology for detecting BCR-ABL transcripts and kinase domain mutations, guidelines for microarray analyses, guidelines on definition of transplant-associated microangiopathy (TAM), recommendations for standardizing indications for SCT, guidelines on prophylaxis and empirical antifungal therapy in neutropenic leukemia patients. The main goals for the first three years have been achieved and the ELN is well prepared for further integration advances in research, diagnosis and treatment of leukemia according to its goals.

0602

PRIMARY PROPHYLAXIS WITH PEGFILGRASTIM WAS MORE COST-EFFECTIVE THAN FILGRASTIM IN PATIENTS WITH NON-HODGKINS LYMPHOMA RECEIVING CHOP-21 IN THE UK

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Background. In the 2006 ASCO and EORTC guidelines, primary prophylaxis with granulocyte colony-stimulating factors (G-CSFs) is recommended when the overall risk of febrile neutropenia (FN) from chemotherapy and patient-related factors is equal to or greater than 20%. Without G-CSF, FN incidence among patients receiving CHOP-21 chemotherapy is 17-50%. Although the daily first-generation G-CSF filgrastim and the long-acting second-generation G-CSF pegfilgrastim are both commonly used, in practice filgrastim is often administered for shorter-than-recommended courses, which has been shown to be associated with less clinical benefit. *Aims.* We evaluated the cost-effectiveness of pegfilgrastim vs. filgrastim used for 11 days (as used in the randomised trials demonstrating efficacy) and 6 days (as often used in clinical practice) in patients with aggressive NHL receiving CHOP-21 in the UK. *Methods.* A decision-analytic model was constructed from the National Health Service's perspective. The study time horizon was life-time. Model inputs, including FN risk (varied by days of filgrastim use), FN case-fatality, relative dose intensity (RDI), impact of RDI on survival, and utility scores were from a comprehensive literature review and expert panel validation. Costs were from official price lists or literature and included drugs, drug administration, FN-related hospitalisations, and subsequent medical costs. NHL mortality data were from literature; all-cause mortality data were from official statistics. The model simulated three clinical scenarios: scenario 1 included the impact of prophylaxis with pegfilgrastim or filgrastim on FN risk; scenario 2 included the impact of a difference in FN risk on FN-related mortality; scenario 3 included a differential impact on RDI and long-term survival. Using data from a meta-analysis (pegfilgrastim vs. 11 days of filgrastim) and observational studies (pegfilgrastim vs. 6 days of filgrastim), we estimated the absolute risk of FN in patients receiving pegfilgrastim decreased by 6.5 percentage points (19.6 vs. 13.1%) vs. 11-day filgrastim, and by 12 percentage points (25.1 vs. 13.1%) vs. 6-day filgrastim. Model robustness was tested using sensitivity analyses. Outcomes were measured as incremental cost-effectiveness ratio (ICER) including £ per percentage (absolute) FN risk decreased, £ per FN event avoided, £ per life-year gained (LYG), and £ per quality-adjusted life-year (QALY) saved. *Results.* Pegfilgrastim was cost saving compared with 11-day filgrastim in all scenarios. Compared with 6-day filgrastim, the ICER was £6,675 per FN avoided or £ 67 per 1% decrease in absolute risk of FN in scenario 1. The ICER was £ 29,438/QALY saved in scenario 2. In scenario 3, when all potential benefits of G-CSF were considered, the ICER became £ 7,699/QALY saved (Table 1).

Table 1. Cost-effectiveness of pegfilgrastim vs. 6-day filgrastim.

	Cost	Scenario 1	Scenario 2		Scenario 3	
	(£)	Risk of FN	LY	QALY	LY	QALY
6-day filgrastim	4,091	25.1%	8.542	7.411	7.249	6.261
Pegfilgrastim	4,892	13.1%	8.572	7.439	7.365	6.365
Difference between pegfilgrastim and 6-day filgrastim	801	-12.0%	0.030	0.027	0.116	0.104
ICER	---	£6,675 per FN event avoided	£26,425 /LYG	£29,438 /QALY	£6,903 /LYG	£7,699 /QALY

Results were sensitive to the relative risk of FN for 6-day filgrastim vs. pegfilgrastim and study time horizon. *Summary and conclusions.* In the UK, pegfilgrastim was cost saving compared with 11-day filgrastim use. The cost of pegfilgrastim vs. 6-day filgrastim at £7,699-£29,438/QALY gained was favourable compared with the £30,000/QALY threshold commonly used in the UK. With primary prophylaxis against FN in NHL patients

using CHOP-21 in the UK, pegfilgrastim was cost-effective compared with filgrastim used for the recommended number of days or fewer.

0603

PREFERENCES TOWARDS COAGULATION FACTOR CONCENTRATES USED TO TREAT HEMOPHILIC PATIENTS WITH INHIBITORS: THE PATIENTS, PHYSICIANS' AND PHARMACISTS PERSPECTIVE

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Background. the management of hemophilia when patients develop inhibitors is particularly complex and costly. Although the advances of modern technologies, no agreement still exists on how to optimally treat these patients. Treatment of haemophilia is the result of interactions between patients, physicians, pharmacists and budget holders, each carrying their own set of preferences. *Aims.* this study was conducted to evaluate preferences toward possible coagulation factor concentrates used in patients with inhibitors from a sample of patients or their caregivers (if patients aged <17 years), physicians specialist in hemophilia care, pharmacists with experience in storing/delivering coagulation factor concentrates. *Methods.* a Discrete Choice Experiment was conducted, by describing possible products with 7 attributes, selected from a previous pilot study: viral safety, risk of anamnestic response, possibility of undergoing major surgery, frequency of infusions in prophylaxis, number of injections to stop bleeding, time to stop bleeding, time to pain recovery. Cost, expressed as increase of health care taxes, was added to estimate willingness to pay towards these attributes. The respondents were asked to choose among 16 sets having 2 hypothetical products each one. A logistic model was used to analyse the data. *Results.* 23 adult patients, 10 caregivers, 37 physicians, 25 pharmacists were interviewed. All the patients had severe hemophilia A, historical median peak titre = 233 BU/mL (5-16,400). Among all the respondents, the hypothesized direction of preferences was confirmed (e.g. lower risk of infections was preferred than higher risk) and the importance of preferences was statistically significant (p values <0.01) for every attribute. Anyhow, each subgroup expressed different strengths of preferences for some attributes. *Conclusions.* this study revealed patients/caregivers', physicians' and pharmacists' preferences towards the products used to treat hemophilic patients with inhibitors. Understanding the preferences from different perspectives can be useful to optimize the benefits deriving form the decisions on treatments for patients with inhibitors.

0604

EFFICACY AND COST ANALYSIS OF PALONOSETRON AND TROPISETRON USE IN CHEMOINDUCED NAUSEA AND VOMITING (CINV) IN HEMATOLOGY PATIENTS

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Background. CINV is the most frequent side effect of chemotherapy. Its incidence is 60-90% with charboplatinum and high dose cytarabine, 30-60% with ifosfamide and idarubicin and 10-30% with cytarabine (inferior/equal to100 mg) and daunorubicin. 5-HT3 receptors antagonists are the most effective drugs against CINV. Among these, tropisetron and palonosetron have a prolonged efficacy (half-life of 8 and 40 hours respectively) and, indeed, require a reduced number of administration. *Aims.*To evaluate clinical efficacy and pharmacoeconomic impact of palonosetron and tropisetron in patients receiving 5-7 days chemotherapy. *Methods.* We evaluated 25 patients treated with 5-7 days chemotherapy for NHL(15) or AML(10). M/F was 10/15, median age was 65 years (R25-81). NHL patients, receiving ifosfamide (IGEV regimen) or high dose cytarabine and charboplatinum (MICMA regimen), were at high-risk of CINV. Instead AML patients, receiving daunorubicin and standard dose cytarabine, were at low-risk CINV. For each patient antibiotic and opioid administration, anticipatory emesis, early or late emesis, mucositis, gastropathy, esophagitis and diarrohea 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 palonosetron and the other tropisetron. This is a monocentric, prospective, randomized study. We administred antiemetic therapy 30 minutes before chemotherapy infusion. Palonosetron was given only at first chemotherapy day, while tropisetron was given from one to three times/day, depending to chemotherapy regimen and patient medical history of CINV. *Results.* In tropisetron group M/F was 5/7, median age 67 years (R25-81), AML/NHL was 4/8. Five patients received antibiotic therapy during antiemetic administration. Three patients showed CINV. In palonosetron group M/F was 5/8, median age 63 years (R31-79), AML/NHL was 6/7. Six patients received antibiotic therapyt during antiemetic administration. Two patients showed CINV (one of them with gastritis and anticipatory nausea responding to methoclopramide administration). Only patients treated with MICMA regimen received steroid infusion as part of chemotherapy administred. Fisher exact test (p=0.6), Odds Ratio (1.8, CI 95%:0.3-11) and relative risk (1.6, CI95%:0.3-8) didn't showed significant difference in CINV incidence between palonosetron and tropisetron group. In palonosetron group, median global antiemetic expense for each patient was 107.25euro (R107.25-834.53), while in tropisetron group was 410.5euro (R30-515). Median antiemetic expense only for real CINV, was 107.25euro (R107.25-167) in palonosetron group, while in tropisetron group was 410.5euro (R30-515). *Summary and conclusions.* Palonosetron efficacy is not inferior to tropisetron in CINV prevention in patients receiving multiple days chemotherapy regimens. In pharmacoeconomic analysis the median cost of palonosetron treatment is about 300euro inferior to tropisetron treatment cost. Nevertheless these data need further confirmation on a larger patient cohort.

0605

RITUXIMAB RAPID INFUSION (90 MINUTES) IS FEASIBLE AFTER THE FIRST DOSE IN AN OUT PATIENT SETTING, A SINGLE CENTER PROSPECTIVE STUDY OF 80 COURSES

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Background. in 2007, Rituximab represent the standard of care for B-cell lymphomas and its use has increased in different settings. The first way to give it was alone with 4 doses weekly, then in combination with chemotherapy and more recently with a maintenance schedule. Due to the risk of cytokine induced toxicity, a premedication is recommended and the first course must be started slowly at 50mg/hour, than 100 mg/h. Aims, with the increasing number of patients treated with rituximab, the administration dropped to outpatient units and the infusion shortened. We, decided to evaluate prospectively a 90 minutes administration after an IV premedication (methylprednisolone 120 mg, paracetamol 1g and Dexchlorpheniramine 4 mg) given 30 minutes before. *Methods.* rituximab (doses of 600 or 700 mg) was begun at 400 mg/h for a total duration of 90 minutes in the absence of adverse reactions. The study was conducted in an outpatient unit including all patients receiving at least the second dose of rituximab from November 2006 to January 2007. Patients who had adverse reactions after the first dose took an oral antihistaminic drug the evening before. Among 100 courses, 20 were not analysed by the nurses due to lack of time to perform the study which consisted of reporting every 30 minutes, blood pressure, fever, pulsations and adverse effects. 80 courses of rituximab were evaluated in 62 patients with a sex ratio M//F of 3.2 and a median age of 59 years [29 to 87 years]. Diagnosis according to the OMS classification of lymphomas were as follows, FCL n = 29, DBCL n = 14, MZL n = 7, B-CLL n = 7, MCL n = 4, NLPHL n = 1. 42 patients were in first line therapy and 20 had relapsed. Results, Rituximab was given alone in 15 patients, or associated with chemotherapy: CHOP n = 21, CVP n = 13, Fludarabine n = 11 others n = 2. Three patients were evaluated 3 times, 15 patients were evaluated 2 times and the remaining had only one evaluation of the rituximab duration. 39 patients were evaluated from the second to the fourth course of rituximab and 41 after the fourth course. The median duration of rituximab perfusion was at 90 minutes with only 10 courses exceeding this time, 2 patients had a transient discontinuation of the perfusion leading to the longest perfusion duration of 170 and 240 minutes related to 2 adverse events (face erythema and throat dysesthesias). At the end, all patients received the total dose of rituximab. No other adverse events were reported, blood pressure and temperatures remained within normal ranges values according to the underlining disease and no patients were hospitalized for the night. Conclusion, rapid perfusion of rituximab after the first dose is safe after IV premedication and can be given in an outpatient setting even in the elderly and represent a less cost effective management of these patients with B-cell malignancies.

Infection and supportive care II

0606

INVASIVE FUNGAL SINUSITIS IN PATIENTS WITH STEM CELL TRANSPLANTS AND HEMATOLOGICAL DISEASE

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Background. Invasive fungal sinusitis (IFS) causes high morbidity and mortality in patients with stem cell transplants and hematological disease. *Aims.* We compared IFS diagnosis using EORTC / MSG-NIAID consensus criteria with physician-diagnosed IFS to determine the applicability in clinical study. The clinical characteristics and prognostic factors were correlated with invasive fungal sinusitis in patients of stem cell transplants and hematological disease. *Methods*. We searched the terms *sinusitis* and *sinonasal infection* in the discharge summary database of all patients admitted to the Department of Internal Medicine between January 1995 and September 2006. The medical records were reviewed retrospectively. *Results.* Totally, 103 hematological patients were enrolled, including 39 with clinically diagnosed IFS and 64 with sinusitis. Forty patients met the criteria by EORTC / MSG-NIAID consensus, including 15 with proven IFS, 16 with probable IFS, and 9 with possible IFS. Patients with acute myeloid leukemia and absolute neutrophil count <500 mm^3 for >10 days had a higher rate of IFS. Aspergillus flavus was isolated in 12 of 20 (60%) patients, and mucormycosis was found in 4 of 20. Bony erosion and extra-sinus infiltration were found in 15 of 39 patients with clinically diagnosed IFS by CT or MRI. Refractory or relapsed disease status was the most important prognostic factor in hematological disease with IFS. Even with anti-fungal therapy and aggressive surgical debridement, 21 of 39 patients (53.8%) with clinically diagnosed IFS and 22 of 40 patients (55%) with IFS diagnosed by EORTC / MSG-NIAID criteria died. *Summary and Conclusions.* IFS diagnostic criteria by EORTC /MSG-NIAID can be applied in the clinical research. IFS is an ominous sign in the patients with refractory / relapsed hematological malignancy. More effective IFS treatment is needed in patients with stem cell transplants and hematological disease, and such treatment should be developed.

0607

SURVIVAL OF PATIENTS WITH HAEMATOLOGICAL MALIGNANCY ADMITTED TO THE INTENSIVE CARE UNIT: PROGNOSTIC FACTORS AND OUTCOME COMPARED TO UNSELECTED MEDICAL ICU ADMISSIONS

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Background. Cancer patients have historically had a very poor outcome following ICU admission. Outcome has however improved over the last decade. *Aims.* To identify factors which predict survival for critically ill patients with haematological malignancy and which can be readily identified prior to admission. This would improve selection of patients suitable for ICU admission, which represents a limited resource. We also assessed the ability of the APACHE II score to predict prognosis in these patients. *Methods.* Since the ICU admission case mix will vary between hospitals, one non-surgical admission within ± 1 week of each haematological admission acted as a control group. Factors which might affect outcome were assessed by multivariate regression analysis. Factors included were age, haematological diagnosis (acute or chronic leukemia, myeloma, lymphoma), time from haematological diagnosis to ICU admission (0-6 months, 6-12 months, >12 months), degree of prior treatment (admission prior to diagnosis, during first line therapy, after first line), remission status, prior stem cell transplant, documented infection and length of neutropenia (none, 1-10 days, >10 days). Predicted hospital mortality was calculated from the APACHE II score by the formula of Knaus et al (Critical Care Medicine 1985) for haematology patients. The APACHE scores of haematology patients were compared to controls by a two-sample t test. Predicted and actual mortalities were compared using a one sample test of proportion. The impact of mechanical ventilation (MV) on mortality was assessed by risk ratios. *Results.* We identified 111 patients with haematological malignancy (acute leukemia n=42, chronic leukemia n=11, myeloma n=19 and lymphoma n=39) admitted to ICU in one teaching and three district general hospitals (November 2000 - January 2006). Median age of haematological patients was 59 years (range 17-84) and M:F ratio 1.22:1. Control patients (n=111) were similar with median age 63 years (range 17-86) and M: F ratio 1.09:1. For control patients, overall ICU and hospital survival rates were 70% and 55% respectively while survival for haematology patients was approximately half at 44% and 24% respectively. In multivariate regression analysis, only increasing age (p=0.016) and documented infection (p=0.016) predicted poor outcome. All other variables were not significant. APACHE scores were significantly higher in haematology patients (median 27) compared to controls (median 19) p<0.001. Predicted hospital mortality for haematology patients was 56%, significantly lower than actual mortality (77%) p<0.001. For controls, hospital survival was slightly reduced for MV v's not receiving MV (risk ratio = 1.37; 95% C.I. =0.91, 2.05). Haematology patients hospital survival was significantly worse for MV - 5/55, 9% v's no MV 20/44, 45% (risk ratio = 5.00; 95% CI = 2.04, 12.50). *Conclusions.* Most pre-admission variables assessed did not predict mortality and should not be used for this purpose. Despite high APACHE II scores, predicted hospital mortality underestimated mortality for patients with haematological malignancy. Need for MV still predicts poor outcome in this group but without MV nearly half survive to hospital discharge.

0608

EMERGING OF MULTIRESISTANT PSEUDOMONAS AND VANCOMYCIN-RESISTANT ENTEROCOCCI: RESULTS OF A PROSPECTIVE SURVEILLANCE STUDY

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Background. Infectious diseases are potentially life-threatening complicances in haematological patients, as a result of their intrinsic and drug-related immunodeficiency. Epidemiological surveillance allows to identify emerging bacterial resistance and to improve empirical antibiotic therapy. Aims. To evaluate epidemiological trends among patients admitted at our Haematology Unit. Methods. Microbiologically documented infections consecutively occurred at our Institution during two sixteen month consecutive periods (A: June 2004-September 2005; B: October 2005-January 2007) were evaluated and correlated with type of infection, state of underlying disease, neutropenia, exposure to prophylaxis with fluoroquinolones (Fq), presence of central venous catheter (CVC), resistance to antibiotics and outcome. Neutropenia was defined as a neutrophil count <0.5×10^9/L. During the two periods, prophylaxis with Fq (levofloxacin 500 mg/d) was carried on in patients with an expected neutropenia lasting more than 7 days; β-lactam plus aminoglycoside ± vancomycin was the regimen adopted for empiric antibiotic therapy in neutropenic patients with fever. *Results.* During the periods A and B, 137 and 146 microbiologically documented infections were recorded and 148 and 149 bacteria responsible for infections were identified respectively. A higher incidence of Gram negative (G^-) with respect to Gram positive (G^+) bacteria was observed in both periods (A: 81/148, 55% vs 67/148, 45%; B: 88/149, 59% vs 61/149, 41%). Overall E. coli was the most frequent microrganism isolated and was similarly represented in the two periods (A: 38/81, 47%; B: 41/88, 47%); whereas Pseudomonas spp infections increased in period B (A: 13/81, 16%; B: 24/88, 27%, p=0.09); four cases were multiresistant (all in B period, 17%). Among G^+ bacteria, Stafilococci were more frequent during period A (42/67, 63%; vs 22/61, 36%, p<0.01), particularly S. aureus (23/67, 34% vs 7/61, 11%, p<0.01); Enterococci increased in period B (A: 13/67, 19%; B: 22/61, 36%, p<0.05) with resistance to vancomicin raising from 23% to 36%. Vancomycin-resistant Enterococci (VRE) and multiresistant Pseudomonas were associated with an uncontrolled haematological underlying disease (10/12), neutropenia (9/12) and hospitalization (12/12 nosocomial infections). VRE were responsible for perianal abscesses in five cases and for bacteraemia in three. Multiresistant Pseudomonas were responsible for pneumonia in one case and for bacteraemia in three. Crude mortality of microbiologically documented infections was 9% (12/137) and 8% (12/146) in period A and B respectively. A bacterial infection was responsible for 8/12 cases in period A (all but one were G^+) and for 6/12 cases in the B period (2 G^+ and 4 G). In two of the 6 cases of the B period, a multiresistant pathogen was implicated (1 vancomicin-resistant E. faecium and 1 multiresistant P. aeruginosa). *Summary and conclusions.* These data show that continuous epidemiological surveillance allows the prompt detection of emergent multiresistant pathogens at haematological units. Antibiotic policy should be guided by it. In particular, our data support a limitation in the use of third generation cephalosporins, of glycopeptide and carbapenems in order to contrast the emergence of resistant strains.

0609

SOFT TISSUE COMPLICATIONS IN PEDIATRIC LEUKEMIA PATIENTS WITH AND WITHOUT CATHETERS

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Background. Skin and soft tissue complications due to invasive procedures for the administration of chemotherapeutic agents, blood sampling and supportive therapy are frequently seen in patients with leukemia. Central venous lines are an essential tool in the management of these patients especially in developed countries. Despite their benefits, catheters are often associated with local or bloodstream infections and a higher rate of infection in developing countries are expected due to low socioeconomic conditions, insufficient training and care. Frequency of skin and soft tissue complications in pediatric leukemia patients with and without catheters have not been compared previously. *Aims.* We wanted to evaluate skin and soft tissue complications secondary to procedures in acute leukemia patients with and without catheters and if the frequency of these complications is less in patients with catheters. *Methods.* Patient records of the children who were diagnosed and treated as acute leukemia between January 1996 and January 2006 in our hematology department were retrospectively evaluated. The following data were collected for each patient: age, gender, insertion of catheter, catheter type, catheter related complication, reason for removal, duration of induction and consolidation treatment, and skin and soft tissue complications in patients without catheters (e.g. sellulitis, abscess, drug extravasation). Patients were treated with ALL BFM 95 or AML BFM 93-98 protocols. *Results.* 87 acute leukemia patients (75 ALL, 12 AML) were included. There were 30 patients with 37 catheter use (6 port, 31 Hickman catheter) and 57 patients without catheter. Median age of all patients was 6 years, in patients with catheters 3.6 years and in patients without catheters 8 years. Median age of patients with catheters were significantly lower than patients without catheter (p=0.004). There was no gender difference between the patients with and without catheters (F/M ratio was 15/15 and 28/29, respectively; p=0.938). 37 catheters were removed for various reasons: 20 (54.1%) end of treatment, 5 (13.5%) infection, 3 (8.1%) unrelated death, 2 (5.4%) thrombosis, 2 (5.4%) tearing, one (2.7%) displacement, and 4 (10.8%) spontaneous removal. In patients with catheters, skin and soft tissue complications were seen in 20 (66%) children. Most frequent complication was cellulitis (n=11, 55%), which was followed by drug extravasation (n=6, 30%), skin ulceration, abscess and hematoma with one patient each. In one patient, coagulase negative Staphylococcus was shown in blood culture. In patients without catheter, skin and soft tissue complications were seen in 37(65%) patients as the following: 14 (37.8%) cellulitis, 14 (37.8%) extravasation, 7 (18.9%) abscess, and 2 (5.5%) skin ulceration. Blood cultures were positive in 6 patients (Klebsiella oxytoca, Staphylococcus aureus, Pseudomonas aureginosa, coagulase negative Staphylococcus, E. coli and E.coli + Pseudomonas aureginosa). When the frequency of skin and soft tissue complications in patients with and without catheters were compared to each other, there was statistically no significant difference (p=0.792). The duration of chemotherapy was significantly longer in patients who developed skin and soft tissue complications with or without catheters when compared to duration of therapy in patients without any skin and soft tissue complications (259.2±36.3 and 218.3±58.3 days, respectively; p<0.0001). *Conclusions.* In pediatric leukemia patients, with or without catheters, skin and soft tissue complications are common and these complications may prolong the duration of chemotherapy. In developing countries, use of catheters does not decrease the frequency of skin and soft tissue complications.

0610

PROGNOSTIC FACTORS OF INTRACRANIAL HEMORRHAGE IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY

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Background. Intracranial hemorrhage is the second leading cause of mortality in patients with hematological disease. Acute promyelocytic leukemia (APL) with DIC has been well controlled with all trans retinoic acid (ATRA). Most patients underwent the safety threshold of prophylactic platelet transfusion. However, the prognostic factors of Intracranial hemorrhage in patients with hematological disease is still under investigated. *Aims.* Identify the clinical characteristics and prognostic factors of intracranial hemorrhage in patients with hematological disease. *Methods.* We searched the term of *intracranial hemorrhage* in the discharge summary database of all patients admitted to the Department of Internal Medicine between January 1995 and September 2006. The adult patients with active hematological disease and spontaneous intracranial hemorrhage were enrolled in this study. The medical records and image study were reviewed retrospectively. *Results*. There were 50 patients (35 men and 15 women) with ICH, the median age was 51 year-old (range 17-86). The hematological diseases subtypes were 30 acute myeloid leukemia (non-APL), 11 APL, 4 acute lymphoblastic leukemia and 5 myelodysplastic syndrome / severe aplastic anemia. Most patients were in relapsed / refractory status of underlying hematological disease except 7 patients were in remission status. Intracranial hemorrhage mainly located at supratentorium (41), basal ganglion (8), cerebellum (5), and brain stem (4). There were 19 patients had multiple sites hemorrhage in the image study. The types of hemorrhage included intracerebral hemorrhage (35), subarachnoid hemorrhage (15), subdural hemorrhage (13), epidural hemorrhage (1) and 11 patients had hemorrhage rupture into ventricles. Most of the patients received supportive care because bleeding tendency. Only 4 patients received surgical intervention and three patients survived. Thirty-three patients (66%) died of intracranial hemorrhage. The age, gender, underlying hypertension, sepsis, hematological subtypes, remission status, hemogram, and surgical intervention had no impaction on survival. Patients with subdural hemorrhage had better prognosis (p=0.021) than the other type of hemorrhage. The hemorrhage located at the basal ganglion (p=0.039), and subarachnoid hemorrhage (p=0.009) had worse prognosis. *Summary / Conclusons.* Intracranial hemorrhage cause high mortality and occurred mainly in advanced stage of hematological disease. Further novel treatment of hematological malignancies maybe effectively reduce the incidence of Intracranial hemorrhage.

0611

HICKMAN LINE REMOVAL IS SUCCESFULL IN TREATING MOST OF THE SEPTIC EPISODES SECONDARY TO RHODOTURULA FUNGAEMIA IN IMMUNOCOMPROMISED HAEMATO-ONCOLOGY PATIENTS

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Background. Rhodotorula species are saprophytic yeasts belonging to the family Cryptococcaceae. They have been isolated from human skin, environmental sources and seem to have strong affinity for plastics. Rhodotorula species show low virulence but have been reported causing serious infections in immunocompromised patients, particularly in the presence of indwelling catheters. *Aims.* To analyse retrospectively incidence, clinical presentation, risk factors, treatment and outcome of Rhodoturula infections within our 15 bedded haemato-oncology and bone marrow transplant unit between August 2003 and September 2006. *Methods.* All Rhodoturula isolates within our unit during the study period were identified by our Microbiology Department. Case notes from the patients with positive isolates were analysed and information collected on clinical presentation, risk factors, treatment and outcome of the infection. *Results*. 21 positive isolates in 10 different patients were identified. All positive cultures were blood cultures from Hickman lines. 12 infective episodes were documented from case notes giving an incidence of 3.78 episodes/year within our unit. In all cases, patients presented with pyrexia secondary to Rhodoturula fungaemia (defined as at least one positive blood culture) but no evidence of any other organ involvement. 7 episodes were in patients within 6 months of an allogenic bone marrow transplant (all conditioned with MabCampath), 4 in patients who had undergone intensive chemotherapy before the infection, and 1 in a patient who had received immunosuppressive medications during the 6 months before the infection. 6 cases happened in patients who had been neutropenic (Absolute Neutrophil count<1) for a prolonged time (range 10 days to 12 months). In 11 episodes the line was removed when preliminary culture results were available. In one episode the line was not removed but the infection recurred within 60 days requiring line removal. Patients were started on intravenous antifungals in 9 of 12 episodes (7 liposomal amphotericin, 1 conventional amphotericin and 1 caspofungin)and treated with oral azoles (voriconazole, itraconazole and fluconazole) in the remaining 3 episodes. All antifungals were started either empirically for persistent pyrexia or on receiving a positive culture, but before classification of yeast isolates was available. As a result, in 2 cases Rhodotorula proved to be resistant to the chosen agent, but, in both cases, antifungals were not changed as patient conditions had improved. All patients survived the septic episode without clinical consequences. *Conclusons.* Most Rhodotorula infections are of low virulence. Presence of an indwelling catheter and immunosuppression are the main risk factors. Treatment with MabCampath appeared to be a possible risk fac-

tor. In 50% of the episodes, patients had a normal neutrophil count, but they were significantly immunocompromised either because of concomitant treatment or underlying disease, thus confirming that a normal neutrophil count does not provide a significant protection against opportunistic infection in aggressively treated haemato-oncology and post bone marrow transplant patients. Line removal is probably sufficient to treat most of the septic episodes secondary to Rhodoturula, therefore saving the patient unnecessary treatment with a potential toxic drug. However when clinical conditions are not improving, despite line removal, the introduction of antifungals may be needed.

0612

PROSPECTIVE CYTOMEGALOVIRUS MONITORING IN ACUTE MYELOID LEUKAEMIA DURING FIRST LINE THERAPY

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Background. Despite the extensive knowledge about the incidence and clinical impact of CMV infection in acute leukaemia patients receiving stem cell transplantation, very little is known about its role in acute myeloid leukaemia (AML) patients at onset and during first line intensive chemotherapy. *Aims.* Aim of our study was to analyse prospectively the incidence of active CMV infection in AML patients, focusing on the role and therapeutic implications of CMV serial monitoring from diagnosis until transplant procedure. *Methods*. Since 8/2002, 68 AML patients at diagnosis (M/F: 33/35; median age: 45 years, range 24-61) were consecutively evaluated. All patients were scheduled to receive induction therapy including standard-dose (SD) or high-dose (HD) Ara-C, Etoposide and Daunorubicin and consolidation therapy with intermediate-dose (ID) Ara-C and Daunorubicin. Active CMV infection was defined as any positivity of pp65 antigenemia in the peripheral blood, while CMV disease was defined as the detection of CMV in tissue samples. Pp65 antigenemia monitoring was scheduled at diagnosis, post-induction and post-consolidation chemotherapy. CMV pre-emptive treatment consisted of Gancyclovir 5 mg/kg/12h for 12-21 days or Cidofovir 5 mg/kg/week for a total of 4 doses. Recovery was defined as negative antigenemia in two consecutive controls. All patients received Acyclovir prophylaxis for Herpesvirus infections during neutropenia after chemotherapy. *Results.* Among the 68 patients enrolled in the study 58 (85,3%) achieved complete remission (CR) (3 refractory; 7 toxic deaths) and 56 received consolidation (2 early relapses). 54/56 patients are evaluable after consolidation. At diagnosis pp65 antigenemia was negative in all patients evaluated . Overall incidence of positive antigenemia in the whole population was 29% (17/58 patients): in particular 17% after induction (10/58 patients in CR) and 12% (9/54) post consolidation. Among patients positive after consolidation only 2 were positive post induction. None of the patients had CMV disease. Gancyclovir treatment was instituted in 8 patients, Cidofovir in 8, while in 3 patients no treatment was given. Recovery of infection was achieved in all cases. The incidence of CMV infection in patients receiving HD or SD Ara-c was of 46%(11/26) and 18%(6/32) respectively (p<0,05). Median follow up from CR was 24 months (range 1-60). Overall relapse rate of acute leukaemia was 44% (26/58). The median interval from diagnosis to consolidation for patients CMV$^+$ and CMV- after induction was of 87 (51-100) and 64 (44-155) days respectively (p<0,05). Overall projected probability of survival at 70 months is 53% (55% vs 52% for CMV$^+$ and CMV- respectively). *Summary.* CMV active infection has an high incidence (29%) in AML patients during chemotherapy. HD Ara-c is associated with a significant increase in CMV positivity and the time interval between induction and consolidation seems to be delayed in CMV$^+$ patients. Our results show that CMV reactivation is not only a problem of stem cell transplantation, and suggest the importance of CMV monitoring and of therapeutic guidelines since diagnosis, in order to prevent the risk of CMV disease without compromising leukaemia treatment.

0613

SEPSIS IN PATIENTS RECEIVING MYELOABLATIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKAEMIA: NO ASSOCIATION WITH MANNOSE-BINDING LECTIN GENE (MBL2) POLYMORPHISM

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Background. Infections after chemotherapy are a major problem in patients with acute myeloid leukaemia (AML). Mannose-binding lectin (MBL) is part of the innate immune system, and deficiency of MBL occurs in patients with common gene variants (AO/OO) compared with the wild-type gene (AA). In addition, polymorphism in the promoter region influence the concentration of MBL. The promoter variant X is associated with low transcription in contrast to the high-transcription promoter variant Y.[1] Variant MBL2 seems to increase the risk of infections in multiple myeloma patients after high-dose melphalan and autologous stem cell transplantation,[2] in children with malignancy treated with chemotherapy,[3] and in patients admitted to intensive care unit.[4] In contrast, no association between the serum level of MBL and infections in AML patients was seen in a smaller study.[5] *Aims.* To investigate the possible associations between MBL2 polymorphism and sepsis in patients treated with high-dose chemotherapy for AML. *Methods.* Patients were included from a single centre between 1/1-1993 and 1/9-2004. The follow-up period was six month from diagnosis. Prophylactic antibiotics were not used after chemotherapy. We included 191 patients treated with myeloablative chemotherapy for AML. Infections were identified retrospectively using clinical records, and microbiological database extractions. MBL2 genotypes were identified by using real-time polymerase chain reactions (PCR) in stored samples of bone marrow aspirates. *Results.* We identified 603 febrile episodes in 191 patients. In 246 episodes (41%) sepsis was present. Thirty-two patients (17%) either died from sepsis, or sepsis was a major concomitant factor for death. Associations between MBL2 polymorphism and sepsis are shown in the table. No significant association with MBL2 polymorphism and fever, sepsis (p=0.79) or death due to sepsis was seen. Furthermore, no significant association with MBL2 and the type of sepsis (whether gram positive, gram negative, or mixed) was found (p=0.16). *Conclusions.* No association between MBL2 polymorphism and the risk of sepsis in AML patients was seen. This result differs from the previous findings in children with malignancy[3] and multiple myeloma patients,[2] but confirms the previous study in AML patients.[5] The severe and long-lasting neutropenia and mucositis after chemotherapy are probable explanatory factors, why the MBL system seems to offer no protection against sepsis in AML patients. Replacement therapy using recombinant human mannan-binding lectin is possible ,and considerations are made, whether replacement therapy could benefit different patients groups.[6] MBL replacement therapy is not likely to decrease the risk of sepsis in AML patients.

Table 1.

	High-functioning Genotypes (YA/YA; YA/XA)	Intermediate-functioning Genotypes (XA/XA; YA/YO)	Low-functioning Genotypes (YO/YO; XA/YO)	
Culture-positive Sepsis episodes	132	74	40	246
Culture-negative Febrile episodes	188	116	53	357
	320	190	93	603

p=0.79 (χ^2-test)

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0614

PREDOMINANCE OF GRAM-NEGATIVE BACTEREMIA IN FEBRILE NEUTROPENIA EPISODES: AN EPIDEMIOLOGIC EVALUATION IN A BRAZILIAN HEMATOLOGIC SERVICE

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Background. Some studies have shown a predominance of gram-positive bacteremia in febrile neutropenia patients. Potential reasons include the use of indwelling catheters, local environmental conditions and the administration of specific antibiotic agents, especially prophylaxis. Some reports have shown that weather's characteristics could influence the patterns of infection. Brazilian weather is very heterogeneous varying a lot along its vast territory. Therefore, there is no consistent data about Brazilian febrile neutropenia's epidemiology. *Methods.* We retrospectively analyzed a total of 92 patients, which were divided in two groups: myeloid neoplasia (69 patients) and lymphoid neoplasia (23 patients). *Results.* Between 2000-2006, we analyzed 266 febrile neutropenia episodes. The median age of the patients was 37.5 (16-81) years-old. In 36% of cases, there was at least one organism (bacteria or fungal) isolated in blood culture. Gram-negative bacteremia was identificated in 65% of these cases and the most frequent gram-negative bacterias were Escherichia coli (31%) and Klebsiella pneumoniae (23%). The resistance of these two bacterias to cefepime, which is the standard empirical treatment in our service, was 13% and 81%, respectively. Gram-positive bacterias and fungal were isolated in blood cultures in 31% and 14% of cases, respectively. Staphylococcus epidermidis was the most frequent gram-positive bacteria (9%). In 8% of cases, more than one organism were isolated and gram-negative bacterias predominated in these episodes (81%). There was in 62% of episodes the presence of at least one identifiable site of infection. Pneumonia and skin infection were the most common site of infection (31% and 24%, respectively). There was no difference in the frequency or resistance of the organisms between the myeloid and lymphoid neoplasia groups. 15% of patients died during the febrile neutropenia episode. The most important factors influencing the overall survival in an univariate analysis were diagnosis of a myeloid neoplasia (OR:5.86, p=0.0002), bacteremia by Klebsiella pneumoniae (OR: 4.40, p=0.012), the presence of pneumonia (OR: 4.37, p=0.0001), age ≥ 65 years-old (OR: 3.6, p=0.015), the presence of a gram-negative bacteremia (OR: 2.27, p=0.04), the presence of at least one identifiable site of infection (OR: 2.23, p=0.049). *Conclusions.* These results differ from other ones coming from developed countries (Kanamuru and Tatsumi CID 2004, Ramphal CID 2004), where the gram-positive bacterias are more frequently isolated in blood culture during febrile neutropenia episodes. One explanation for our findings is the fact that we do not usually use ciprofloxacin as a primary prophylaxis. However, recently, some studies showed a shift towards gram-negative bacteremia during febrile neutropenia (Guven Support Care Cancer 2006). Therefore, our results reinforce the concept that antibiotic therapy for febrile neutropenia should be adapted to the local epidemiologic data.

0615

COST-EFFECTIVENESS OF PEGFILGRASTIM VS. FILGRASTIM PRIMARY PROPHYLAXIS IN PATIENTS WITH NON-HODGKINS LYMPHOMA RECEIVING CHOP-21 IN FRANCE

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Background. Primary prophylaxis (starting in the first chemotherapy cycle and continuing for all subsequent cycles) with granulocyte-colony stimulating factors (G-CSF) is recommended in the 2006 ASCO and EORTC guidelines for patients with a risk of febrile neutropenia (FN) equal to or greater than 20%. Both filgrastim (the first-generation G-CSF, daily injections) and pegfilgrastim (the second-generation, pegylated version of G-CSF, once per cycle) are commonly used. However, data from certain clinical trials suggest that pegfilgrastim was associated with a lower risk of FN compared with filgrastim. Moreover, in clinical practice filgrastim has often been used for shorter-than-recommended courses of administration; this has been shown to be associated with less clinical benefit. *Aims.* We evaluated the cost-effectiveness of pegfilgrastim vs. filgrastim used for 11 days (as used in the randomised trials demonstrating efficacy) and 6 days (as often used in clinical practice) in patients with aggressive NHL receiving CHOP-21 in France. *Methods.* A decision-analytic model was constructed from a healthcare payer's perspective. The study time horizon was life-time. Model inputs, including FN risk (varied by days of filgrastim use), FN case-fatality, relative dose intensity (RDI), and impact of RDI on survival were based on a comprehensive literature review and expert panel validation. Costs were acquired from official price lists or literature and included drugs, drug administration, FN-related hospitalisations, and subsequent medical costs. NHL mortality and all-cause mortality were obtained from official statistics. Using data from a meta-analysis (pegfilgrastim vs. 11 days of filgrastim) and from observational studies (pegfilgrastim vs. 6 days of filgrastim), we estimated that the absolute risk of FN in patients receiving pegfilgrastim decreased by 6.5 percentage points (19.6% vs. 13.1%) vs. 11-day filgrastim, and by 12 percentage points (25.1% vs. 13.1%) vs. 6-day filgrastim. Next, we estimated the impact of a difference in FN risk on FN-related mortality, RDI, and long-term survival. Sensitivity analyses were used to assess the model robustness. Outcomes were measured as incremental cost-effectiveness ratio (ICER) including € per percentage (absolute) FN risk decreased, € per life-year gained (LYG), and € per quality-adjusted life-year (QALY) saved. *Results.* Pegfilgrastim was cost saving compared with 11-day filgrastim (€10,457 vs. €13,081 for pegfilgrastim vs. filgrastim). Compared with 6-day filgrastim, pegfilgrastim was associated with ¢226 per additional 1% decrease in absolute risk of FN. Pegfilgrastim achieved 7.57 LY (6.51 QALY) as compared to 7.45 LY (6.41 QALY) from filgrastim at a moderate cost increase of €2,707 per person, yielding an ICER of €24,387/LYG (€27,214/QALY saved) (Table 1). Results were sensitive to the assumption of RDI impact, relative risk of FN for 6-day filgrastim vs. pegfilgrastim and study time horizon. *Summary and conclusions.* In France, pegfilgrastim was less expensive than 11-day filgrastim. Pegfilgrastim resulted in a gain in life-years with a moderate cost increase compared with filgrastim used for 6 days per CHOP-21 chemotherapy cycle.

Table 1. Cost-effectiveness of pegfilgrastim vs. 6-day filgrastim.

0616

CAPILLARY LEAK SYNDROME IN HEMATOLOGICAL PATIENTS: UNICENTRIC PROTOCOL FOR MANAGEMENT AND TREATMENT

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Capillary leak syndrome (CLS) is an uncommon (< 10%) early complication of hematopoietic stem cell transplantation (HSCT) or intensive chemotherapy caused by the injury of the cytokines against the vascular endothelium, with loss of sodium, albumin and fluids into the interstitial space. The clinical features of CLS are: weight gain (>4% in 24h), generalized oedema, hypotension and renal insufficiency of pre-renal origin. The CLS is related to a high risk of mortality due to frequent progression in a multiorgan failure (MOF). In order to evaluate if an early treatment has a prognostic impact, we designed a prospective protocol: all patients undergoing autologous or allogeneic HSCT or intensive chemotherapy for acute leukemia or bulky lymphoma were monitored every 6 hours for weight, arterial pressure and fluid balance from the beginning of chemotherapy until 16 days after the end of chemotherapy or pre-transplant conditioning. In presence of > 2% weight gain and/or 1000 cc positive fluid balance at 6 h, furosemide was given at dose of 0.4 mg/kg. After 1 hour, if patient didn't responde to furosemide (normalization of weight and fluid balance), 100 cc of 18 % mannitolo and after 30 minutes 125 mg of furosemide were administered. In case of a persisting negative response, we began CLS therapy consisting of dopamine (4 ug/kg/min), prednisolone (1 gm/kg) and furosemide (250 mg by continuous infusion) for 24 hours. After 24 hours, if the response to therapy was positive, the pt continued the same therapy until the stabilization of the clinical picture; in the other case we began the continuous hemofiltration until the normalization of clinical parameters. From January 2004 to July 2006, 10 patients met the diagnostic criteria for CLS (2 HSCT: allogeneic n=2, autologous n=3; bulky NHL n= 2; AML: n=3) . The 5 transplanted patients responded to the treatment with dopamine and prednisolone (PDN) with resolution of all symptoms within 24 hours. Two of 3 AML patients not responding to dopamine and PDN underwent hemofiltration within 24 hours with complete resolution of symptoms after a median time of 72 hours (range 48-96 h) . Three patients (2 LNH and 1 AML), not responding to the first line therapy, for clinical and logistic reasons, underwent hemofiltration later than 24 h: all 3 patients progressed in a MOF and died. Altought limited to a small number of cases, from our experience we can draw the preliminary conclusion that our protocol seems to be effective 1) in monitoring patients at risk for developing CLS and 2) in permitting an early diagnosis and treatment of CLS. Furthermore, our study suggests that hemofiltration within 24 hours could be a crucial intervention for patients not responding to the first line therapy.

0617

INFECTIOUS COMPLICATIONS IN REDUCED INTENSITY STEM CELL TRANSPLANTATION PATIENTS

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Background. Infections are a common complication of allogeneic stem cells transplantation (SCT) and contribute significantly to transplant-related morbidity and mortality. It has been hypothesized that transplantation following reduced intensity conditioning (RIC) would result in fewer infections by causing less mucositis, shorter duration of neutropenia and allowing earlier immune reconstitution. *Aims.* We aimed to evaluate the infectious transplant-related mortality in patients which received a RIC in our center. *Methods*. We have retrospectively reviewed the data of 117 consecutive patients (pts) with hematological malignancies or solid tumors that underwent an RIC allogeneic transplantation from November 1999 until November 2006. The conditioning regimen included Fludarabine in 109 pts (93,16 %)+TBI2Gy, Busulfan, ATG, Endoxan, Ida, Ara-C or Melphalan. 81,19% pts received SCT from an identical sibling and 18,8% pts from an MUD. Post transplant immunosuppression consisted in CSA and/or MMF or short course MTX. The patients did not receive antibacterial prophylaxis or systematic oral digestive decontamination. Pneumocystis carinii prophylaxis consisted of trimethoprim/sulfamethoxazole and systemic antifungal prophylaxis by fluconazole. The CMVpp65 antigenemia assay was used to monitor the pts. All patients with a positive antigenemia received pre-emptive therapy (ganciclovir, foscavir or valganciclovir). *Results.* Cytomegalovirus (CMV) infection occurred in 32 pts (36.78% of the 87 pts at risk of reactivation - donor, recipient or both having a positive CMV serology) and CMV pulmonary disease in only 1 pt (1.14%). There is a strong relationship between CMV infection and GVHD. The rate of CMV reactivation was of 50% for the pts at risk that developed a GVHD, and only 27% in the absence of GVHD. First episode of CMV infection occurred at day 39(range 30-180), and 7 pts presented more than one episode. Only one pt died because of CMV pulmonary disease. We noted a trend to a lower lymphocyte count at day 30 and at day 100 in pts reactivating CMV compared to the pts at risk that did not reactivate de CMV. A sustained absolute neutrophil count of >0,5 x 109/l was reached at a median of 18(range 1-37) days. 29(24,8%) patients experienced one or more episodes of bacteriemia - 10 pts (8,5%) during the first 30 days post transplant, 14 pts (12%) from D 30 to D 100, 15 pts (12,8 %) from D100 to one year post transplant, most of them due to coagulase-negative staphylococci (55%). Gram negative bacteraemia accounted for only 21,6% of the events. Pneumocystis carinii pneumonia occured in 3 pts, cerebral toxoplasmosis in 1 pt, and were concomitant with GVHD. Five patients (4,2%) presented new onset invasive aspergillosis in the post transplant period (1 proven, 4 probable). Of note, two other pts that had documented fungal infection before SCT (1 proven pulmonary and intestinal aspergillosis, and 1 probable pulmonary aspergillosis) and received voriconazole prophylaxis did not reactivate the aspergillosis after the transplant. At the time of analysis 50 patients died during the follow-up period and 67 are still alive with a median follow up of 23 months (3-88,5). The majority of deaths (34%) were directly attributed to disease progression or relapse. Only 6 deaths (5.12%) were directly attributed to infection as the principal cause of death. 6 patients died because of uncontrolled GVH and documented infection was a contributively cause of death for 2 patients in the setting of the GVHD. *Conclusion*. Our results suggest that RIC SCT is associated to a low incidence of bacterial, viral and fungal infection. The incidence of documented fungal infections compares favorably with the already published data. In our patients the infectious transplant-related mortality was low. Whether these findings would translate into an improved overall survival needs to be confirmed in further prospective studies.

0618

ADDITION OF CLADRIBINE TO DAUNORUBICIN AND CYTARABINE HAS NO IMPACT ON INFECTIOUS COMPLICATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA. A MULTICENTER, RETROSPECTIVE STUDY BY THE POLISH ADULT LEUKEMIA GROUP

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Background. Infectious complications occur in most of the patients with acute myeloid leukemia (AML) receiving the induction therapy. It was reported that addition of cladribine to daunorubicin and cytarabine increases complete remission rate after a single course of induction treatment in AML patients (Leukemia 2004). However, the use of purine analog cladribine may increase the risk of infection due to the prolonged T-lymphocyte depletion produced by this agent. *Aims*. We sought to determine whether the induction therapy with cladribine, daunorubicin and cytarabine (DAC-7 regimen) compared to the standard induction treatment with daunorubicin and cytarabine (DA-7 regimen) had an impact on the incidence and spectrum of infections among a series of untreated adult patients with AML. *Methods.* Three hundred nine AML patients were enrolled into a multicenter, retrospective study. Patients were randomized either to DA-7 (n=157) or DAC-7 (n=152) induction treatment. Differences in the infection rate among treatment arms were tested with the Kruskal-Wallis, Wilcoxon, and chi2 tests. *Results.* A total episodes of 422 infections occurred in 309 patients over the period of the induction treatment. Two hundred eight infections were of grade III/IV according to the WHO classification. There was no difference in over-

all infection rate (p=0.12) as well as grade III/IV infection frequency (p=0.23) among patients treated with DA-7 or DAC-7 regimen. Microbiologically or clinically documented bacterial infections occurred in 127 (41%) patients, including 67 (43%) DA-7 patients and 60 (39%) DAC-7 patients (p=0.5). A total of 71 (23%) episodes of bacteremia were found, including 60% Gram-positive, 30% Gram-negative, and 10% mixed blood cultures. Forty-two (27%) patients in the DA-7 group and 29 (19%) patients in the DAC-7 group had bacteremia (p=0.06). Microbiologically documented fungal infections occurred in 42 (13%) patients, including 16 (10%) DA-7 patients and 26 (17%) DAC-7 patients (p=0.06). A total of 5 (2%) episodes of fungemia were reported and its frequency was similar in both study arms. Viral infections were observed in 35 (11%) patients and it did not occur more often in patients receiving DAC-7 therapy when compared to patients treated with DA-7 regimen (p=0.6). At univariate analysis, AML subtypes according to FAB classification, preceding myelodysplastic syndrome, age, sex, induction treatment arm, response to induction therapy, the presence of Hickman's catheter, and the use of antimicrobial prophylaxis were not significantly associated with the risk of infection. In all, 11 (7%) patients died of infection'related complications in the DA-7 arm compared to 17 (11%) in the DAC-7 arm (p=0.4) *Conclusions.* Addition of cladribine to daunorubicin and cytarabine did not result in significantly more infectious complications than standard treatment with daunorubicin and cytarabine in adult AML patients.

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ACICLOVIR PROPHYLAXIS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Herpes viruses establish a latent phase following primary infection. Viral reactivation occurs in more than 30% patients following allogeneic stem cell transplant. Most of cases occur between 3-12 months after transplantation. Current evidence suggests that infection rates can be decreased by giving prophylaxis however there is no consensus on the duration of prophylaxis and doses. Our previous audit study (2001 -2003) showed that the administration of prophylaxis until neutrophil engraftment left patients at risk of viral reactivation occurring in 76% of allograft recipients leading to considerable morbidity and healthcare cost. It was shown that these reactivations occurred during the period of time prior to the recovery if CD4 count to a level of >300/mm^3. The audit outcome led to a decision to extend the duration of prophylaxis until CD4>300/mm^3, by doing so we hoped to considerably decrease infection rates. *Aim*. The first aim of the study was to assess compliance in prescribing to our own standard.We also needed to re-audit to assess whether the change in standard resulted in reduction in herpes virus reactivation rates as predicted from our previous study. *Methods.* This was a single centre, retrospective study conducted at Birmingham Heartlands Hospital with a study period between January2004 -January 2006. All patients who survived >100days post-transplant were included. The standard against which we assessed our practice was our own JACIE standard operating protocol for prevention of infection post transplantation (Joint accreditation committee of institute of cellular biology and EBMT). The protocol stated to continue aciclovir prophylaxis at a dose of 200mg bd until CD4 count reaches 300/mm^3.Source data was obtained from the transplant database, case notes, drug charts and confirmatory virology reports.

Figure 1. Shows CD4 and HSV/VZV infection.

Results. In the current study there were twenty eight patients who underwent allogenic transplant during the study period, five were excluded due to transplant related mortality (n=23). Sixteen were Sibling transplant and seven MUD.The median duration for of follow up was 442 days and median duration of prophylaxis was 348 days. During this period there were 6/23 (26%) episodes of HSV/VZV infections (Figure 1). Compliance was seen in 21/23 patients (91%). Non-compliance with prescribing resulted in one episodes each of HSV and VZV infection prior to the target CD4 count being achieved. Four episodes of viral reactivation occurred in patients with CD4>300/mm^3. Of these two patients had disseminated VZV infections resulting in hospitalisation and intravenous aciclovir therapy. Three individuals have since suffered from recurrent episodes of HSV/VZV and are on long term prophylaxis in spite of attaining a CD4>300/mm^3. *Summary and Conclusions*. In our previous study there were sixteen episodes of HSV /VZV reactivation (n=21). In comparison we had only six in our present study. There was also a reduction in recurrent episodes from six (n=21) to four (n=23). We have achieved a significant reduction in morbidity due to re-activation of VZV and HSV infections in allogeneic bone marrow transplant patients by our approach.

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HAEMATOLOGICAL FEATURES OF LEISHMANIASIS RESEMBLING BLOOD-RELATED MALIGNANCIES: THE CONCERN OF A DIFFERENTIAL DIAGNOSIS THROUGH THE PRESENTATION OF SIX CASES

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Background. Leishmaniasis is a chronic infectious disease from the group of anthropozoonoses. It is caused by protozoa in the genus leishmania flagellate. Infection is transmitted by insects; Mediterranean countries are one of the major foci of this disease in the world. The course of the disease may be acute, subacute and chronic and several forms are differentiated such as visceral, cutaneous and mucocutaneous. The diagnosis is established by serologic tests; however, parasitological findings in macrophages of the bone marrow (BM) can be observed. Several signs of disease, such as elevated temperature, gastrointestinal disorders, splenomegaly and hepatomegaly, generalized lymphadenomegaly and pancytopenia, may resemble several haematologic malignancies, for which some challenging concerns may arise in the differential diagnosis, as recently observed by us in a series of six cases that is hereby reported. *Case series.* There were 6 patients (3 male) with a median age of 34 (20-60) years. In all patients the symptomatolgy started gradually with uncharacteristic manifestations, such as fatigue, gastrointestinal discomforts, loss of appetite, nausea, and vomiting, accompanied by fever. Temperature increased twice a day and was followed by shuddering and very often by nocturnal sweating. Antibiotics and antipyretics were used without any benefits. Subjective discomforts were increasingly pronounced, so that due to unclear febrile state and in addition to the present pancytopenia for which patients kept under our attention. On the physic examination all presented splenomegaly and three presented lymphadenopathies. The laboratory findings pointed to pancytopenia with lymphocytosis and monocytosis; moreover, important polyclonal hypergammaglobulinemia ranging from 2 to 6 gr (median 4 gr.) was found. BM aspirates and trephine biopsies were performed in all patients. Hypocellularity was found in all cases; moreover, in three patients the examination of BM smears revealed 15% to 25% polyclonal lymphocytes infiltrating the BM. Lastly, both intra- and extracellular protozoa resembling leishmaniasis were detectable in three cases. In the remaining cases, no intramacrophagic or extracellular protozoa were found for which the diagnosis was suspected and then confirmed by serologic tests after the evaluation of all other diagnostic tests in order to exclude an hematologic malignancy that were into normal values. After the establishment of diagnosis, patients received causal therapy with liposomal amphotericin B and rapidly recovered; the decrease of splenomegaly as well the reduction of hypergammaglobulinemia and the improvement in haematological findings were observed. *Conclusions.* Hematologic findings which are characteristic for leishmaniasis may resemble some of those associated with haematologic malignancy, such as lymphomas and acute leukemias; however, in the presence of polyclonal hypergammaglobulinemia, fever, splenomegaly and pancytopenia, this infectious disease should be suspected and specific serologic tests should be done together a BM examination.

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ACICLOVIR-INDUCED NEUROTOXICITY IN HEMATOLOGICAL PATIENTS. MEASUREMENT OF THE ACICLOVIR MAIN METABOLITE CMMG MAY DIFFERENTIATE BETWEEN ADVERSE DRUG REACTIONS AND HERPES INFECTIONS

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Background. The antiviral drug aciclovir and its prodrug valaciclovir is commonly used to treat herpes virus infections in hematological patients. Both drugs have in general low toxicity with GI-disturbances and headache as common side-effects. However, CNS-symptoms such as tremor, confusion, hallucinosis and coma have been reported, mainly in patients with chronic or acute renal failure (ARF). The CNS side-effects may confuse the clinical picture and make it difficult to distinguish between adverse drug reactions (ADRs) and viral infection. It may result in increased doses of aciclovir instead of withdrawal of the drug. Other drugs used in hematology patients may also interact on aciclovir excretion in the kidneys and increase the risk for high aciclovir concentrations, resulting in acute renal failure, followed by CNS-symptoms. We have previously shown that the aciclovir main metabolite 9-carboxymethoxymethylguanine (CMMG) is consistently increased in serum and CSF in patients with aciclovir-related CNS-symptoms and that CMMG might have importance as a marker of aciclovir-induced neurotoxicity. *Aims.* To increase the knowledge on aciclovir-induced toxicity in hematological patients. *Methods.* We present 3 haematological patients, one with myeloma, one with a mantel cell lymphoma and one bone marrow transplanted (BMT) patient with chronic myeloid leukaemia which developed severe aciclovir-related CNS-toxicity despite doses according to the Swedish Physicians Desk Reference. Aciclovir and CMMG concentrations were measured in two of the patients. *Results.* The BMT patient developed acute renal failure, nightmares and depression after institution of valaciclovir 500 mg b.i.d. Aciclovir and CMMG concentrations 16 hrs after last dose were 39 and 29 μmol/L, respectively (normally <10 μmol/L and <6 μmol/L 8 hrs after dose). One myeloma patient on hemodialysis developed confusion, agitation, slurred speech, somnolence and myoclonus on oral aciclovir 200 mg q.i.d. No concentrations was analysed in this patient. The patient with mantel cell lymphoma was treated with IV aciclovir 250 mg b.i.d. and developed ARF, confusion, disorientation, psychosis and somnolence. The aciclovir and CMMG concentrations 72 hrs after last dose were 6.0 and 6.0 μmol/L, respectively, indicating high concentrations 8 hours after last dose. One patient improved after a hemodialysis session, one developed multiorgan failure and died of AMI a few days after the aciclovir-intoxication despite hemodialysis and the last patient improved three days after withdrawal of aciclovir. *Summary and Conclusions.* Aciclovir- or valaciclovir-induced CNS-symptoms and ARF are important ADRs in hematological patients and may be more frequent than previously recognized. Measurement of CMMG seems to be a useful marker of aciclovir toxicity and might function as a diagnostic tool to discriminate between aciclovir-induced ADRs and herpes virus infections.

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MINI EPIDEMICS OF SYSTEMIC TRICHOSPORON MUCOIDES INFECTION IN NEUTROPENIC PATIENTS WITH LEUKEMIA

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Background. Disseminated infection caused by Trichosporon spp. is one of the emerging mycoses in neutropenic patients. Several case reports so far describe individual cases of systemic Trichosporon spp. infections. Generally this infection carries a poor prognosis. Here we reported five consecutive cases of systemic Trichosporon mucoides infection in a short period of time in neutropenic patients. At our knowledge this is the first report of epidemic appearance of systemic Trichosporon infections. Patients: During the 4 month period Trichosporon mucoides were isolated from blood cultures in 5 patients at the same ward. There were 3 males and 2 females, 42-57 years old. Four patients were diagnosed with AML, and one had CML in transformation. All patients received induction chemotherapy and were severely neutropenic with ANC $<0.1\times10^9$/L. All but one patient received antifungal prophylaxis with 300 mg fluconazole orally. Patients presented with general symptoms of severe infection that was not responding to standard antibiotic therapy three of them with severe leg pain. Four patients had characteristic papulous skin lesions with central necrosis. Biopsy of lesions yielded granulomatous inflammation with marked central necrosis and microbiology was positive for T. mucoides in all patients. Four out of five patients had pulmonary infiltrates, and two of them had pleural effusions. Three patients had liver focal lesions, the largest measuring up to 1.8 cm, accompanied with elevated liver enzymes; two of them had also spleen infiltrates. Liver biopsy, done in one patient showed only inflammatory mononuclear infiltration, microbiology remained negative. In all patients initial therapy consisted of Amphotericin B, one patient responded favorably. In other four patients signs of infections or blood cultures remained positive despite Ampho B or Caspofungin therapy in one patient. According to data on *in vitro* susceptibility of T. mucoides voriconazole (6mg/kg loading dose, 4 mg/kg continuing) was introduced. Four to 12 days later patients became afebrile with the regression of pulmonary infiltrates and pleural effusion. The recovery of neutrophil count correlate with regression if infection. Patients continue to receive voriconazole orally until discharge. All patients survived. They all received further intensive chemotherapy, one of them allergenic stem cell transplant without developing Trichosporon infection with the secondary prophylactic use of voriconazole therapy. Although the source of the epidemic at the hematology ward remained obscure, it seems that water or sewage system could be responsible because Trichosporon mucoides were revealed in almost all washstands in reverse isolation rooms were patients were maintained. The department was closed and cleaned after that no cases of systemic Trichosporon infections appeared. *Conclusions.* This report indicates possibility of epidemic appearance of systemic Trichosporon spp. infections and illustrates that voriconazole is efficient in the treatment of disseminated Trichosporon mucoides infections in neutropenic patients as well as in secondary prophylaxis in these patients.

Myelodysplastic syndromes II

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MDS PATIENTS WITH ANTI ERYTHROBLAST AUTOIMMUNITY: EFFECT OF BM CULTURE SUPERNATANTS ON CLONOGENIC ACTIVITY OF NORMAL BONE MARROW

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Background. Myelodysplatic syndromes (MDS) are characterised by ineffective and dysplastic hematopoiesis, bone marrow hypercellularity and, paradoxically, progressive peripheral blood cytopenias. Autoimmune phenomena, mainly directed against RBC, are thought to play a role in bone marrow failure of early MDS, i.e. refractory anemia (RA) and RA with ringed sideroblasts (RARS). We showed an autoimmune reactivity against erythroblasts in roughly half patients with RA and RARS by a method named mitogen-stimulated-direct antiglobulin test (MS-DAT). The same patients displayed increased erythroblast counts together with peripheral signs of hemolysis. *Aims.* To study the effect of BM culture supernatants from MDS patients with anti erythroblast autoimmunity on clonogenic activity of normal BM *Methods.* MS-DAT was performed by stimulating BM cells with PMA and PHA and antibodies were detected in supernatants by competitive solid phase ELISA. Culture supernatants from BM MS-DAT positive and negative patients were tested on the growth of normal BM progenitors on methylcellulose medium. Colony forming units (CFU) count and morphological evaluation were performed after 14 days of culture. Smears were obtained from CFU and differential counts performed. *Results.* The morphology and the number of colony forming units granulocyte-monocyte (CFU-GM) and burst forming units erythroid (BFU-E) were comparable in cultures performed with or without BM MS-DAT positive supernatants. Likewise, control experiments with BM MS-DAT negative supernatants gave negligible effect on the number of CFU-GM and BFU-E colonies. Addition of BM MS-DAT positive supernatants increased the overall CFU smears cellularity along with the appearance of diserythropoietic 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. Regarding the differential BM counts (percentage of myeloid and erythroid precursors) we did not observe major differences in the presence or absence of various supernatants. *Conclusions.* BM culture supernatants from MDS patients with an autoimmune reactivity against erythroblasts induced normal BM progenitors to an hyperplastic and diserythropoietic growth *in vitro*, without a preferential destruction of a cell subset or a blockade in red cell hematopoietic maturation.

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RELATIVE DISTRIBUTION OF DIFFERENT COMPARTMENTS OF CD34+ CELLS IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Introduction. Despite the value of the overall number of CD34$^+$ cells in the bone marrow (BM) of patients with myelodysplastic syndromes (MDS), currently information about the distribution and behaviour of the different compartments of CD34$^+$ cells in MDS patients remains limited. *Aims.* The aim of the present study is to analyze the relative distribution of different compartments of immature, myeloid and lymphoid CD34$^+$ cell precursors in the BM of patients with MDS and correlate it with the different diagnostic and prognostic subtypes of the disease. METHODS. Overall, 73 BM samples were analyzed corresponding to 23 normal BM samples (NBM), and 50 from patients with newly diagnosed MDS classified according to WHO and IPSS criteria into: refractory anemia (RA) (n=11), refractory cytopenia with multilineage dysplasia (RCMD) (n=9), RA with excess of blasts (RAEB)-1 (n=13), RAEB-2 (n=10), unclassifiable MDS (UNC) (n=2) and myelodysplastic/myeloproliferative disorders (MDS/MPD) (n=5). Low risk (LOW) (n=12), intermediate risk (INT)-1 (n=14), INT-2 (n=9), and high risk (HIGH) (n=4). Analysis of CD34$^+$ cells was performed using a standard 4-color flow cytometry approach for the discrimination of immature precursors (ImP), neutrophil lineage committed CD34$^+$ cells (NP), and B-lymphoid precursors (BCP) within CD34$^+$ BM cells, based on their different light scatter characteristics and expression of CD45, CD34, and cytoplasmatic Myeloperoxidase (cyMPO). In addition, CD34$^+$ precursors phenotypically committed to other less represented cell lineages were also identified using a large panel of monoclonal antibodies: CD34$^+$ plasmacytoid dendritic cell (pDCP), monocytic, basophyl, erythroid and mast cell precursors. *Results.* Overall, the three major compartments of CD34$^+$ cells were identified in every NBM sample analyzed, whereas ImP and NP were detected in 59% and 100% of low grade MDS and in 15% and 85% of high grade MDS, respectively. As compared to NBM, an increased percentage of ImP and a significant decreased number of BCP was detected in RAEB-1 (p=0.003 and p<0.001, respectively), RAEB-2 (p=0.009 and p<0.001, respectively), INT-1 (p=0.02 y p=0.004), INT-2 (p=0.004 y p<0.001) and HIGH risk (p=0.02 y p=0.002) categories of MDS. In addition, the percentage of NP was also significantly decreased among AREB-1(p=0.01) patients. In turn, CD34$^+$ plasmacytoid dendritic cell precursors were significantly decreased in all MDS subgroups and lower percentages of CD34$^+$ monocytic, basophyl and erythroid lineage precursors were also detected in advanced MDS. *Conclusions.* In summary, our results show the existence of significant abnormalities in the distribution of the different major and minor compartments of CD34$^+$ cells in the BM of MDS patients. The decreased frequency of CD34$^+$ precursors committed into different hematopoietic cell lineages among advanced MDS patients points to the occurrence of a more pronounced maturation blockade of CD34+ cells in the BM of these patients.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME AND AML AFTER MDS (SAML): SUPERIOR RESULTS WITH REDUCED INTENSITY VERSUS STANDARD CONDITIONING

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Background. Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal stem cell disorders characterized by peripheral cytopenias which can progress into secondary acute myelogenous leukemia (sAML). Allogeneic stem cell transplantation (SCT) is considered the most effective treatment for patients with MDS and sAML, but regimen-related toxicities and comorbidities of these generally elderly patients have limited conventional conditioning regimens to selected patients. This limitation has partially been overcome by the development of reduced-intensity conditioning. *Aims.* Here we retrospectively evaluated efficacy and toxicity of SCT after standard myeloablative (S) or FLAMSA-reduced intensity conditioning (F-RIC) for MDS and sAML. F-RIC was chosen as a reduced, but still myeloablative regimen. *Patients and Methods.* Between 1986 and 2006, 66 patients with MDS (n=21) or sAML (n=45) were transplanted at our institution. 32 patients received standard conditioning (Bu/Cy+/-other or TBI/Cy+/-other) whereas 34 patients were treated according to the FLAMSA protocol combining chemotherapy with reduced intensity conditioning (Schmid et al.; J Clin Oncol 2005;23:5675-5687). 7 MDS patients had an IPSS score of intermediate-2 or high, and 7 had secondary MDS mostly with unfavorable cytogenetics. Of the 45 sAML patients, 17 were transplanted in 1st or higher CR, 19 patients were refractory (relapse or induction failure) and 9 patients untreated. *Results.* The F-RIC group included patients with a higher risk profile according to age (S: median 43, range 21-61; F-RIC: 57, 30-71), refractory status of sAML prior to SCT (S 9%; F-RIC 47%), unrelated donors (S 25%; F-RIC 79%) and mismatch donors (S 6%, F-RIC 26%). Median follow-up in the S group was 14 months, range 0,1-236 and in the F-RIC group 10 months, range 0,6-27. At 1 year, overall survival (OS) was 69% and 54% in the F-RIC and S group, respectively. Also it seems after this short follow-up that in the F-RIC group OS was superior after HLA-identical sibling SCT (100%) than after MUD-SCT (60%), however, both groups were heterogeneous with regard to risk factors. Treatment related mortality was higher after standard therapy (S 56%; F-RIC 3%) but relapse rates were similar for both regimens so far (S 16%; F-RIC 18%). Since both groups differed according to the percentage of unrelated donors, all relapses which occurred in the standard group were in HLA-identical sibling SCTs vs. no relapse in the F-RIC group. In addition, engraftment in F-RIC was 100%, whereas in standard group early death (n=4) or engraftment failure (n=3) was seen in 22%. With the limited follow-up, F-RIC also compared favorably to standard conditioning for aGvHD (44% vs. 53%) and severity of aGvHD grade III-IV (7% vs. 53%). *Conclusions.* F-RIC for allogeneic SCT apparently has acceptable toxicity and a sufficient antileukemic efficacy for high risk pts. with MDS and sAML. Longer follow-up and prospective trials are needed to define its therapeutic benefit and the optimal timing for MDS and sAML patients.

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FLT3 EXPRESSION AS A MARKER OF PROGRESSION AND RESPONSE TO TREATMENT IN MYELODYSPLASTIC SYNDROMES

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Upon acute myeloid leukemia (AML) progression one third of MDS patients acquire an internal tandem duplication (ITD) or a point mutation of the fms-like tyrosine kinase 3 (FLT3) gene and both these mutations determine an increase of FLT3 expression. Based on this data, the present study is aimed at determining any correlation betyween FLT3 expression and clinical parameters and at estimating the influence of FLT3 expression on the risk of MDS/AML progression and on the probability of achieving any response after intensive chemotherapy. The 22 patients entered in the study were 9 females and 13 males with a median age of 61 years (range 18-76). According to FAB classification five patients were classified as RARS, eleven as RA, six as RAEB. When WHO classification was applied, four patients were diagnosed as RARS, four as RA, two as 5q- syndrome, six as RCMD-RCMDS, two as RAEB-1 and four as RAEB-2. According to IPSS score, eleven patients were classified as low-risk, six as intermediate-1 risk, three as intermediate-2 risk and two as high-risk. Eleven patients (2 RA, 3 CRMD, 2 RAEB-1 and 4 RAEB-2) progressed into AML after a median follow-up time of 21 months (range 8-43). Cytogenetic analyses and real-time PCR were performed on clinical diagnosis and during the follow-up. Chromosome analyses revealed a normal karyotype in sixteen patients, del(5q) in two, del(20q) in three and del(12p) in one. The PCR for FLT3 relative quantification employed SybrGreen I as DNA-binding fluorescent dye. Standard curve for real-time quantification was obtained by serial dilution of total RNA isolated from mononuclear cells collected from an AML patient, exhibiting FLT3 ITD and an increased expression of FLT3 mRNA. Gene expression was calculated by the delta-deltaCt method. FLT3 levels were normalised to ABL and calibrated on a normal sample. On clinical diagnosis the expression levels of the FLT3 gene were similar to that of normal sample in seventeen patients and were two-four times higher than that of the normal sample in four patients (one RAEB-1 and three RAEB-2). When AML progression occurred, a total of eight patients (72.7%) presented an increase of FLT3 expression which was two-seventeen times higher than that of the normal sample. So, four patients developed high FLT3 expression just on AML evolution. Five of these eight patients were submitted to intensive chemotherapy which was able to induce a complete remission (CR) in three patients. In these last FLT3 expression reverted to normal values, whereas in non responsive patients it remained constantly elevated. All the three CR patients relapsed after twenty, eighteen and eight months respectively and again showed a quick rise in FLT3 expression which was six-eight times higher than that of the normal sample. In conclusion, i) 13.6% of our patients presented high FLT3 expression on clinical diagnosis; ii) high FLT3 expression was associated with no peculiar clinical finding; iii) a quick increase of FLT3 expression occurred in 72.7% patients in AML progression; iv) CR achievement was correlated with a normal FLT3 expression which increased upon relapse.

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FAMILIAL MYELODYSPLASIA WITH MONOSOMY 7

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Introduction. Familial occurrence of myelodysplasia (MDS) or acute myeloid leukaemia (AML) is rare but has provided a useful resource for investigation of predisposing mutations in these diseases. Germline mutations have been reported in RUNX1 in several familial MDS/AML pedigrees; but the cause remains obscure in many other families. Monosomy 7 as a cause of familial MDS/AML has been formally reported in 12 pedigrees (Minelli et al. Cancer Genetics and Cytogenetics 2001). In these cases, MDS developed at young ages with most patients presenting at less than 18 years. Most individuals were first-degree relatives and males and females were equally affected, suggesting an autosomal dominant mode of transmission. Though many of these families were investigated before the era of advanced molecular diagnostics, several groups demonstrated different parental origins of monosomy 7 in individuals within the same family. This non-preferential deletion of parental chromosomes suggests that the predisposing locus does not reside on chromosome 7. We describe an unusual case of familial MDS with monosomy 7 in 2 first-cousins who presented in their early 20s with no other family history of MDS. The individuals were investigated for a predisposing germline mutation. *Methods.* Direct PCR-sequencing (DNA based) was performed to exclude mutations in the RUNX1 gene. A genomewide Single Nucleotide Polymorphism (SNP) analysis was then performed using the Affymetrix GeneChip Human Mapping 10K Array Set. The individual samples were examined for evidence of copy number loss or gain as well as loss of heterozygosity (LOH). *Results.* Individual 1 presented at age 23 with symptomatic cytopenias. A bone marrow aspirate and biopsy demonstrated dysplasia in the erythroid, myeloid and megakaryocytic lineages and the presence of 17% myeloblasts, consistent with a diagnosis of Refractory Anemia with Excess Blasts (RAEB). Cytogenetic analysis demonstrated an abnormal clone containing 45 chromosomes with loss of one copy of chromosome 7. No other cytogenetic abnormalities were detected. Treatment was commenced with intensive chemotherapy and a complete remission (CR) was achieved but with persistence of dysplastic features in the marrow. The bone marrow sample from the time of CR revealed normal cytogenetics with no evidence of the monosomy 7 clone. Seven months following presentation, the patient relapsed with reemergence of the monosomy 7 clone. He received re-induction chemotherapy and a matched-unrelated donor haematopoeitic stem cell transplantation (MUD-HSCT) but died early of transplant-related complications. Individual 2, the first cousin of Individual 1, presented 1 week after his cousin and was similarly diagnosed with RAEB and monosomy 7. His bone marrow revealed 7% myeloblasts and monosomy 7 with no other cytogenetic abnormalities. He also underwent a MUD-HSCT but died from relapsed disease subsequently. Stored diagnostic peripheral blood samples from Individual 1 and bone marrow samples from Individual 2 were examined. No mutations were observed in exons 3-8 of RUNX1 in either individual. In order to look for accompanying events, which are not detected by conventional cytogenetics, a 10K SNP analysis was performed. The SNP genotyping demonstrated a reduced copy number for chromosome 7, confirming monosomy 7 in both cases. Additionally, an area of homozygosity of 10.5 MB was noted for Individual 1 on chromosome 6 in the region 6p22.1-6p21.2. No other deletions/amplifications or areas of LOH were noted in either individual. *Conclusions.* Monosomy 7 is a poor prognostic factor in MDS and AML. Evidence suggests that the disease-causing gene is not located on chromosome 7 but is instead thought to be a mutator gene(s) located elsewhere in the genome. We describe two cousins who presented with high-grade MDS and monosomy 7 at young ages. Though this family differs from previously reported pedigrees with Familial MDS with monosomy 7, the occurrence of MDS in young adults in uncommon and suggests an inherited predisposition to disease in this family. Our preliminary studies have excluded mutations in RUNX1. 10K SNP analysis confirms the presence of monosomy 7 and identified a region of homozygosity on 6p. Chromosomal abnormalities of 6p have been previously reported in MDS and AML. Recurrent duplications of 6p21 have also been described in secondary MDS/AML (La Starza *et al.,* Leukemia 2006). The predisposing locus in this family is now being sought.

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COMMON DNA AMPLIFICATION PATTERNS IS OBSERVED AT MDS TRANSFORMATION TO AML: A CH-CGH STUDY ON SEQUENTIAL BONE MARROW SAMPLES AT MDS DIAGNOSIS AND AML EVOLUTION

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Background. Abnormal karyotypes are identified in about 50% of *de novo* MDS patients while 30-40% of all MDS evolve to AML. Studies comparing karyotypes from patients at both MDS diagnosis and AML evolution are limited and suggest that chromosomal gains/losses rarher than translocations are implicated in AML transformation. Oncogene mutations (N-Ras, P53, FLT3) and P15ink4b hypermethylation have been associated with progression of MDS to AML but in a limited number of cases. *Aims.* To identify if a common pattern of genomic changes occur in different MDS patients during their transformation to AML. *Materials and Methods.* Bone marrow DNA was extracted from 14 patients at MDS diagnosis (RAEB I: 5, RAEB II: 8, Unclassified:1) and at AML trans-

formation (in total 28 samples). Time to AML progression was 2-40 months (mean 13 months). The methodology utilized for CGH was a variation of the basic protocol described by Kallioniemi et al (Genes Chrom C 1994;10:231-43). DNA copy number changes were described according to the ISCN (2005) guidelines. *Results.* Average number of chromosome imbalances per MDS sample was 6 (range 0-17). Average number of chromosome imbalances per AML sample was 12 (range 3-21). X and Y chromosomes were excluded from analysis. Unexpectently, a common pattern of chromosomal loci gains was observed in different MDS patients during AML transformation (Table 1) namely 20p11.2, 21q11.2, 21q21, 4q12, 16p11.2, 7q11.2, 18p11.2, 5q11.2, 20q11.2, 18q11.2, 14q13, 16q13. Chromosomal losses were less frequently detected in this series. Confirmation of Ch-CGH *Results.* In 2 patients with abnormal karyotypes (1 patient with trisomy 8 and 1 with monosomy 7) Ch-CGH showed amplification/loss of the relevant chromosomal loci. In 2 patients with 5q31 deletion in Ch-CGH was verified by FISH analysis using a 5q31 probe hybridised to bone marrow smears. In a patient with amplification of 22q11.2 in Ch-CGH 3 signals were obsereved in 44% of cell nuclei when hybridised with 22q11.2 probe. Ch-CGH results were also verified by array-CGH in a sample (Gene Chip Affymetrix). *Discussions.* The finding of the existence of common chromosomal loci gains who appear only during the transition of MDS to AML is extremely important, given the extreme clinical and genetic heterogeneity of MDS patients. Identification of oncogene(s) located in the amplified chromosomal areas that may contribute to the transformation of MDS to AML will give new therapeutic targets.

Table 1.

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INCREASED BONE MARROW ENDOTHELIAL PROGENITOR CONTENT IN MDS PATIENTS CORRELATES WITH DISEASE STAGE, AND MAY BE INVOLVED IN THE ANGIOGENESIS RESPONSE LEADING TO ACUTE LEUKEMIA

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Myelodysplastic syndromes (MDS) are bone marrow (BM) disorders considered as pre-leukemic stages; an increase in BM angiogenesis has been suggested to contribute towards leukaemia progression. However, the mechanisms that regulate the vascular increase in MDS are poorly understood. In the present study we hypothesized changes within the MDS BM microenvironment might contribute to increased BM angiogenesis, and eventually result in AML onset and progression. For this purpose, we determined the cellular content and cell apoptosis of MDS BM samples (at diagnosis), and compared these to BM samples from patients with other BM diseases (lymphomas, CLL, others). In parallel, we analysed the BM expression of factors that might contribute towards increased vascularisation and/or altered cell turnover, such as VEGF, PlGF, TNF-α and TGF-β, respectively. Our criteria for BM collection and analysis, restricted to MDS BM samples collected at diagnosis (before any therapeutic intervention), allowed us to study these parameters in 12 MDS patients and in 10 samples from patients with other BM diseases. Flow cytometry analysis of BM biopsies revealed a significant increase in the percentage of $CD34^+$, $CD117^+$ and $AC133^+$ progenitor cells which were related with MDS stages. Although MDS BM had globally a lower BM cellular content, the proportion of other cell lineages remained largely unchanged between MDS BM (at diagnosis) and other diseases. In detail,$AC133^+$ and KDR^+ cells percentage was significantly higher in MDS patients (5,6 and 1,7-fold respectively), suggesting an increase in endothelial cells or progenitors (EPC) BM content. Accordingly, the apoptotic index of $AC133^+$ cells was lower in MDS marrows (8% apoptotic $AC133^+$ cells in MDS versus 30% in other bone marrow malignancies). To understand how the MDS BM microenvironment might contribute to towards an increase in BM vasculature, the total level of BM VEGF was determined (by ELISA), and the proportion of the different VEGF isoforms was assessed (by RQ-PCR). Although the bone marrow mononuclear cell number was lower in MDS BM, VEGF levels per cell were 2-fold higher in MDS marrows than in the other disases group. Interestingly, besides a clear increase in total VEGF, it was also observed that the proportion between the VEGF isoforms changed: isoforms VEGF145 and VEGF189 increased significantly in MDS BM, and with MDS progression. The quantification of factors that might be involved in the changes in cell turnover within the MDS BM samples, favouring endothelial cell and EPC expansion in relation to other lineages, revealed that TNF-α expression was lower in MDS patients while TGF-β levels were similar in both groups, and PlGF was marginally expressed. Although obtained from a restricted group of patients, these findings suggest that an increase in the EPC pool is directly related with MDS stages, may contribute to the angiogenic profile in MDS, which in turn may lead to disrupted BM homeostasis, eventually resulting in leukemia onset and disease progression.

Myelodysplastic syndromes (MDS) are bone marrow (BM) disorders considered as pre-leukemic stages; an increase in BM angiogenesis has been suggested to contribute towards leukaemia progression. However, the mechanisms that regulate the vascular increase in MDS are poorly understood. In the present study we hypothesized changes within the MDS BM microenvironment might contribute to increased BM angiogenesis, and eventually result in AML onset and progression. For this purpose, we determined the cellular content and cell apoptosis of MDS BM samples (at diagnosis), and compared these to BM samples from patients with other BM diseases (lymphomas, CLL, others). In parallel, we analysed the BM expression of factors that might contribute towards increased vascularisation and/or altered cell turnover, such as VEGF, PlGF, TNF-α and TGF-β, respectively. Our criteria for BM collection and analysis, restricted to MDS BM samples collected at diagnosis (before any therapeutic intervention), allowed us to study these parameters in 12 MDS patients and in 10 samples from patients with other BM diseases. Flow cytometry analysis of BM biopsies revealed a significant increase in the percentage of $CD34^+$, $CD117^+$ and $AC133^+$ progenitor cells which were related with MDS stages. Although MDS BM had globally a lower BM cellular content, the proportion of other cell lineages remained largely unchanged between MDS BM (at diagnosis) and other diseases. In detail, $AC133^+$ and KDR^+ cells percentage was significantly higher in MDS patients (5,6 and 1,7-fold respectively), suggesting an increase in endothelial cells or progenitors (EPC) BM content. Accordingly, the apoptotic index of $AC133^+$ cells was lower in MDS marrows (8% apoptotic $AC133^+$ cells in MDS versus 30% in other bone marrow malignancies). To understand how the MDS BM microenvironment might contribute to towards an increase in BM vasculature, the total level of BM VEGF was determined (by ELISA), and the proportion of the different VEGF isoforms was assessed (by RQ-PCR). Although the bone marrow mononuclear cell number was lower in MDS BM, VEGF levels per cell were 2-fold higher in MDS marrows than in the other disases group. Interestingly, besides a clear increase in total VEGF, it was also observed that the proportion between the VEGF isoforms changed: isoforms VEGF145 and VEGF189 increased significantly in MDS BM, and with MDS progression. The quantification of factors that might be involved in the changes in cell turnover within the MDS BM samples, favouring endothelial cell and EPC expansion in relation to other lineages, revealed that TNF-α expression was lower in MDS patients while TGF-β levels were similar in both groups, and PlGF was marginally expressed. Although obtained from a restricted group of patients, these findings suggest that an increase in the EPC pool is directly related with MDS stages, may contribute to the angiogenic profile in MDS, which in turn may lead to disrupted BM homeostasis, eventually resulting in leukemia onset and disease progression.

0630

AUGMENTED *IN VITRO* SENSITIVITY OF MDS PROGENITORS TO THE FARNESYLTRANSFERASE INHIBITOR TIPIFARNIB

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Background. Farnesyltransferase inhibitors (FTIs) target, amongst other proteins needing prenylation for functioning, activating ras mutations which occur in up to 20% of myelodysplastic syndrome (MDS) patients. Tipifarnib (Zarnestra®), a potent and specific inhibitor of farnesyltransferase R115777 showed activity in two phase II studies in MDS, but the optimal dose to avoid significant myelossupression remains to be determined. Moreover, the exact mechanism of FTI action is not fully clarified, whereas a direct effect on human MDS progenitors *in vitro* has not yet been shown, neither is known if FTI exhibit selective toxicity against clonal MDS hematopoiesis. *Patients and methods.* Bone marrow aspirates of 16 MDS patients, 9 men and 7 women (5 with refractory anemia, 9 refractory anemia with multilineage dysplasia, 2 refractory anemia with excess blasts I, according to WHO classification), were taken prior to either cytotoxic or growth factor treatment. The median age was 68 years (range 62-77). MDS was diagnosed on the basis of morphologic and cytogenetic criteria. The control group consisted of 6 age matched individuals all of which had anemia of chronic disease (ACD). Mononuclear BM cells, were enriched for $CD34^+$ cells with positive selection by using immunomagnetic beads and plated for short term cultures in semisolid media, or liquid cultures for assessment of apoptosis, in the presence of either DMSO or 2.5, 10, 25 and 50 nM of FTI. The percentage of apoptotic, annexin V positive, mature ($CD34^+CD38^+$) and immature ($CD34^+CD38^-$) progenitor cells was assessed by flow cytometry after 48 and 72 hours. The significance of the differences was assessed by paired or unpaired Student t test as appropriate. IC_{50} values were determined by linear interpolation. *Results*. FTI inhibited the colony growth of total committed MDS progenitors at concentrations of 2.5nM (p=0.017) and higher, in contrast to normal, non clonal, $CD34^+$ progenitors which were sensitive only in concentrations of 25 nM and above. Interestingly, the effect was more prominent in the MDS CFU-GM which significantly reduced their plating efficiency at 2.5 nM (p=0.018), whereas erythroid and CFU-GEMM progenitors exhibited significant inhibition at 10nM (p=0.01) and above. Indicative of the augmented susceptibility of the MDS $CD34^+$ cells was also the lower IC_{50} value for MDS (14 nM) compared to the normals (33 nM). No differences were observed in either early $CD34^+CD38^-$ or mature $CD34^+CD38^+$ MDS progenitors when cultured in DMSO or various concentrations of tipifarnib for 48 and 72 hours. Likewise, the same subsets in normal controls displayed no evidence for increased apoptosis in the presence of tipifarnib. *Conclusions.* Commited MDS progenitors are more sensitive to tipifarnib *in vitro* than their normal counterparts, the effect being more evident in white cell progenitors even at low tipifarnib concentrations, whereas this action is not due to apoptosis induction. Since myelosuppression represents the main obstacle in the clinical use, in MDS careful determination of the appropriate tipifarnib dose will help in dissecting the desired control of the more susceptible leukemic clone from the unwanted inhibition of normal hematopoiesis.

0631

PREVALENCE OF ERYTHROCYTE HEMOGLOBIN H INCLUSIONS IN UNSELECTED PATIENTS WITH MYELODYSPLASTIC SYNDROMES OR MYELOPROLIFERATIVE DISORDERS

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Background. Patients with clonal chronic myeloid disorders (CMD), especially myelodysplastic syndrome (MDS), may develop an acquired form of α thalassemia - a phenomenon recently linked to somatic mutations in the chromatin remodeling factor ATRX. The prevalence of erythrocyte hemoglobin H (Hb H) inclusions, a sensitive marker for α thalassemia, in a general population of patients with CMD is unknown. Most reported patients with acquired thalassemia arising in the context of myeloid neoplasia have exhibited microcytic, hypochromic red cell indices. However, in the era when reticulocytes were routinely quantified by supravital staining of peripheral blood - the most sensitive assay for Hb H inclusions - rather than by modern flow cytometric methods, patients with CMD and normal erythrocyte indices were sometimes incidentally discovered to have Hb H inclusions. *Aims.* We sought to estimate the prevalence of Hb H inclusions in a general population of patients with CMD, regardless of red cell indices, in order to better understand the clonal evolution of thalassemic erythoid cells in this setting. *Methods.* We prepared fresh brilliant cresyl blue-stained peripheral blood smears from 355 individuals: 130 patients with CMD (95 with MDS and 35 with myeloproliferative disorders (MPD) - including idiopathic myelofibrosis, where unexplained microcytosis is common); 200 patients with an abnormal blood count not associated with a clonal myeloid disorder or an inherited form of thalassemia; and 25 healthy persons. Patients were not selected on the basis of red cell indices. Smears were examined at 4 and 24 hrs for Hb H inclusions. *Results.* Among the 95 MDS patients (all WHO subtypes represented), 7 had small numbers of Hb H inclusions (<1/500 cells), while 1 had 14% Hb H-containing cells (prevalence 8/95, 8%). Among MPD patients, 2/35 (6%) had Hb H-containing cells. All MDS patients with HbH cells except the patient with 14% cells had normal erythrocyte indices. None of the healthy persons or those with anemia due to non-clonal causes had detectable Hb H inclusions. *Conclusions.* These findings indicate that emergence of small thalassemic clones may be relatively common in the disordered marrow milieu of CMD, with the more striking α thalassemia-MDS (ATMDS) phenotype only resulting when additional mutations confer a selective survival advantage to a thalassemic clone. This observation suggests that ATRX mutations in themselves do not facilitate MDS progression, but are neutral markers of clonality, similar to acquired loss of the Y chromosome in males. However, the potential for ATRX alterations to interact with the already disturbed epigenetic patterning in MDS (e.g. CpG island methylation) is a related question that deserves further exploration.

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ARHGAP21 IS DOWNREGULATED BY DECITABINE TREATMENT IN BONE MARROW MONONUCLEAR CELLS FROM PATIENTS WITH MYELODYSPLATIC SYNDROMES

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Background. RhoGTPases mediate many aspects of cell biology including proliferation and adhesion. We identified a human gene, termed ARHGAP21, a negative regulator of RhoGTPase signaling pathways, which is upregulated during myeloid differentiation. Recently, we observed that ARHGAP21 levels are upregulated in patients with Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL), suggesting that this protein could be involved in the malignant process of hematopoietic cells. The Myelodysplatic Syndromes (MDS) encloses a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis and by a high incidence of progression to AML. The demethylating agent 5-aza-2'-deoxycytidine (DAC) has significant therapeutic value for the treatment of patients with MDS and AML, being able to induce differentiation and inhibition of growth of leukemic myeloid cells. However, the molecular events induced by DAC are not completely characterized. *Aims.* The aim of this work is to evaluate the differential expression of ARHGAP21 during myelodysplastic syndromes progression, analyzing ARHGAP21 gene as a target for DAC treatment. *Methods.* Myeloid cell lines (HL60, KG1, K562) and a total of 13 samples of bone marrow cells of patients with MDS, including 5 refractory anemia (RA), 1 refractory anemia with ringed sideroblasts (RARS), 5 refractory anemia with excess blasts (RAEB), 2 refractory anemia with excess blasts in transformation (RAEB-t) and 5 secondary leukemia (LMA) were analysed in this study. Normal hematopoietic tissues were obtained from healthy donors (n=5). The National Ethical Committee Board approved the study and informed-written consent was obtained from all subjects. Real-time PCR analyses, normalized by β-actin control, were used to determine the differential expression of ARHGAP21. Using a functional *in vitro* model of hematopoiesis with an adherent layer of stromal cells providing a suitable microenvironment for the survival and differentiation of precursor cells we evaluated the modulation of ARHGAP21 expression of mononuclear cells (MNCs) of two patients with MDS (1 refractory anemia-RA and 1 refractory anemia with excess blasts-RAEB), treated or not with DAC. Localization of the ARHGAP21 protein was obtained using confocal microscopic analysis. *Results.* ARHGAP21 levels were upregulated in some myeloid cell lines (KG1, K562). In addition, we observed a tendency to higher expression of ARHGAP21 transcripts in high-risk MDS (medians; RAEB/RAEB-t: 1.30 vs 0.25, p>0.05; Mann-Whitney test). When MNCs were co-cultured or not with stroma cells and treated with DAC we observed a downregulation of ARHGAP21 expres-

sion. In addition, confocal analysis showed that ARHGAP21 is preferentially localized in the cytoplasm of the MNCs, but after DAC treatment this protein translocates into the nucleus. *Conclusions.* ARHGAP21 is overexpressed in AML and ALL cells and our data show a tendency to higher expression of ARHGAP21 in high-risk MDS cells. The down-regulation of ARHGAP21 in MNCs co-cultured or not with stroma cells by DAC treatment, in parallel with its translocation cytoplasm-nucleus, suggest that ARHGAP21 may be involved in the abnormal phenotype of MDS cells and might be a target of DAC treatment, aiming this gene an important candidate for anti-tumor therapy. Supported by: FAPESP and CNPq

0633

SEQUENTIAL CYTOGENETIC STUDIES IN 79 PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. While karyotype and cytopenias are useful in desease monitoring in myelodysplastic syndrome (MDS), little data exist on the prognostic impact of cytogenetic changes on outcome and survival. *Aims.* To analyse the usefulness of adding karyotypic studies to the marrow cytological follow-up during the course of myelodysplastic syndromes (MDS). *Methods.* In 79 MDS pts aged 68 ys (range 15-91), F/M 31/48, a karyotypic study was performed whenever pts underwent marrow morphological reevaluation. Their median follow-up was 13 months (range 0-97). All cases were treated with supportive measures ± growth factors, corticosteroids. Low-dose hydroxyurea was used in 4 pts with CMML. Pts treated with aggressive chemotherapy were excluded. MDS were defined according to FAB classification and karyotypes were classified according to IPSS risk classes.

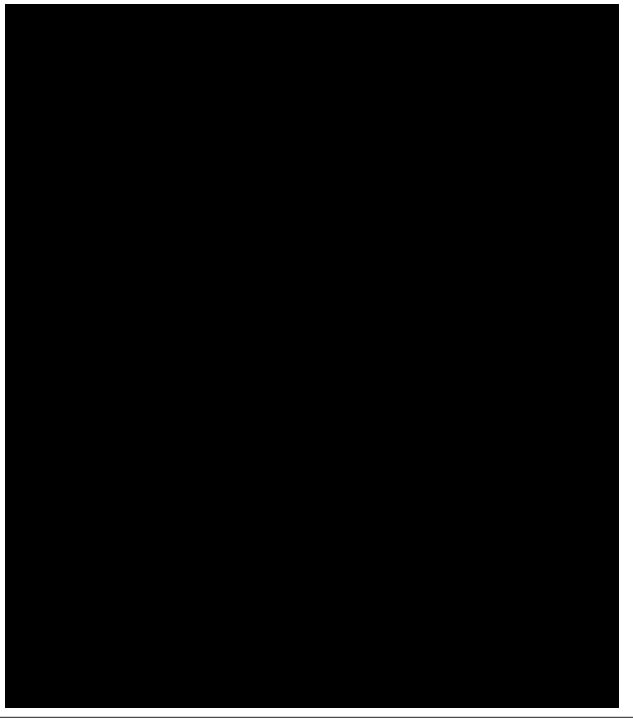

Figure 1.

Results. A total of 119 combined morphological/cytogenetic analyses were performed. There were 46 RA, 4 RARS, 38 RAEB, 10 RAEB-T, 10 CMML and 11 MDS not specified. Distribution among IPSS karyotype groups was: 66 good, 26 intermediate and 27 poor. At baseline no significant correlation was found between FAB morphology and IPSS cytogenetic risk class. FAB morphology remained *stable* in 49 cases (41%); it regressed to a lower risk FAB class in 11 cases (*better morphology*: 9%). Morphological evolution occurred in 59 cases (50%), respectively to a higher risk MDS FAB class in 19 (*worse MDS morphology*: 16%), or to acute leukaemia in 40 (*leukaemic evolution*: 34%). IPSS karyotype risk class was stable in 84 cases (70%). It regressed to a lower IPSS risk in 14 (*better karyotype*: 12%) and progressed to a higher IPSS risk in 21 (*worse karyotype*: 18%). No significant correlations were found between the morphological and cytogenetic variations. The incidence of both favourable (IPSS good) or unfavourable karyotype (IPSS intermediate+poor) progressively decrease with increasing marrow blasts percentage: respectively, for RA, RARS, CMML, NAS: 59% favourable vs. 58,5% unfavourable; for RAEB: 32% vs 34% and for RAEB-T: 9% vs 7,5%. Morphological changes during follow-up had a significant impact on median survival, which was 12 months (range: 0-68) in pts both with *better* and *stable* morphology, 5 months (0.5-97) in pts with *worse MDS* and 3 months in pts with *leukaemic evolution* (p<0.009). Conversely no significant effect of karyotypic changes was demonstrated on survival, which was 5, 6.5 and 7 months for pts with *better*, *stable* and *worse* karyotype, respectively (p=0.9). (Figure 1). Cytogenetic changes failed to predict survival even considering only the subgroup of pts with *stable* morphology. *Conclusions.* Routine cytogenetic analysis during the morphological follow-up of patients with MDS does not add useful prognostic informations and cannot be recommended on a routine basis.

0634

MYELODYSPLASTIC SYNDROMES AND PATIENT QUALITY OF LIFE

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Background. As is the case with many diseases, MDS affects patients physically, psychologically, emotionally, spiritually, and socially. Along with the diagnosis of MDS, symptom management and the administration and complications of specific treatments significantly impact QoL. The physical burden alone is challenging for adult MDS patients and is compounded by factors such as age and comorbidities. Overwhelming fatigue is reported to have the greatest negative impact on QoL. Emotional and psychosocial dimensions of the disease influence daily functioning, work, and social and family life. Caregivers often express feelings of helplessness, depression, and anxiety as well. *Aims.* The MDS Foundation has convened a series of patient forums to systematically study QoL of adult MDS patients. Analysis of qualitative and quantitative QoL data from these forums is being used to develop educational programs for physicians/healthcare providers and patients/caregivers with the specific aim of improving patient knowledge, patient-provider relationships, and individualized care of MDS patients. *Methods.* Twenty-two patient forums have been conducted at MDS Foundation Centers of Excellence in Europe (n=11) and U.S. (n=11) through February 2007. Nurse specialists with expertise in QoL assessment in MDS patients conducted the forums, which included open dialogue and question-and-answer sessions. Anonymous questionnaires were used to assess patients' knowledge about their disease, feelings about their relationships with their physicians, perceptions of the attitude of and support offered by healthcare providers, and the effect of specific disease management or treatments strategies on QoL. The patient forums were audiotaped and qualitative data were extracted from the verbatim transcriptions. (Quantitative data from survey responses have been presented elsewhere). *Results.* A total of 437 MDS patients and caregivers, spouses, or friends participated in the forums (274 patients; 163 caregivers). A range of emotions was articulated in the dialogue sessions as patients and caregivers, spouses, or friends described the burden of MDS from their personal experience and in their own words. Patients expressed feelings of depression, anger, and helplessness due to the fatigue that affects their ability to perform daily activities and participate in social and family life as they did prior to their diagnosis. Patients also expressed frustration with what they called their declining health and loss of independence. The vast majority of patients who spoke said that they were looking forward to retirement, but their expectations changed when they were diagnosed with MDS. In addition to the negative impact of MDS, some patients reported positive changes, stating that the diagnosis forced them to reassess their life priorities and take a fresh look at their relationships. Several patients talked about their renewed spiritual life since being diagnosed. *Conclusions.* A diagnosis of MDS has a much greater impact on QoL than generally appreciated, affecting not only the physical, but the emotional, psychological, and social as well. Qualitative QoL data provided by MDS patients can be used to help physicians and other healthcare professionals better understand the challenges of living with MDS and the impact associated with the initial diagnosis, routine doctor visits and tests, and specific management approaches and treatments.

0635

DAP-KINASE HYPERMETHYLATION AND APOPTOSIS IN MYELODYSPLASTIC SYNDROMES

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Background. Aberrant CpG island methylation in the context of the promoter region of multiple genes, leading to their silencing, have gained increasing recognition as important factors in development and progression of myelodysplastic syndromes (MDS). Among genes regulated by promoter hypermethylation, death-associated protein kinase (DAP-kinase) is a proapoptotic calcium/calmodulin-regulated serine/threonine kinase that participates in several apoptotic pathways. *Aims.* We studied the frequency of DAP-kinase promoter hypermethylation in a group of 106 MDS patients and analyzed its effect on apoptotic cell levels in their bone marrow samples. The contribution to apoptotis of DAP-kinase re-activation due to demethylating agents, as decitabine and azacitidine, was studied in cell line models. *Methods*. DNA was extracted from bone marrow mononuclear cells of MDS patients at the time of diagnosis and during the disease follow-up. DAP-kinase promoter methylation and expression were analyzed by methylation-specific PCR and real time RT-PCR, respectively. $CD34^+$ cells were isolated from bone marrow samples by immunomagnetic beads. Apoptosis was evaluated by 7-amino-actinomycin-D (7-AAD) incorporation and quantified by a FACScan flow cytometer. *Results.* DAP-kinase was methylated in 37% of MDS patients. No association with patients' characteristics was found. Sequential samples from 11 MDS patients were studied during follow-up, at a median of 12.4 months (range 1.8-40.6 months) from initial diagnosis. Eight patients, 7 of whom with disease progression, maintained the same DAP-kinase unmethylated (n=6) or methylated (2 patients) status, while only 2 patients gained DAP-kinase hypermethylation, and one patient became unmethylated. Freshly isolated $CD34^+$ cells from MDS samples showed a DAP-kinase methylation status similar to the $CD34^-$ cell fractions. Moreover, DAP-kinase hypermethylation was associated to reduced mRNA expression in $CD34^+$ cells, when compared to unmethylated samples. Apoptosis was higher in bone marrow samples from MDS patients compared to normal controls. In MDS patients, the apoptosis rate increased in bone marrow mononuclear cells and $CD34^+$ cells of patients *unmethylated* for DAP-kinase, when compared to *methylated* patients (1.8±0.5% vs 0.4±0.1% in MNC, p=0.068, and 0.5±0.2% vs 0.08±0.04% in $CD34^+$ cells, p=0.1, respectively). We used the HL-60 cell line to study the effect of demethylating agents on DAP-kinase function. Restoration of the unmethylated state of DAP-kinase occurred after 72-96 hours of exposure to 1 micromolar decitabine, and was associated to re-expression of DAP-kinase transcripts. Moreover, treatment was associated to cell growth inhibition and significant increase of apoptotic cells. In particular, following 4 days of treatment of HL60 cells with azacitidine, trichostatin A, or both drugs, we found a cell growth inhibition of 65%, 32% and 82%, respectively, compared to mock treated cells. Correspondingly, mean apoptosis was 2.2%±0.3% in the control, 11.3±2.8% in the presence of azacitidine (p=0.02), 5.7%±2.3% in the presence of trichostatin A (p=0.13), while the combination of the two drugs led to a synergistic effect, with apoptosis increasing to 41.9%±9.8% (p=0.009). *Summary and conclusions.* DAP-kinase promoter hypermethylation reduces apoptosis in MDS and its re-expression may have an important role in the response to epigenetic treatment.

0636

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

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Background. 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-κB through TNF♀ release. Recently it was demonstrated that NF-κB is abnormally activated not only in acute leukemias but also in MDS patients *Aims.* The aim of the study was to evaluate the effects of the oral chelator ICL670 (Novartis) on NK-kB activity in order to identify a possible mechanism responsible for the observed reduced transfusion requirements during chelation therapy. *Methods.* 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 3 hrs. Moreover, K562 and HL60 cells were analyzed as control. Incubated and control cells were evaluated for NF-κB activity using both EMSA and ELISA method. *Results.* We detected an increased activation of NF-κB 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 NF-κB activity comparing patients with or without iron overload (p=0,5). The levels of NF-κB activity increase during disease progression being higher in RAEB and s-AML as compared to RA (p=0,003). Regression analysis demonstrated a correlation between NF-κB activity and blast percentage (r=0,75) but no correlation between NF-κB and ferritin levels (r=0,5). Among patients with increased NF-κB (n=14) the incubation with ICL670 induced a significant reduction of NF-κB activity (p=0,0002). No significant difference was detected in NF-κB inhibition comparing patients with or without iron overload. In addition, ICL670 also inhibits NF-κB activity in HL60 and K562 cells. *Conclusions.* NF-κB 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-κB 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 strorage induced by oral chelation.

Myeloproliferative disorders - Clinical

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PULMONARY HYPERTENSION IN ESSENTIAL THROMBOCYTHEMIA

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Increased incidence of pulmonary hypertension (PH) has been reported in patients with chronic myeloproliferative disorders (MPDs). However, the exact incidence of PH in essential thrombocythemia (ET) is unknown and most of the reported literature consists of case reports or small studies. Previously or newly diagnosed 46 patients with ET and 40 patients with reactive thrombocytosis secondary to iron deficiency anemia were eligible for this study in between January 2004 to February 2007. Diagnosis of PH was established if right ventricular systolic pressure > 35 mmHg by transthoracic echocardiography. Diagnosis of PH was established in 22 (47.8%) of 46 patients with ET. Seven patients with PH were newly diagnosed ET. Five patients with PH were in low, and the other patients with PH were in intermediate or high risk category. However, none of the patients with reactive thrombocytosis with iron deficiency anemia had PH. In conclusion, PH appears to be common in patients with previously or newly diagnosed ET. All patients with ET should be evaluated for development of PH at the time of diagnosis and during their follow-up period. Larger and prospective designed studies are needed to clarify the long-term impact of PH on the survival of these patients. It should illuminated whether cytoreductive treatment and use of aspirin prevent development of PH, and their effects on progress of PH and prognosis. In addition, especially in low risk group patients who are not recommended treatment but aspirin the role of PH on prognosis should be determined.

0638

SERUM VEGF LEVELS ARE RELATED WITH METALLOPROTEINASE-9 (MMP-9) LEVELS IN PATIENTS WITH POLYCYTHAEMIA VERA

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The release of angiogenic factors by physiological stress or malignant cells is known to mobilize hematopoiesis. The mechanism by which these factors recruit stem cells is unknown. It has been shown that VEGF (vascular endothelial growth factor) and PlGF (placenta growth factor) promote hematopoiesis through the up regulation of MMP-9 (metalloproteinase 9) levels. MMP-9 induces the cycling of Go hematopoietic stem cells. In polycythemia vera (PV) there is evidence of increased angiogenesis and several angiogenic factors and microvessel density of the bone marrow have been examined and found increased. However the aetiology of the bone marrow hyperplasia is unidentified. We tried to investigate the role of the two angiogenic factors (VEGF and MMP-9) that are known to synergize and regulate hematopoiesis, in PV patients. A total of 38 patients with PV (mean age 56,1±2,5 years) were included. Δwenty five patients were managed with phlebotomy, four received hydroxyurea, eight were managed with hydroxyurea and phlebotoby and one was treated with interferon. Three had clinically detected spleen enlargement. The control group consisted of 22 healthy subjects (mean age 55,3±1,3 years). Serum VEGF levels were found increased in PV patients in comparison to control group (510,6±87,8 pg/mL vs 182,7±27,2 pg/mL respectively, p=0,032). Although serum MMP-9 concentrations did not differ among polycythaemic patients and the control group (316±40,4 pg/mL and 446,2±72,6 pg/mL, respectively, p=0,086) we found a statistically significant positive correlation in the patients group between serum MMP-9 levels and VEGF levels (r=0,36, p=0,03). VEGF plays a prominent role as an angiogenesis inducer and was found very significantly increased in the group of polycythaemic patients. The role of MMP-9 in angiogenesis and hematopoietic cell stimulation is well established. It has been shown that MMP-9 has the capacity to generate sKitL from membrane bound KitL and mobilizes stem cells. The positive correlation (although slight) of VEGF and MMP-9 serum levels in PV patients may reflect the autocrine signaling pathways that exist between the two factors in the bone marrow microenviroment. The role of angiogenic factors is under investigation in PV and myeloproliferative syndromes and further studies are needed to determine their role in the pathogenesis of these disorders.

0639

IS RED CELL MASS DEFINITELY USELESS TO CLASSIFY MYELOPROLIFERATIVE DISORDERS IN THE JAK2 ERA?

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Background. Since the discovery of JAK2V617F mutation, new algorithms to diagnose and classify MPD are still debated. The current WHO criteria for PV require either an elevated RCM above 125% of predicted value, or Hb level above 18.5 g in men and 16.5 g in women. Some investigators recently proposed to set the threshold for Hematocrit (Ht) at 52% in men and 48% in women to diagnose PV in JAK2V617F patients (Green & Campbell, NEJM, 2006). RCM analysis could therefore become obsolete in JAKV617F patients, if one considers that mutated PV and ET are almost similar conditions. However, no prospective clinical data support this plausible assumption, and distinguishing PV from ET still seems necessary, at least until those hypotheses are validated. *Aims.* To assess the diagnostic value of RCM measurement in the diagnosis of PV in patients with Hb or Ht below values used in currently validated or proposed criteria. *Methods.* We reviewed all RCM measurements performed in patients suspected of MPD in a single nuclear medicine laboratory during a one-year period. *Results.* 66 patients were referred for RCM measurement between January 05 and March 06. Among them, 19 had isolated thrombocytosis, i.e. both Hb below WHO, and Ht below Greens' proposed criteria, respectively. JAK2V617F mutation was found in 10 pts, while 9 pts had wild-type (wt) JAK2. RCM was below 125% of predicted value in all the 9 wt-JAK2 pts. Among the 10 patients with JAKV617F, 7 had measured RCM above and 3 below 125% of the predicted value, respectively. Therefore, 7/19 (37%) patients presenting with isolated thrombocytosis had PV based on RCM measurement, but they would have been diagnosed as ET based on Hb or Ht values only. All those patients had JAK2V617F mutation, while all wt-JAK2 ET had normal RCM. *Conclusions.* In this series of unselected consecutive patients with isolated thrombocytosis referred for RCM determination, we found that 37% of cases would have been misdiagnosed as ET instead of PV in the absence of RCM measurement, this proportion reaching 70% in the group of JAKV617F patients. Those results suggest that RCM should be performed in JAK2V617F patients with isolated thrombocytosis, for proper MPD classification and management. On the other hand, RCM is useless in wt-JAK2, as PV is extremely rare in such patients.

0640

IMATINIB AT 100MG ONCE WEEKLY IS SUFFICIENT TO MAINTAIN HAEMATOLOGIC AND MOLECULAR REMISSION IN PATIENTS WITH IDIOPATHIC HYPEREOSINOPHILIC SYNDROME

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Background. Persistent blood eosinohilia is usually associated with a large number of clonal and non-clonal disorders. Idiopathic hypereosinophilic syndrome (HES) is a condition of unknown cause with overproduction of eosinophils resulting in organ damage. In a small subset of patients (pts) previously regarded as having HES, the disease is caused by the FIP1L1-PDGFRA fusion tyrosine kinase. In these pts the tyrosine kinase blocker- imatinib is highly effective inducing a haematologic and molecular remission. Some pts lacking this transcript can also benefit from imatinib. Of crucial importance is to define the policy of long term treatment and this issue is adressed in this report. Material and methods. 21 pts affected by HES were studied for FIP1L1-PDGFRA by RT-PCR and this fusion was detectable in 2 pts (9%). 10 out of 21 pts were treated with imatinib at 100 to 300 mg daily, because of advanced organ damage and/or marked hypereosinophilia. The dose was reduced to 100mg once weekly after a complete haematologic remission (CHR) was achieved. *Results.* Median age at diagnosis was 54 years (range 6-67 yrs) with male/female ratio 1.5/1. Heart and spleen were most frequent involved. Cytogenetics was obtained in 7 out of 10 pts and it was normal. Median number of prior lines of therapy was 3 (range 1-8). Median time from diagnosis to imatinib was 55 months (range 6-145). Troponin level was not increased before imatinib commencement. 3 pts, all male, showed rapid response to imatinib at 100 mg daily. In 2 FIP1L1-PDGFRA positive pts, CHR was achieved after 14 and 67 days respec-

tively whereas molecular remission defined as a negative RT-PCR for FIP1L1-PDGFRA fusion gene was recorded after 6 and 24 months respectively. One patient, who was not initially studied for FIP1L1-PDGFRA transcript, revelaed CHR after 13 days. In this pt, imatinib was stopped after 7 months while in CHR and after 5 months off, eosinophilia recurred. Imatinib at 100 mg daily was resumed and CHR was obtained in next 2 weeks. Of note, a FIP1L1-PDGFRA fusion gene was not detectable at relapse. The median follow-up is +24 months (range +24 to+36) and pts receive imatinib at 100mg once weekly with sustained remission. In 7 pts resulted negative for FIP1L1-PDGFRA fusion gene we observed 2 transient and short-term haematologic improvement. 2 out of those 7 pts during follow-up, developed leukemia, one pt was diagnosed as having chronic eosinophilic leukemia with 10% of blasts in peripheral blood and he was positive for the JAK2V617F mutation. The second patient- a 6-year old boy developed acute lymphoblastic leukemia. Three patients who responded to therapy were younger (54 vs 56yrs), had higher blood eosinophilia count (5.1 vs 3.6), higher bone marrow eosinophilia infiltration (32 vs 29), lower B12 serum level (288 vs 649 pg/mL), serum IL-5 level (12 vs 23 pg/mL) and serum IgE level (22 vs 89 IU/mL) if compare to 7 pts who failed imatinib. Time to start imatinib was shorter in responders (50 vs 56 mo). *Conclusions.* We confirm high efficacy of imatinib in patients carrying FIP1L1-PDGFRA fusion gene. In imatinib responders, the dose can be safely reduced to 100 mg once weekly and it is sufficient to maintain remission.

0641

THE V617F JAK2 MUTATION INCIDENCE IS HIGHLY REPRESENTED IN BCR/ABL-NEGATIVE CHRONIC MYELOFERATIVE DISORDERS AND PREDICTABLE BY SIMPLE HAEMATOLOGICAL PARAMETERS IN PATIENTS WITH ERYTHROCYTOSIS

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Background. 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 chronic myeloproliferative disorders (cMPD), including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). Estimates, using different detection methods, indicate that JAK2V617F is present in 65-97% of PV, 23-57% of ET, and 35-57% of IMF patients, respectively. 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 wonted to establish the V617F JAK2 mutation incidence in bcr/abl-negative cMPD and to predict JK2 mutational status, by using simple haematological parameters, in those cases presenting with erythrocytosis. *Methods*.We used an allele-specific polymerase chain reaction (PCR) assay (designated as the AS-PCR assay) to detect JAK2V617F. Briefly, a mutation-specific forward primer containing a fluorescent tag (FAM) was used in a PCR reaction with a wild-type sequence reverse primer. Only mutated DNA will be amplified (203bp), if present, and the PCR reaction product was analyzed using capillary electrophoresis in an automated genetic analyzer. To evaluate the sensitivity of the technique employed, either bone marrow (BM) or peripheral blood (PB) were analyzed, while the background signal was examined by using 50 normal PB samples. *Results.* All normal samples resulted negative, showing a median signal one log lower than cases belonging to positive PB samples obtained from cMPD. JAK2V617F mutated cMPD BM samples showed a fairly expected two logs higher signal than normal controls. We analyzed 38 PV, 43 TE, 24 MIF, 20 bcr/abl cMPD and 22 secondary polyglobulia (secPoly). Out of 147 cases analysed, 100 resulted JAK2 mutated. In particular, none of secPoly cases resulted mutated, while 94.7%, 76.7%, 70.8% and 70.0% showed JAK2V617F mutation in PV, TE, MIF and cMPD, respectively. Next, we pooled PV and secPoly trying to predict JK2 mutational status by using 3 simple parameters, i.e. Ht level, and WBC and PLT count. By using ROC curve analysis, we determined 320×10^9/L (AUC=0.925, $p<0.0001$) and 10×10^9/L (AUC=0.867, $p<0.0001$) as the best cut-off values for PLT and WBC, respectively. Moreover, 54% was identified as the best cut-off for Ht (AUC=0.738, $p=0.004$). On the basis of these results, cases were clustered accordingly. Thus, we designed a predicted model based on these results, giving a score 1 to each *abnormal* variable. The devised predictive model allowed us to split cases in low risk (score 0), intermediate risk (score 1-2) and high risk (score 3) to be JAK2 mutated. In terms of incidence, we observed 100%, 62.5% and 10.5% of cases with the presence of JAK2V617F in high, intermediate and low risk categories, respectively. *Conclusion*. It is conceivable that the detection of JAK2 V617F should be used as an initial tool in the diagnosis of chronic hyperleukocytosis, thrombocytosis and erythrocytosis. Moreover, JAK2 mutational status is highly predictable by clustering cases using 3 simple hematological parameters.

0642

PROSPECTIVE EVALUATION OF PULMONARY VASCULAR INVOLVEMENT IN ASYMPTOMATIC PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background. Pulmonary hypertension (PH) has been recognized as a severe complication of myeloproliferative disorders (MPD), essentially in primary myelofibrosis. In a retrospective study, 26 cases of unexplained PH associated with MPD (including 6 PV and 5 ET) were identified in the referral population of the Mayo Clinic (Chest, 2001;120:801). More recently, a prospective study found that 10 (including 2 PV and 14 ET) of 24 MPD patients had PH (Haematologica, 2004;89:245). Yet, pulmonary microcirculation has never been investigated in such a context. *Aims.* Prospective evaluation of pulmonary artery systolic pressure (PASP) and pulmonary microcirculation in asymptomatic patients with PV and ET. *Methods.* 53 consecutive patients (26 PV and 27 ET) with no associated lung disease were included. Doppler transthoracic echocardiography was used to estimate PASP, PH being defined as PASP > 35 mmHg. Pulmonary function tests included vital capacity, total lung capacity, flow-volume curves, diffusing capacity for carbon monoxide (DLCO) and gas exchange at moderate exercise including arterialized blood gas, alveolar-arterial oxygen pressure difference (P(A-a)O2) and dead space. *Results.* Patent PH was never observed (mean PASP: 29±2.6 mmHg). Pulmonary volumes and flow were normal in all MPD patients. Patent decrease in DLCO was found in 4 PV (15%) and 8 ET (30%), respectively. Furthermore, mean DLCO adjusted for hemoglobin was significantly decreased (compared to predicted value based on sex, age, height and weight) both in PV (92.3% of predicted value±14; $p=0.035$) and ET (86.5%±16.0; $p=0.005$) groups, suggesting pulmonary microvascular abnormalities. Accordingly, mean PASP was significantly higher in patients with decreased DLCO ($p=0.03$). This decreased DLCO was observed independently of JAK2 mutational status. DLCO was negatively related to exercise dead space ($p<0.01$) in ET, providing further evidence for vascular involvement, as increased dead space reveals pulmonary regions that are ventilated but not perfused. 14 patients (7 PV and 7 ET) at diagnosis were tested prior and after the initiation of low dose aspirin only. A significant decrease of dead space after aspirin was demonstrated in PV ($p=0.02$) but not in ET. *Conclusions.* Contrary to previous reports, in this large prospective study in unselected MPD patients, no case of patent PH was found. Yet, pulmonary function studies revealed anomalies suggesting presence of pulmonary vascular disease a minima, with increased dead space at exercise correlated with decreased DLCO. These anomalies were similarly observed in PV and ET, and in patients with or without JAK2V617F mutation. A possible mechanism could be occlusion of the pulmonary micro-vascular bed, a phenomenon well described in terminal arterial circulation of MPD patients. To support this hypothesis, we have shown that aspirin alone allowed normalizing dead space in PV. Longer follow-up will show if abnormal dead space and DLCO are predictive of overt PH development in MPD patients and could justify preventive measures.

0643

INVESTIGATION OF THE SAFETY AND PHARMACOKINETICS OF ANAGRELIDE IN A PAEDIATRIC POPULATION WITH THROMBOCYTHAEMIA

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Background. Myeloproliferative disorders (MPDs) occur less frequently in children than in adults. As only limited safety data have been published in children, this phase II study helps to build the body of evidence for the management of MPDs in this patient population. *Aims.* The primary objective of the study was to assess the safety and tolerability of anagrelide in a younger (″15 years) compared with an older (≥16 years) group of subjects with thrombocythaemia secondary to MPDs. The secondary objectives were to assess the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of anagrelide in these groups. *Methods*. The

open-label, multi-centre study enrolled 35 patients (all provided informed consent) who were either naïve or currently receiving anagrelide therapy. For those on anagrelide prior to the study, the dosing regimen was maintained at the same pre-study level. For naïve patients, anagrelide was initiated at 0.5 mg daily. Patients were treated with anagrelide for 3 months. Safety parameters and a range of PK and PD endpoints were assessed. *Results*. A total of 35 patients were enrolled in the study; 8 paediatric (≤11 years) patients with essential thrombocythaemia (ET), 9 adolescents (age 12-15 years) with ET and 18 adolescents/adults (age ≥16 years) with ET (12), polycythemia vera (5) or another MPD (1). The majority of the patients had experienced anagrelide prior to the study (94% in ≤15 years group, 72% in ≥16 years group) and exposure prior to the study was similar in the two groups. The median starting total daily dose was 1.0 mg in both groups, increasing to median final total daily doses of 1.5 mg and 2.0mg in the older and younger groups, respectively. The incidence of on-study AEs and related AEs in those aged ≤15 years (53% and 18%, respectively) was lower than for those in the ≥16 years group (67% and 34%). There were no apparent differences in the types of AEs observed in the two groups and no patients discontinued the study due to AEs. The three most frequent AEs were palpitations, fatigue and headache. The majority of events were considered drug-related. There were no clinically significant abnormalities in terms of 24-hour ECGs, 12-lead ECGs or cardiovascular function (as assessed by echocardiogram). A comparison of PK data (normalised to 1mg dose and 70kg bodyweight) showed that exposure to anagrelide (maximum observed plasma concentration [Cmax] and area under the plasma concentration-time curve during the dosing interval [AUCt]) was substantially lower (48% and 55%, respectively) in those ≤15 years than in those ≥16 years. No differences in the exposure to the active metabolite BCH24426 were observed. A number of correlations were analysed, and for example, when the age groups were analysed together, there was a good correlation between anagrelide and BCH24426 daily plasma exposure (Cmax and AUCt) and decreases in platelet count. *Conclusions*. The safety findings were as expected and were consistent with the pharmacological profile of anagrelide and underlying diseases. Acceptable differences in PK profiles were observed and from this study current dosing regimens for anagrelide appear appropriate for use in children.

0644

PROGNOSTIC FACTORS IN IDIOPATHIC MYELOFIBROSIS: 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 requires some prognostic factors, in order to orientate the therapy choice in this cohort of patients. The Lille scoring system, proposed for patient risk stratification, seems unable to clearly discriminate between intermediate and high risk patients.

Figure 1. Risk stratification in 65 patients with IM.

The aim of our 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 patients (43 males and 22 females; median age 62 years, range 35-80) diagnosed between 1990 and 2000. Fifty-three cases were *de novo* IM, while twelve were secondary disease (policytemia 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), as shown in Figure 1. The median survival for each group was respectively 145, 62 and 50 months, with a very significant p value (<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 to acute leukemia. Obviously a wider population is needed to validate the results of this study.

0645

PROGNOSTIC RELEVANCE OF JAK2 V617F MUTATION IN PRIMARY MYELOFIBROSIS

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Background. An acquired point mutation in JAK2 V617F is reported in about half of patients with primary myelofibrosis (PMF). However, whether the presence of the mutation is associated with distinct prognosis is still a matter of debate. *Aims.* To study the onset of the relevant outcomes of the disease. To exclude time and selection biases, i.e. the effect of different time periods before genotyping and the effect of patients who died before genotyping, we analyzed only patients who had the JAK2 genotype determined at their diagnosis. *Methods.* A cohort of patients with a diagnosis of PMF who had received the JAK2 V617F mutational study at the time of diagnosis or no later than 3 months from the diagnosis were analyzed in a longitudinal study. The primary outcome events analyzed were total mortality, development of severe anemia (Hb less than 10 g/dL), development of large splenomegaly (spleen larger than 10 cm from the left costal margin), development of thrombocytopenia (platelet count lower than 150×10^9/L), development of leukopenia (WBC lower than 4×10^9/L), major thrombosis (i.e. non-fatal myocardial infarction, stroke, deep vein thrombosis, including splanchnic vein thrombosis, pulmonary embolism or cardiovascular death), and development of blast transformation (peripheral blood blasts greater than 20% of WBC and/or blasts in the bone marrow greater than 40%). The censoring for the development of severe anemia, large splenomegaly, thrombocytopenia and leukopenia was considered at the beginning of cytostatic, thalidomide, steroid, or androgen treatment. The distribution for overall and progression-free survival was estimated using the method of Kaplan and Meier. The log-rank test was used to test for differences in survival between groups. *Results.* These were 111 patients (66 males, 45 females), aged 52 years (median, range 11 to 89 years). In 14 of them, a diagnosis of prefibrotic myelofibrosis was done. The mean follow up was 111 months (range, 1-266 months). Over the course of follow-up, 39 patients (35.1%) developed severe anemia, 40 (36%) large splenomegaly, 25 (22.5%) thrombocytopenia, 23 (21%) leukopenia, 25 (22.5%) thrombosis. Only 3 patients develop blast transformation and only 5 died, so these events were not used as major analyzable outcomes. Baseline V617F mutated status was not significantly associated with increase rate of development of anemia, splenomegaly, thrombocytopenia and leukopenia (log-rank test = NS). The thrombosis-free survival was longer in non-mutated patients, but the difference did not reach a statistical significance. *Conclusions.* We conclude that the influence of having V617F JAK2 mutation on the natural history of the disease is not visible at a median follow-up of 111 months.

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JAK2 MUTATIONS IN EXON 12 AND EXON 14 IN POLYCYTHEMIA VERA PATIENTS

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Background. The JAK2 V617F mutation, which is located in exon 14, is found in 90-97% of polycythemia vera (PV) patients, being a small pro-

portion of cases negative for this mutation. Recently, it has been described that JAK2V617F negative PV patients have mutations in exon 12 of the JAK2 gene. *Aims.* To determine the JAK2 mutational status analysing both exon 12 and exon 14 in a series of PV patients. *Patients and Methods.* In 86 patients (44M/42F) from a single institution diagnosed with PV, at a median age of 62 years (range 25-87), analysis of JAK2 exons 12 and 14 was performed by direct sequencing using granulocyte RNA. At the time the JAK2 analysis was performed, 24/86 PV patients were receiving therapy with platelet-lowering agents: hydroxyurea±ASA (n=23); anagrelide±ASA (n=1). Twenty-eight patients only received ASA and 34 patients did not receive any specific treatment. *Results.* The JAK2 mutational status of our cohort is summarized in the Table. The V617F JAK2 mutation was detected by direct sequencing in 77 cases. In two of these cases, additional mutations to the V617F in the same exon 14 were found: a C616C silent mutation in one patient and a C618R exchange in the second one. In this latter patient, V617F was a consequence of two heterozygous nucleotide substitutions at positions 1849 and 1851 resulting in a GTC>TTT exchange at codon 617. Exon 12 mutations were detected in 3 out of 8 patients negative for the JAK2 V617F mutation (37%). These three mutations in exon 12 were different from each other (Table 1). Moreover, exon 12 was analyzed in the whole cohort, but no mutations were detected among JAK2V617F positive patients. In 5 patients no mutations were found in either exon 12 or exon 14. *Conclusions.* Mutations in exon 12 are detected in a percentage of JAK2V617F negative patients, but not in all cases. Exon 12 and exon 14 mutations are mutually exclusive, but the V617F mutation may coexist with other mutations in exon 14.

Table 1.

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THE NATURAL HISTORY OF FAMILIAL CHRONIC MYELOPROLIFERATIVE DISORDERS: CLINICAL PRESENTATION, OUTCOME, AND ANTICIPATION

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Background. Chronic myeloproliferative disorders (CMD) appear to have a sporadic occurrence in most instances. However, familial clustering of CMD has been recently reported. Our group has demonstrated that JAK2 (V617F) represents an acquired somatic mutation in familial CMD and that the mutation occurs as a secondary genetic event in the background of a pre-existing clonal hematopoiesis. To date, no definite data are available on the clinical presentation and disease progression in patients with familial CMD. *Aims.* The aim of this study was to assess the clinical presentation and outcome of patients with familial CMD and to provide a comparison with sporadic cases. In addition, we studied the phenomenon of anticipation in two-generation pairs. *Patients and Methods.* We interviewed on family history for CMD 348 patients with apparently sporadic CMD followed at our Department. Patients were grouped in two categories: familial cases, and sporadic cases as controls. *Results.* Among 348 patients, 30 pedigrees (8.6%) have been identified with one or more relatives affected with CMD. In familial cases, the diagnosis of CMD in a member of the family did not prompt specific investigations within relatives. Pedigrees included 64 patients: 31 with polycythemia vera (PV; 253 person-years of follow-up), 19 with essential thrombocythemia (ET; 144 person-years of follow-up), and 14 with primary myelofibrosis (PM; 84 person-years of follow-up). Nineteen families had an homogeneous clinical phenotype (12 families with PV, 5 with ET, 2 with PM), while 11 families had a mixed CMD phenotype. Kolmogorov-Smirnov test did not reveal statistically significant differences in clinical presentation between patients with familial CMD and those with sporadic CMD. During follow-up of familial CMD, the incidence of thrombosis was 26.8×1000 person-years (95% CI 12-60) in PV and 14.6×1000 person-years (95% CI 3.7-58.7) in ET; the incidence of leukemia was 7.9 ×1000 person-years (95% CI 1.9-31.7) in PV and 48.6×1000 person-years (95% CI 18.2-129.5) in PM; the incidence of secondary myelofibrosis was 4×1000 person-years (95% CI 0.5-28.4) in PV and 10.1×1000 person-years (95% CI 3.5-56.3) in ET. In familial cases, 10-year survival was 91.5% for patients with PV, 100% for those with ET and 30% for those with PM. Finally, we studied the anticipation of disease onset in 15 families with two generation pairs. At diagnosis, the median age was 61 years (range, 43-78) for the first generation and 37 years (range 23-57) for the second generation. Wilcoxon matched pair test showed a significant difference between these values (p=0.0004). Applying Nelson Aalen estimator, we compared the cumulative hazard of CMD onset between patients of the first and the second generation adopting age as a time scale: a significantly different hazard was obtained (p=0.00001). *Conclusions.* This study provides evidence that patients with familial CMD have a clinical phenotype at diagnosis similar to that of sporadic CMD. Similarly to sporadic cases, patients with familial CMD may develop thrombosis or may progress to myelofibrosis or leukemia. Our data are in favour of the anticipation of disease onset in familial CMD.

0648

CLINICAL AND MOLECULAR RESPONSE DURING PEGYLATED INTERFERON ALPHA THERAPY IN PRIMARY AND POST-POLYCYTHEMIC MYELOFIBROSIS

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Background. Based on the absence of specific treatment in primary myelofibrosis (PMF) or postpolycythemic myelofibrosis (post-PV-MF) myelosuppressive therapy with hydroxyurea is considered as standard therapy in order to delay a further deterioration of splenomegaly and to prevent progression of bone marrow fibrosis. An increased release of platelet derived growth factor (PDGF) and transforming growth factor β (TGF-β) seem to play an importatant pathogenetic role. *Aims.*Interferon α (IFN) has been shown to suppress the proliferation of megakaryocyte cell lines and *in vivo* studies have shown that IFN inhibits the production and release of PDGF and TGF-β from megakaryocytes. An early therapeutic intervention with IFN may therefore prevent disease progression to myeloid metaplasia in these patients. *Patients/Methods.* Twenty-seven patients (10 female, 17 male; median age 58 years, 34-81 years) with PMF (n=25) or post-PV-MF (n=2) were assigned to be included in a phase II study with PegIntron.One patient had to be excluded after screening phase. *Results.* The treatment was initiated in 25 patients with 50 µg and in one with 80 µg PegIntron/week. In 7 patients the dose was increased to 80 µg/week. In all patients this dosage had to be decreased at least to 50 µg/week due to intolerance during long-term treatment. The treatment had to be withdrawn in 6 patients after a median treatment duration of 24 weeks. The reason for withdrawal was treatment failure or disease progression in three patients, severe abdominal pain after dose increase to 100µg/week in one patient, heart failure in one patient, psychosis in one patient, and one patient did decide to stop treatment. The most frequent side effects were fatigue, fever, arthralgia, nausea, anemia, mild thrombopenia and leukopenia and psychological alterations. After a median treatment follow-up of 12 months platelet counts dropped significantly (p<0.0001) and improved in 13 of 14 thrombocythemic patients by at least 30% and reached normal values in 8 patients (Figure A). In 8 patients a quantitative JAK2-V617F analysis was performed. In 7 patients, a statistically significant (p=0.0069) decrease of JAK2-V617F allelic ratio was observed after PegIntron therapy (Figure B) In 6 patients spleen size decreased by at least 30% and in 7 patients LDH levels also decreased at least by 25% of the initial value. An improvement of hemoglobin level by 2 g/dL was observed in one patient whereas hemoglobin levels dropped in 7 patients by 0,7 to 4,3 g/dL. Grade 1 leukopenia was observed in one patient and one patient with very advanced disease had further disease progression with an increase of white cell blood count and a stricking increase of immature precursors in the differential count. There were no signs of an increase of bone marrow fibrosis during follow-up

biopsies after 6 and 12 months treatment in 24 patients. *Summary.* Our study reveals beneficial effects of PegIntron in PMF or post-PV-MF patients. The most stricking effect of IFN was observed on lowering of platelet counts and on a decrease of the JAK2 expression in bone marrow cells. Pegylated IFN may be reconsidered as treatment alternative in the early stage of PMF associated to thrombocytosis.

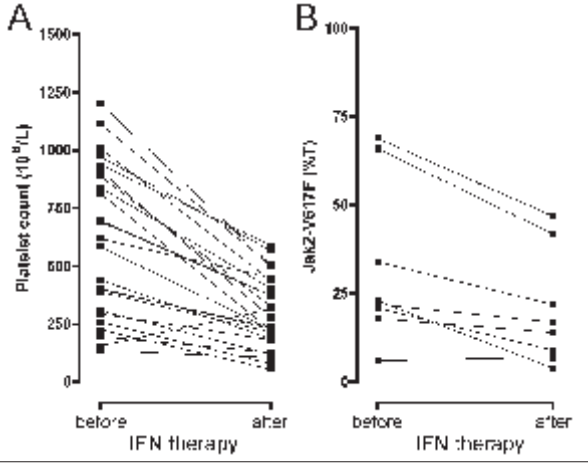

Figure 1. Platelet counts and JAK2-V617F allelic ratios.

0649

JAK2 (V617F) MUTATION BURDEN, PHENOTYPE, AND CLINICAL COURSE OF CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. An identical mutation (V617F) of JAK2 is found in most patients with polycythemia vera (PV) and in about half of those with essential thrombocythemia (ET) and primary myelofibrosis (PM). Clonal cells can be either heterozygous or homozygous for the mutation, and the JAK2 (V617F) burden can vary considerably in myeloid cells. Previous reports suggested that this variable burden might influence both phenotype and disease severity. *Aims.* To define the relationship between granulocyte JAK2 (V617F) mutation burden, phenotype, and clinical course of chronic myeloproliferative disorders (CMD). Methods. We studied 608 patients with CMD classified according to the diagnostic criteria of the World Health Organization (WHO). The JAK2 (V617F) mutation burden was assessed through a quantitative evaluation of granulocyte mutant alleles by real-time polymerase chain reaction. *Results.* Overall, 423 out of 608 (69.5%) patients carried JAK2 (V617F) and showed variable proportions of mutant alleles in circulating granulocytes (1-100%). Relationships were observed between JAK2 (V617F) mutation burden and hematologic parameters. In particular, the higher the percentage of granulocyte mutant alleles, the higher the hemoglobin level and the lower the platelet count. The majority of patients with high mutation burden (granulocyte mutant alleles in excess of 50%) had splenomegaly, increased LDH levels, leukocytosis and elevated circulating CD34-positive cell counts. The JAK2 (V617F) mutation burden was found to increase in untreated patients and phenotypic conversions (from essential thrombocythemia to polycythemia vera, and from this latter to myelofibrosis) were observed. Patients with more than 50% mutant alleles had worse event-free survival (p=0.039) and overall survival (p=0.042) as compared with those with lower mutation burden (1-50% mutant alleles). *Conclusions.* The JAK2 (V617F) mutation burden, as assessed by the proportion of granulocyte mutant alleles, influences the clinical phenotype of myeloproliferative disorders and contributes to determining their severity. Patients with high mutation burden tend to have more advanced disease with abnormal stem cell trafficking. They also have worse prognosis both in terms of disease-related complications and overall survival.

0650

PREGNANCY IN THROMBOCYTHEMIA: 118 CASES FROM THE REGISTRO ITALIANO TROMBOCITEMIA

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Essential Thrombocythaemia (ET) is diagnosed in the childbearing age in about 20% of patients. Fertility reduction and adverse outcome of pregnancy due to thrombotic or haemorrhagic complications are a matter of concern. The pregnancies observed in ET patients in 17 Italian Hematological Centres from 1998 to 2006 and registered in the RIT are object of this retrospective study. One hundred-eighteen pregnancies occurring in 91 women with ET, diagnosed according to the PVSG or WHO criteria, are considered. The median age at the conception was 31 yr (21-45). Median platelet count was at diagnosis 996 (range 489-2140) and at delivery 520 (232-1530). 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 mg/day) was reported, associated in 18 cases to prophylactic LMWH one week before delivery and for six weeks post-partum. A cytoreductive treatment at conception was registered in 36 pregnancies (Interferon α 20, Anagrelide 9, Hydroxyurea 6, and Busulphan 1). The Interferon α, associated or not to aspirin, was administered during 18 pregnancies considered at high thrombotic risk. Fifteen of these pregnancies were valuable (2 ongoing and 1 elective abortion) and, interestingly, all cases ended in live births. These data confirm that fetal morbidity and mortality rate is not negligible in ET. Cytoreductive therapy with interferon α 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).

0651

MK-0457 IS A NOVEL AURORA KINASE AND JANUS KINASE 2 INHIBITOR WITH ACTIVITY IN TRANSFORMED JAK2-POSITIVE MYELOPROLIFERATIVE DISEASE

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Background. MK-0457 (VX-680) was developed as a small-molecule inhibitor of Aurora kinases A, B, and C (Ki,app = 0.66-18 nM). Recent screening for additional kinase activity shows that MK-0457 inhibits JAK-2 with an IC_{50} of 123 nM for wild type JAK2 and 295 nM for the JAK2 V617F activating mutation. *Aims.* The aim of this study was to determine the tolerability of MK-0457 in patients with hematological malignancies, including patients with myeloproliferative disorders (MPD). *Methods.* After IRB approval, eleven consenting patients with documented JAK2-mutated MPD, including 2 patients with prior MPD progressing to AML, were treated with MK-0457 at either 20, 24 or 28 mg/m^2/hr administered by a continuous 5-day intravenous infusion every 3 weeks. The age range was 37- 83 (median 64); 5 (45%) patients were male and performance status was 0 or 1 in 10 (91%) patients. Five (45%) patients were previously splenectomized and 2 (33%) of the non-splenectomized patients had splenomegaly present at baseline. Two (18%) patients had diploid cytogenetics, eight (73%) were pseudodiploid with varying abnormalities and one (9%) had a -17 abnormality. *Results.* One patient with hepatosplenomegaly at enrollment experienced complete resolution of hepatomegaly and >50% reduction in splenomegaly with a single cycle of therapy. Of the 10 (91%) patients starting therapy with a normal or increased absolute neutrophil count, all experienced grade 3 or 4 neutropenia during the study period. Transient reductions in platelet counts of patients with normal or elevated baseline platelets were also seen. Six of 7 (86%) patients with serial JAK2 testing had grad-

ual, yet steady reductions in percentage of PCR product with the V617F mutation. *Conclusions.* MK-0457 is worthy of further exploration at lower doses (20 mg/m²/hr) which are more suitable for chronic administration in patients with less aggressive disease, in contrast to patients with AML transformation of JAK2-mutated MPD in which higher, more myelosuppressive doses may be warranted.

0652

THE JAK2-V617F MUTATION IS A RISK FACTOR FOR VENOUS BUT NOT FOR ARTERIAL THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA

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Background. Essential thrombocythemia (ET), the most benign disorder of the group of chronic myeloproliferative disorders (MPDs) is considered as a prothrombotic state without any significant reduction of life expectancy. The morbidity in this disorder is characterized by the occurrence of thromboembolic events or bleeding. Previous thrombotic events, older age or high platelet counts (>1000G/L) are associated with an increased risk for thrombosis or bleeding. An additional occurrence of thrombophilic parameters in ET like a factor V Leiden mutation or the prothrombin mutation may potentiate the risk for thrombosis. The pathophysiology of thrombophilia and bleeding in ET is not elucidated. A major step towards understanding the ethiology of MPDs was the discovery of a somatic mutation in the Janus kinase 2 (JAK2-V617F) gene that can be found in about 50% of ET patients. Recent studies have postulated that JAK2-V617F might be considered as a risk factor for thromboembolic events. The aim of this study was to evaluate the influence of the JAK2-V617F mutation on different types of thrombotic events (venous versus arterial thrombotic events) in a well characterized cohort of ET patients.

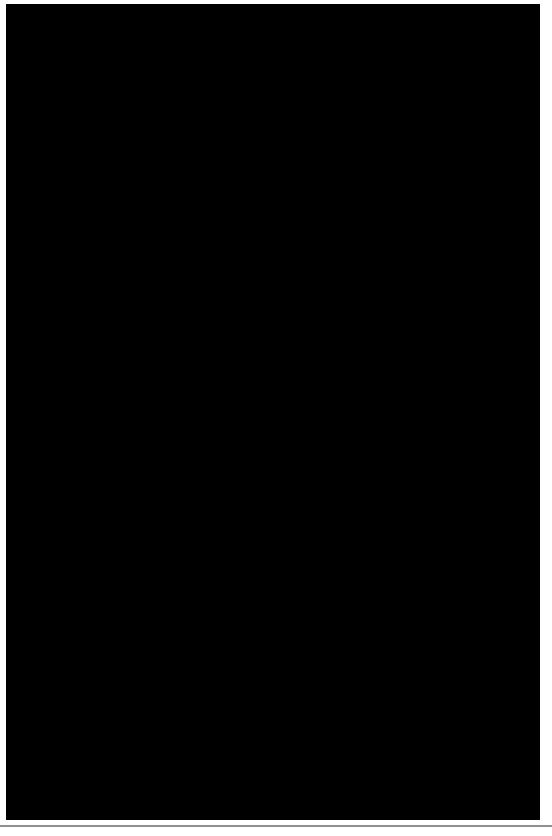

Figure 1. Association of JAK2-V617F mutation with thrombosis.

Patients and Methods. We investigated a cohort of 97 ET patients (55 female, 42 male; median age 66 years, range 25-93 years) and analyzed the influence of the JAK2-V617F mutation on arterial thrombosis, venous thrombosis and bleeding complications. The statistical analysis was done using Kaplan-Meier survival estimates. *Results.* The presence of the JAK2-V617F mutation shows a trend towards higher mortality in patients with the mutation (p=0.098), however, the relatively small number of patients might diminish a statistically significant difference between the two groups. Considering all venous thrombotic events from the time from birth, there was a statistically highly significant difference between those patients who had the JAK2-V617F mutation compared to the wild-type patients (p=0.0008). This highly significant statistical difference was diminished when the patients were observed only during the time from diagnosis (p=0.1). There was no statistical significant difference for arterial thrombosis taking the observation time from birth as well as from diagnosis (p=0.52 and 0.57 respectively). The same was true for the bleeding events as there was no difference between the frequency of bleeding in patients with the JAK2-V617F mutation compared to the wild-type patients. The combined analysis of arterial and venous thrombosis diminished the statistically highly significant difference and did only show a trend (p=0.07) towards more thrombotic events in the patient group who had the mutation.

0653

DIFFERENCES IN TOLERABILITY OF TWO COMMERCIAL ANAGRELIDE FORMULATIONS MAY BE CAUSED BY DIFFERENCES IN BIOAVAILABILITY

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Background. Anagrelide is an important drug for the reduction of platelets in patients with thrombocythemia. It is commercially available from two manufacturers (Xagrid/Agrylin®, Shire, England and Thromboreductin®, AOP, Austria). Although most clinical studies investigating the platelet-reducing efficacy of anagrelide have revealed similar results, there is a striking divergence concerning adverse effects with discontinuation rates from 8 to about 28% This may be explained by the different experience of hematologists handling the drug, patient selection and study conduct. Alternatively, the difference in tolerability may be due to the fact that either Xagrid/Agrylin® or Thromboreductin® were used in these studies (e.g. PT-1 and ANAHYDRET). Adverse effects of anagrelide are mediated through the phosphodiesterase 3 (PDE 3) system which is present in platelets and in the cardiovascular and cerebral system. Whereas anagrelide and its active metabolite 3-hydroxyanagrelide are equipotent with regard to their action on the megakaryocyte, 3-hydroxyanagrelide is 40 times more potent as an inhibitor of PDE 3. *Aims.* 1. to analyze the pharmacokinetic parameters of anagrelide and its metabolites in healthy volunteers on Xagrid/Agrylin® or Thromboreductin? and relate these to the adverse event profiles and 2. to compare the platelet reducing activity of both anagrelide formulations in the same patient cohort. *Methods.* In a bioequivalence study with 42 healthy volunteers on either Agrylin/Xagrid® or Thromboreductin® anagrelide and metabolites (3-hydroxyanagrelide and FL603) were determined by a combined HPLC-MS procedure. In addition to the pharmacokinetic parameters adverse events were recorded. We also compared *in vitro* dissolution profiles of Agrylin/Xagrid® and Thromboreductin® in an assay mimicking the intragastric milieu. The platelet reducing efficacy of Xagrid/Agrylin® and Thromboreductin® was studied in 33 patients with thrombocythemia which were switched from one formulation to the other. *Results.* The raise and fall of the major metabolite 3-hydroxyanagrelide is delayed by about 45 minutes when compared to anagrelide. The cmax and AUC values of 3-hydroxyanagrelide represent about 50 and 65% of the corresponding values for anagrelide. When the anagrelide plasma levels after the ingestion of either Xagrid/Agrylin® or Thromboreductin® are compared in healthy volunteers different profiles become evident: Cmax and AUC are significantly lower for Thromboreductin® when compared to Xagrid/Agrylin®. In the group taking Xagrid/Agrylin® there was an increased rate of adverse events (particularly headache and dizziness). In the *in vitro* dissolution assay anagrelide from the Xagrid/Agrylin® capsule is released within 5 minutes to reach the 90% level whereas it takes over 30 minutes for anagrelide in the Thromboreductin® capsule to reach this level. Both anagrelide formulations showed equal efficacy in maintaining platelet counts in patients with thrombocythemia within the predefined margin of clinical equivalence. *Conclusions.* Xagrid/Agrylin® and Thromboreductin® show different pharmacokinetics which are caused by different intragastric dissolution profiles and hence intestinal absorption rates. In particular, the different pharmacokinetics of 3-hydroxyanagrelide may explain why they have an equal platelet reducing activity but differ in tolerability in various clinical trials.

Myeloproliferative disorders
Chronic myeloid leukemia

0654

SKIN CHANGES ON CONTINUED IMATINIB THERAPY IN PATIENTS WITH PH+ CML

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Background. We were the first to report the skin changes (generalized hypopigmentation) caused by imatinib mesylate (IM) in patients with Ph+ CML or GIST (EHA 2004). Subsequent reports from MDACC confirmed such findings. IM appears to interfere with the melanin metabolism through the tyrosine kinase pathway and such changes could be different and more profound among the brown skinned people of India. *Aims.* To document the dynamics of skin changes among the Indian patients who were on regular IM therapy for Ph+ CML. *Methods.* A cohort of 200 Ph+ CML patients at various phases of the disease receiving IM in a daily dose of 300 mg - 800 mg (children 200 mg daily) were studied over a median period of 12 months (range 3-72 months). *Results.* Skin changes of any kind or severity were noted in 120 (60%) patients. Most changes were evident within first 6 months of IM and in majority within first 6 weeks of initiation of therapy. Hypopigmentation alone (usually generalized) was seen in 75 patients (32.5%), combined hypopigmentation and hyperpigmentation in 39 (19.5%), hypopigmentation + extreme thinning of the skin in 3 (1.5%) and the classical skin rash of grade 3/4 in 2 (1%). Skin changes occurred at all dose levels. Characteristically the hyperpigmented patches were localized to the facial area over the forehead and/or in a butterfly pattern across the malar areas and bridge of the nose. Cosmetically these were disturbing for most of the patients. There was no sex predilection and lesions were non-itchy. On continued IM therapy the skin changes remained unchanged. IM dose was not modified among patients with pigment changes alone. However, in the 3 patients with extreme thinning of the skin (generalized) causing easy bruising or desquamation at the nail bases, the dose of IM decreased to 400 mg (from 600 mg) in 2 and to 300 mg (from 400 mg) in one. In the 2 patients with classical IM skin rash of grade 3/4, recommended guideline for therapy was followed. *Conclusions.* IM causes dose-independent hypo- or hyperpigmentation of the skin in a significant number of Indian patients with Ph+ CML. This apparently happens due to modified melanin metabolism through the tyrosine pathway. Some of these changes (hyperpigmentation and extreme thinning) are bothersome from cosmetic viewpoint.

0655

NILOTINIB THERAPY INDUCES RESPONSE IN ADVANCED STAGE CML PATIENTS INCLUDING THOSE IN BLAST CRISIS AND FAILING PRIOR DASATINIB

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Background. The second generation Tyrosine kinase inhibitor (TKI) nilotinib is 30-50 times more potent than imatinib *in vitro* against CML cell lines and is active against most clones with BCR-ABL kinase domain (KD) mutations; therefore it has been studied in patients (pts) with CML failing imatinib therapy. *Aims.* Here, we report the results of thirteen CML pts with resistance or intolerance to imatinib treated with nilotinib 400 mg bid. Pts and *Methods.* Mean age of the pts was 55 years (range 26-77), there were 8 men and 5 women, 6 with chronic phase (CP), 5 with blast crisis (BC) (myeloid-3 and lymphoid-2) and 2 with accelerated phase (AP) CML. Eleven pts were imatinib resistant and 2 intolerant to imatinib. Four pts were treated with dasatinib prior to nilotinib and started on nilotinib due to resistance (3 pts) or intolerance to dasatinib (1 pts). All pts were screened for ABL KD mutations at baseline using a novel, sensitive, MALDI-TOF based assay (SEQUENOM MassARRAY® system, Sequenom, San Diego, CA) designed to detect 27 of the common ABL KD mutations (Leukemia 2007, in press). Mutation status was verified by sequencing. Three mutations were found in 2 pts at baseline including F359V, M244V (same patient, AP) and Y253H (BC). *Results.* After a median follow up of 5 months (range 1-9) CHR was achieved in 5/10 pts (BC-3, CP-1, AP-1), 2 BC pts have returned to CP and 1 pt has maintained a previously achieved CHR (overall hematological response rate 80%). Pts not achieving CHR included a CP pt with primary resistance to imatinib and a pt with AP with a transient prior CHR. For 3 CP pts it is too early to assess response. CCyR was achieved in 3/10 pts without a CyR at baseline, one pt has maintained a previously achieved CCyR and one pt has achieved a major CyR (overall CyR 50%). Nilotinib was discontinued in 5 pts due to progressive disease (BC-1), performance of an Allo SCT (BC-1), administration of additional chemotherapy (AP-1, CP-1) and side effects (CP-1, grade 2 bone pain). One BC pt has died due to disease progression. Among the four pts with prior dasatinib therapy, one pt with disease progression on dasatinib and AP before starting nilotinib have reached a CCyR, one pt intolerant to dasatinib is maintaining a previously achieved MMR on nilotinib, and two other pts are too early to evaluate. Following 6 months nilotinib therapy the pt with AP harboring 2 mutations has not reached a CHR. We observed a disappearance of the M244V mutation while F359V mutation level has remained unchanged. An additional pt with myeloid BC achieving CHR with nilotinib has subsequently acquired the T315I mutation at disease progression. The most common non-hematological side effects were bone pain and rash in 5 and 3 pts, respectively, necessitating drug withdrawal in 1. *Conclusions.* Nilotinib therapy may result in CHR and CCyR in advanced stage CML pts including BC, and in those failing prior Dasatinib therapy.

0656

PEGYLATED IFN-ALFA 2A COMBINED TO IMATINIB MESYLATE (IM) 600 MG/DAY CAN INDUCE COMPLETE CYTOGENETIC AND MOLECULAR RESPONSES IN A SUBFRACTION OF CHRONIC PHASE (CP) CML PATIENTS REFRACTORY TO IFN ALONE AND TO IM 600 ALONE

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Most CP CML patients are sensitive to IM, but some resist at different levels. Higher doses (≥600 mg/d) of IM may restore disease sensitivity of resistant patients to 400 mg/d, but usually fail. In the past, IFN-alfa has demonstrated its usefulness and is able to induce long-term disease control, despite important side-effects. Here, we prospectively combined IM 600 mg/day to PegIFN-alfa2a 90 microg/wk for ≥12 months in patients exhibiting a CHR but <MCyR to IM, after being exposed to IM≥600 mg/d for ≥3 months, and lacking a histocompatible donor. Patients were assessed every month clinically and every 3 month for BM karyotype, centralised BCR-ABL RQ-PCR, VEGF plasma value for a year. Fifteen patients were enrolled [9 M and 6 F, median age 50 (34-63) years]. Sokal scores were high for 3, intermediate for 8, low for 3 and unknown for 1. All patients had a common Ph1 at diagnosis except 2 [Ph1 duplication, additional t(2;5)]. Eleven patients had IFN prior to IM for a median of 16 (4-119) months with 4 patients achieving PCyR, 1 minor CyR, 4 CHR, and 2 no response. All IFN patients had IM because of insufficient response to IFN except 1 (intolerance). Three patients had had an autograft. The median time diagnosis-IM 400 was 28 (0.2-132) months, and 4 patients achieved CCR, 2 PCyR, 4 minor CyR, 5 CHR only, to IM. The median interval between IM 400 and IM 600 was 15 (0-42.4) months. At entry, 7/15 patients had a clonal evolution [+8 (3 patients), Ph1 duplication (2 patients), deletions (3 patients: del(7), del(20), del(3))], and 6 patients harbored a BCR-ABL mutation (M244V, D276G, L298V, M351T, 2 H396R), accounting for 100% of total BCR-ABL transcripts. Median% of Ph1+ BM mitosis was 88 (35-100)%, median BCR-ABL/ABL international normalized ratio was 26 (2.6-106.5)%, and plasma concentration of VEGF 242 (12.8-1650.2) ng/ml. Median follow-up was 27 (13.5-30.4) months. Eight patients were withdrawn from the study at various times for lack of efficacy or recurrent Grade 3-4 hematological toxicity. A gradual decline with median values at 12 months. for BM Ph1+ mitosis, BCR-ABL/ABL ratios and VEGF concentrations of 76 (0-100)%, 15 (0.03-66)% and 145 (83-171) ng/ml respectively. At 12 months, 2 patients were in CCR, 1 in MCyR and 4 in minor CyR, of whom at latest time-point with the same treatment 2 remain in CMolR. Among patients with BCR-ABL mutations at screening, 2/6 experienced transient cytogenetic improvent, 2 patients with clonal evolution did as well. One patient developed a T315I mutation (75% of the transcripts at M9) and was immediately withdrawn from the study. The combination of drugs was mostly well tolerated with 86% of the intended 600 mg/day of IM and 78% of the 90 microg/wk of PegIFN-alfa delivered, over a year. Limiting toxicities were mostly hematologic grade 3-4, particularly in autografted patients. In conclusion, even in cytogenetically refractory patients, the combination of high doses of IM+PegIFNalfa-2a is able to induce significant responses with an acceptable safety profile

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ASSESSING THE LONGTERM EFFECT OF IMATINIB MESYLATE IN BONE METABOLISM OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background and Aims. Imatinib mesylate (IM) inhibits several tyrosine kinases, including BCR-ABL, the C-KIT receptor, and the platelet-derived growth factor receptors α and β, all of which are associated with disease. Recently, IM has been shown to alter bone metabolism by inhibiting osteoclast differentiation and function in *in vitro* models. In the present study, bone metabolism markers were determined in a cohort of chronic myeloid leukaemia (CML) patients who were extensively treated with IM. *Patients and Methods.* Seventeen CML patients [M/F: 11/6, median age: 62(48-74) years, median time on IM: 48(10-56) months, median daily dose of IM: 400(400-800) mg] and 10 healthy volunteers [M/F: 7/3, median age: 57(38-64) years] were studied. All CML patients were in chronic phase when IM was started. At the time of study, 13/17 patients were in complete cytogenetic response (cR), one patient was in partial cR and 3 patients were in hematological remission. We measured the plasma levels of intact parathyroid hormone (PTH) and the serum levels of total calcium (Ca) and phosphate (P). Evaluation of the bone formation was made by measuring total alkaline phosphatase (ALP), procollagen type I N-terminal propeptide (PINP) and osteocalcin (OSC). Bone resorption was assessed by determination of the plasma C-telopeptide of collagen cross-links (CTX). None of the patients had metabolic bone disease, and none was receiving medications known to influence the metabolism of calcium. *Results.* Hypocalcemia (total Ca<8.8 mg/dL) was detected in 3/17 (18%) patients and hypophosphatemia (p<2.8 mg/dL) in 9/17 (53%) patients. Hyperparathyroidism (intact PTH>65 pg/mL) was revealed in 6/17 (35%) patients. Patients with high PTH levels, when compared to the subgroup of patients with normal PTH levels, had also significantly elevated OSC [median level: 24.3(8.5-44) ng/ml vs 10.2(4.6-15.2) ng/mL, p:0.015] and CTX [median level: 0.276(0.1-0.747) ng/ml vs 0.087(0.021-0.237) ng/mL, p:0.003]. There was no difference in the time of administration and the dosage of IM between the two subgroups. IM patients, in comparison to healthy volunteers, had higher PTH [45.5(32-194) pg/mL vs 34.3(24-60) pg/mL, p: 0.05] and total Ca [9.5(6.7-13.6) mg/mL vs 8.2(6.9-10) mg/ml, p: 0.05] and lower p [2.7(2-3.9) mg/mL vs 3.5(3-4) mg/mL, p:0.001]. Bone formation/resorption markers' levels were similar in the two groups. *Conclusions.* In the present study, secondary hyperparathyroidism and subsequent hypophosphatemia were detected in a significant proportion of IM pretreated patients. Determination of P and PTH levels at baseline and regularly during administration seems a reasonable measure in patients treated with IM.

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COMPARISON OF GRADE 3/4 ADVERSE EVENTS (AES) OF SECOND GENERATION TYROSINE KINASE INHIBITORS (TKIS) FOR IMATINIB RESISTANT/INTOLERANT PATIENTS IN ACCELERATED PHASE CML (CML-AP)

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Background. Toxicity profiles of targeted therapies for CML may impact the resources required to manage patients, as well as the ability to maintain the planned treatment regimen. *Aims.* The objective of this study was to systematically compare the incidence rates of Grade 3/4 AEs reported for nilotinib and dasatinib in imatinib resistant/intolerant CML-AP in preparation for development of an economic analysis. *Methods.* A systematic literature review was conducted using online databases to identify published articles, registration studies, or abstracts of trials for nilotinib 400 mg BID or dasatinib 70 mg BID in CML-AP. The base case incidence rates were based on accelerated phase patient data from the dasatinib product label and the 120 day update of the nilotinib phase II pivotal trial. Grade 3/4 AEs were selected because of likely economic impact for medical treatment/monitoring. The ranges (if available) for incidence of AEs were summarized from other publications to provide for sensitivity analyses. *Results.* The incidence of Grade 3/4 AEs having likely economic impact and occurring in > 5% of patients are reported in the table. Grade 3/4 hematologic AEs for dasatinib were 2-3 times greater compared to those for nilotinib. Biochemistry abnormalities occurring in > 5% of nilotinib patients included lipase increase in 16.8%, elevated bilirubin in 10.3%, and hypophosphatemia in 9.7%; dasatinib patients experienced hypophosphatemia in 13% and hypocalcemia in 9%. Grade 3/4 non-hematologic AEs such as pleural/pericardial effusions were also higher for dasatinib than nilotinib. *Summary and conclusions.* The toxicity profiles of the second generation TKIs for imatinib resistant/intolerant CML-AP are different. Nilotinib's lower rates of Grade 3/4 hematologic AEs and GI hemorrhage may result in reduced resource utilization. Further research is warranted to assess the economic impact of the differences in safety profiles.

Table 1. Grade 3/4 AE rates: nilotinib v. dasatinib.

	Base AE Occurrence Rate % (Range)	
Grade 3/4 Adverse Event	Nilotinib	Dasatinib
Anemia	22.9 (13.5-30.4)	70.0 (67.3-80.0)
Neutropenia	37.1 (14.6-43.8)	74 (71.0-81.8)
Febrile Neutropenia	2.5	11
Thrombocytopenia	37.4 (28.0 -39.4)	83 (79.4-83.0)
Diarrhea	1.7 (1.7-2.2)	10
GI Hemorrhage	1.5 (1.1-1.5)	12 (0.0-12.0)
Pneumonia	4.2 (4.2-5.6)	8 (3.4 – 8.0)
Infection	6.6 (4.5-6.6)	8

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IMATINIB EFFICACY AND TOLERABILITY IN ADVANCED AGE (>70 YEARS) CML PATIENTS

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In the last years, CML prognosis had improved after introduction of tyrosine-kinase inhibitor imatinib-mesylate, that induces a complete cytogenetic response in about 80% of patients when used as a front-line therapy in chronic phase (O'Brien SG *et al.* N Engl J Med. 2003;348: 994-1004) However, the optimal CML treatment in advanced age has not been definitely established. Before imatinib advent, old CML patients were handled just with palliative chemotherapy because of the impossibility for such patients to tolerate bone marrow transplantation or α interferon. Moreover, age is a prognostic negative factor for survival in CML (Sokal JE et al.: Blood 1984; 63: 789-99); therefore, most of old CML patients likely died of blastic phase CML. Imatinib has opened new therapy opportunities for advanced age patients. However, in spite of a median age for CML patients around 60, most of Imatinib studies mainly involved patients below that age. Two papers actually reported similar tolerance and efficacy of imatinib below and above the age of 60 (Cortes J et al.: Cancer. 2003; 98:1105-13; Latagliata R et al.: Leuk Res. 2005; 29:287-91) but no information has been provided about older patients, above the age of 70. Indeed, median age was 66 in one of the two papers and not reported in the other. Therefore, some doubts still exist about imatinib tolerability and cost effectiveness in old patients. In this study, we evaluate Imatinib tolerance and efficacy in 37 CML patients at or above the age of 70 (median age at the beginning of imatinib treatment was 76, range 70-87). One patient was treated in blastic phase, four in accelerated, one in second chronic and 31 in first chronic phase. Sokal score of first chronic phase patients was: low risk in 6 patients, intermediate in 18 and and high risk in 6. Seventeen chronic phase patients received imatinib as a front-line treatment, the other had received different previous therapies. The great majority presented with associated comorbidities. Grade 3-4 fluid retention was significantly more frequent (13%) than previously reported for younger patients. Conversely, the frequency of other hematological (neutropenia and thrombocytopenia) and extra-hematological toxic effects (cutaneous

rash, muscolar cramps, diarrhoea, hepatotoxicity), was not different from that reported in literature. Only 5 of 37 patients with severe comorbidities discontinued imatinib therapy for toxicity. Response rate (87% complete hematological and 56% complete cytogenetical remission in chronic phase patients) was just a little lower than reported for younger patients. All patients treated in 1st chronic phase are alive after a median 4 year follow up. Five of the 6 patients treated in advanced phase died (4 of CML progression, 1 of second neoplasm) at 3-25 months from diagnosis. In conclusion, this study shows that Imatinib therapy can be fairly tolerated and provide cytogenetic complete responses and long-term survival also in advanced age patients in chronic phase CML. It can be regarded as the treatment of choice for very old patients too, unless affected by severe comorbidities with short life expectancy.

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HYPOPHOSPHATAEMIA IN PATIENTS RECEIVING IMATINIB THERAPY

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Background. A recent report found a high incidence of hypophosphataemia and changes in bone metabolism in patients receiving imatinib therapy.[1] *Aims.* To determine whether there is a similar significant incidence of hypophosphataemia in patients receiving long-term imatinib therapy for haematological disorders within our institution and identify any factors that may be linked to development of hypophosphataemia. *Methods.* The records and biochemistry results of patients receiving imatinib therapy for haematological disorders for >1 month duration were reviewed to identify development of hypophosphataemia (phosphate <0.80 mmol/L) and determine whether there were any differences with respect to age, sex, dose of imatinib, calcium levels, pre-imatinib phosphate levels and duration of therapy between the hypophosphataemic and normophosphataemic patients. *Results.* Thirty-one of forty-seven (66%) patients (30 males, 17 females) receiving imatinib were identified as having at least one low serum phosphate level (median 0.71 mmol/L, range 0.50-0.79 mmol/L) with one female patient with pre-existing hyperparathyroidism excluded from further analysis. Imatinib therapy was predominantly for chronic myeloid leukaemia but also Philadelphia positive acute lymphoblastic leukaemia (2 patients) and hypereosinophilic syndrome (1 patient). Median time to onset of hypophosphataemia was 7 months (range <1-20). The hypophosphataemic group had a significantly higher proportion of males (25 males: 5 females) compared to the normophosphataemic group (5 males: 11 females, $p<0.0008$). There were no differences with respect to imatinib dose (median 400 mg/day both groups, range 100-800 mg/day), age (median 50.5 versus 52 years, range 16-81 years) or calcium levels pre-imatinib therapy between the groups (2.28 mmol/L both groups) although there was a trend to longer duration of therapy in the hypophosphataemic group (38 months versus 22 months, $p=0.07$). All patients had lower phosphate levels post -imatinib with median 0.95 mmol/L compared to pre-imatinib level of 1.20 mmol/L ($p<0.0001$). Phosphate levels were lower pre-imatinib in the hypophosphataemic group with median 1.18 mmol/L compared to 1.27mmol/L in the normophosphataemic group ($p=0.004$). There was no difference in phosphate levels between males and females pre-imatinib therapy, but males had lower median phosphate levels 0.92 mmol/L compared to 0.99 mmol/L in females post-imatinib ($p=0.0009$). Pre-imatinib calcium levels were lower in males 2.27 mmol/L compared to 2.29 mmol/L in females ($p=0.04$). Post-imatinib calcium levels were lower in the hypophosphataemic group 2.26 mmol/L compared to 2.28 mmol/L in the normophosphataemic group ($p=0.007$) and in males 2.25 mmol/L compared to 2.31 mmol/L in females ($p<0.0001$). *Conclusions.* Hypophosphataemia is a common finding associated with lowering of calcium levels in patients receiving imatinib for haematological disorders and may warrant treatment and surveillance for effects on bone metabolism when imatinib is used long-term. Risk factors identified for development of hypophosphataemia include male sex and relatively lower phosphate levels pre-imatinib therapy.

Reference

1. Berman E et al. Altered Bone and Mineral Metabolism in Patients Receiving Imatinib Mesylate. N Engl J Med 2006;354:2006-13.

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FIBROBLASTOID CONVERSION IS OBSERVED IN CHRONIC MYELOGENOUS LEUKEMIA CELLS WITH THE LONG-TERM LIQUID CULTURE

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Introduction. On chronic myelogenous leukemia, especially in the accelerated phase or blast phase, fibroblast cells are proliferated and bone marrow fibrosis is sometimes observed. These growing fibroblasts are reported to be originated from the normal clone and reactive. In this report we cultured non-adherent mononuclear cells obtained from chronic myelogenous leukemia patients, and characterized the obtained stromal fibroblastoid cells biologically and molecularly. *Materials and Methods.* Bone marrow cells were collected from 11 patients with chronic myelogenous leukemia (6 of chronic phase, 2 of accelerated phase, 2 of lymphoblast phase and 1 of myeloblast phase), which were separated with gravity sedimentation. Obtained non-adherent mononuclear cells were cultured in DMEM with 10% FCS in the humidified 5% CO2 incubator. When cells showed morphological changes into stromal fibroblastoid cells, they were treated with trypsin and further cultured. When whole cells were stromal fibroblastoid cells, cells were sub cloned into culturing in a 96 well plate, in which RNAs were extracted. cDNAs were synthesized from each clone, and RT-PCR was performed to identify Bcr-Abl fusion products. The selected clones were further analyzed on DNA levels with FISH to identify BCR-ABL translocation. The obtained clones were further analyzed on their expression of cell-surface molecules, activities of producing cytokines and characterization of their biological activities for additional effects on the growth of leukemia cells or normal bone marrow cells. *Results and Discussions*. After culturing 1 month a few BCR-ABL translocated fibroblastoid cells were identified in all chronic, accelerated and myeloblast phase but not in 2 lymphoblast-phase cases. These cells expressed CD 106, fibronectin, smooth muscle actin, and FSP1 that is expressed specifically in myofibroblasts but not in macrophages. Also the expression of myeloperoxidase, CD 13 and 33 were observed which are expressed in myeloid lineage cells. As the stem cell marker, CD 34 and 133 were also observed in these fibroblastoid cells. These cells produced cytokines such as VEGF, IL-6 and G-CSF more than that observed in normal bone marrow-derived fibroblasts. When the generated fibroblastoid cells were cultured with chronic myeloid leukemia cells or normal bone marrow-derived mononuclear cells, significant additive effects were observed with 3H-thymidine incorporation assays. These data indicate that in chronic myelogenous leukemia, a part of fibroblasts are from leukemia origin, and create the microenvironment to proliferate their own leukemia cells.

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THE EUROPEAN LEUKEMIANET CML REGISTRY - OBJECTIVES, ACHIEVEMENTS AND FIRST RESULTS

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Background. In 2004/2005, the European Chronic Myeloid Leukemia (CML) Registry was established within the European Leukemia Net (ELN) which is funded by the EU. *Aims.* The purpose is the prospective, standardized collection of baseline, follow-up and outcome data of CML patients treated with imatinib or with more recent tyrosine kinase inhibitors. Among the objectives are the development and validation of prognostic scores, the analysis of various prespecified subgroups, e.g. of patients with imatinib failure or of patients who discontinued imatinib after complete cytogenetic remission and to support the design of future clinical trials. *Results.* At present, individual patient data of 1872 bcr/abl positive CML patients from Denmark, Finland, France, Germany, Israel, Italy, Norway, Poland, Romania, Sweden, Spain, Switzerland and the Czech Republic are part of the data bank. Patients were treated with 400 or 800 mg imatinib/day as mono or combination treatment. About 41%

of the patients are female and median age at diagnosis was 50 years for males and 53 years for females. Using the New CML Score the proportion of low-risk patients varies between 34-53% (high risk: 5-23%) among the participating study groups. First analyses of outcome data indicate that the results of the IRIS trial can be reproduced. Having excluded patients with less than 20 evaluated metaphases or with FISH, 72.3% of 535 patients treated with imatinib 400 mg with or without co-medication had a complete cytogenetic response up to month 12 compared to 69.0% of the IRIS study. *Summary and Conclusions.* With more countries participating and longer observation times, the European CML Registry promises to provide highly interesting data.

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STABLE MOLECULAR REMISSION IN CHRONIC MYELOID LEUKEMIA PATIENTS MAINTAINED WITH LOWER DOSES OF IMATINIB MESYLATE BECAUSE OF INTOLERANCE

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Imatinib mesylate (IM) has became the standard of care for chronic myeloid leukaemia (CML) patients but it is unclear whether this therapy can be discontinued in patients with undetectable BCR-ABL transcripts by RQ-PCR and whether IM can cure CML. Between 2000 and 2005, 60 CML patients were treated with IM in our department. Twenty-seven patients were in late chronic phase refractory to interferon-α and 33 were newly diagnosed chronic phase patients. Forty-five (75%) patients achieved complete cytogenetic remission (CCyR) and in 11 (25%) patients BCR-ABL transcripts became undetectable by RQ-PCR. In this report, we want to address the question if the reduction of standard dosage of IM in CML patients with undetectable residual disease by RQ-PCR may impair their outcome. We describe the clinical history of 4 CML patients where the IM treatment was tapered to a lower dosage due to intolerance after those patients had achieved a molecular remission documented by RQ-PCR. The median follow-up from the beginning of IM therapy was 45 months (33-59). The median duration of undetectable transcripts levels by RQ-PCR on IM 200 mg daily was 18 months (3-36). At the time of this report, all patients are in molecular remission. We hypothesize that in IM intolerant CML patients in molecular remission the compound dosage might be safely reduced to a lower other than standard dose without to lose the response. In these cases, the amount of leukemic transcripts is low and lower doses might control the disease. Those patients should be monitored closely because the selection of resistant clones after prolonged IM exposure and the emergence of Philadelphia-negative clones with secondary cytogenetic abnormalities could be a matter of concern. However, it is important to underline that the probability to develop drug resistance is directly related to the level of BCR-ABL transcripts, the higher is its level the higher is the risk to become resistant. The improved quality of life in our patients, after reduction of IM dosage, suggests that the subset of intolerant patients who have a sustained molecular remission might be candidates for lower doses of IM. Our observation can promote a clinical trial to determine when, how and where it is possible to reduce IM standard dose in imatinib-intolerant CML patients.

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TREATMENT AND RESISTANCE TO IMATINIB IN CML PATIENTS: EXPERIENCE OF A PORTUGUESE CENTER

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Background. Chronic myeloid leukaemia (CML) is a myeloproliferative disorder characterized by identification of the Philadelphia chromosome (Ph) or the product of the fusion gene resulting from t(9;22), the hybrid BCR/ABL mRNA which encodes a oncoprotein with a constitutively active tyrosine kinase. Before Imatinib (IM), Interferon ± Cytarabine was considered standard therapy for the disease. Nowadays Imatinib, a potent and specific inhibitor of the Bcr-Abl tyrosine kinase, is the first choice treatment in CML. We decided to carry out a retrospective analysis of our patients treated with this tyrosine kinase inhibitor (TKi) emphasising disease monitoring. *Material and Methods.* Seventy two patients (29 female; 43 male; median age 47 years) were evaluated for haematological, cytogenetic and molecular response, and progression to accelerated phase or blast crisis. Patients were treated with IM standard doses; 30 of these 72 patients were treated with Interferon ± Cytarabine before IM. Twelve patients who failed IM were analysed for resistance, namely mutation analysis. *Results.* Patients were divided in two treatment groups: Imatinib as first line (Group A; 42 patients; follow up 5'50 months, median 42) and IM as second line (Group B; 30 patients; follow up 20-68 months, median 45). Complete haematological response was achieved in all patients in Group A and in 76% in Group B after 3 months of treatment. Major cytogenetic response was 94,1% (Group A) and 73% (Group B). Complete cytogenetic response was 79,4% (Group A) and 53,8% (Group B) at 12 months. Complete cytogenetic responders were monitorized by real time quantitative PCR. Major molecular response (Q-PCR<0,5%) was achieved in 68% of group A (BCR/ABL levels still decreasing after cytogenetic response, because of the short follow-up) and 81% of group B. Only 2 patients who had a complete cytogenetic response and a major molecular response by 18 months progressed to accelerated phase or blast crisis (one of them was in a second line treatment after a blast crisis). Six patients who failed IM were studied by DNA sequencing for mutation of BCR/ABL kinase domain. We identified mutation in 3 of them (p.M351T/p.E450K; p.F486S; p.D276G). In this group, 4 patients received IM 600 mg, 1 received Dasatinib 70 mg bid and 1 is on palliative treatment. *Conclusions.* IM is a highly effective in CML with impressive responses, mostly when used as front line therapy. Most patients achieved complete remission (hematologic and cytogenetic remission), but a great number retain variable levels of residual molecular disease. A subset of patients has failed or responded poorly because they have been developing distinct patterns of resistance, namely mutations in the BCR/ABL kinase. Other therapies incorporating new TKi or their combination would optimize IM response and eventually may erradicate the malignant disease.

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EPHA3 IS ABNORMALLY EXPRESSED IN CHRONIC MYELOPROLIPHERATIVE DISORDERS AND CAN BE TARGETED BY DASATINIB OR BY MONOCLONAL ANTIBODIES

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Background. Eph receptors tyrosine kinase are involved in many key developmental 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 different TKs *Aims.* 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. *Methods.* 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 CMML, 8 HES, 50 CML and 36 Ph- CML. In 10 patients and 10 healthy subjects $CD34^+$ cells were selected and analyzed by FACS for the presence of EphA3 protein. Protein expression and 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 45 EphA3+ patients. Finally samples were incubated with Dasatinib 20 nM for 6 hrs and evaluated for cell proliferation by incorporation of 3H timidine and MTT assay, apoptosis was analyzed by FACS (Annexin V) and colony growth by methylcellulose culture. *Results.* Normal BM, PB and $CD34^+$ cells are negative for EphA3 expression.(mean value of ΔΔ Ct=19) By contrast EphA3 was found significantly increased in 45% of PV (mean value of ΔΔ Ct= 11) in 55% of ET (mean value of ΔΔCt= 10,5) in 90% of CMML (mean value of ΔΔ=5), in 100% of IM (mean value of ΔΔ Ct=4), in 30% of CML (mean value of ΔΔ Ct=9), 15% of HES (mean value of ΔΔCt=12) and 80% of Ph-CML (mean value of ΔΔCt= 9). $CD34^+$ cells presented significantly higher levels as compared to the corresponding unfractioned sample (p=0,001). Western Blot and immunoflourecence confirmed the presence of EphA3 protein in EphA3 overexpressing cells and revealed abnormal phophorylation 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%±17) 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

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AUTOMATED FISH ANALYSIS PREDICTS GOOD OUTCOME IN CML PATIENTS WITH IMATINIB-INDUCED REMISSION AND DETECTS VERY LOW LEVEL LEUKAEMIC CELL POPULATION WITH DOUBLE BCR-ABL REARRANGEMENT

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*Background.*Minimal residual disease (MRD) analysis is essential for establishing the efficacy of therapy in chronic myeloid leukemia (CML) patients. *Aims.* Seventy-six patients with CML, 69 in chronic phase, 1 in accelerate phase, and 6 in blastic crisis, were treated with imatinib mesylate (IM). We used automated FISH analysis to evaluate the MRD as a predictor of efficacy of IM therapy. *Methods.* The patients were studied for a 18-66 months follow up period using cytogenetics and FISH analysis with a dual-fusion BCR-ABL probe, and an automated FISH imaging system for rare cell events (BioView-Duet). Before IM treatment 50 patients showed Ph chromosome in all examined cells while in the remaining 26 patients a normal clone was also found. *Results.* Complete or partial cytogenetic remission rate [CCR or PCR: t(9;22) absent or <35% of cells, respectively] overlapped complete or partial FISH remission rate (CFR or PFR: BCR-ABL absent or <35% of cells, respectively) in 74% of patients during the follow up period. However, CFR achievement within 12 months of treatment resulted in a disease-free second year of treatment in 97% of patients as previously reported using QF-PCR. Forty-three percent of CCR samples actually showed >0.5% leukaemic cells by FISH. Two cases showed 0.08% cells with double BCR-ABL (i.e. double Ph), undetectable by QF-PCR, which disappeared after increasing IM dosage. Moreover, FISH unravelled leukaemic cells in 21% of samples unsuitable for cytogenetic investigation from 9 patients who subsequently developed haematological relapse. In 11 patients with partial deletion on der(9)(q34) at treatment start, no CCR/CFR was achieved. Clinical and haematological relapse occurred in 5 cases, while in the remaining 6 a PCR/PFR was observed only after 30 months of treatment supporting a negative role of der(9) deletion on IM effect in CML patients. *Conclusions.* Our experience underlines the importance of automated FISH analysis in predicting the outcome of CML patients treated with imatinib, and detecting very low level of leukaemic cells.

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EFFECTS OF HYPOMETHYLATING AGENTS AND HDAC INHIBITORS ON THE EXPRESSION OF CXCR4 IN CD34+ CELLS OF IDIOPATHIC MYELOFIBROSIS

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Background. Primary Myelofibrosis (PMF) is characterized by increased of CD34+ hematopoietic stem/progenitor cells in the peripheral blood (PB). The mechanisms inplicated in CD34+ cell release in PMF are not completely clarified but the involvement of the SDF-1/CXCR4 axis is supported by several observations. More recently, 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. *Aims.* To characterize the possible role of CXCR4 abnormalities in the constitutive CD34+ cell mobilization in PMF. *Methods.* CD34+ cells, isolated from the PB of PMF patients, were analysed by FACS analysis to study CXCR4 RNA regulation. *Results.* As supported by previous study (Guglielmelli *et al.*, Stem Cells, 2007:165-73) using a global gene transcriptional profiling approach, we have 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. Using Real Time PCR, we found that RNA levels for CXCR4 were reduced compared to normal BM-derived cells ($p<0.001$). CXCR4 RNA were also significantly lower in the granulocytes from PMF compared to healthy subjects ($p=0.01$). Furthermore, CXCR4 RNA levels 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 SDF-1, G-CSF, TGF-β and Interferon-γ, that all have been supposed to play some role in PMF pathogenesis and/or in CD34+ mobilization. We found that only SDF-1 was able to down-regulate the membrane content of CXCR4, while the mRNA levels were unchanged, to suggest ligand-induced receptor internalization but no effect on gene transcription. We hypothesized that epigenetic modifications might be involved in the down-regulation of CXCR4. As a cell model, HEL cells were treated with 5-azacytydine (5-AZA) and SAHA, as well as the CD34+ purified from 10 PMF patients. In both these models, we observed significant up-regulation of CXCR4 expression on the membrane; the combination of 5-AZA and SAHA determined only minor increase over the levels obtained with AZA only. The increase of CXCR4 could be detected as soon as after 24 to 48 hrs of incubation, and was not due to mobilization to the membrane of the intracellular pool of CXCR4 as demonstrated with confocal microscopy. However, the effects on CXCR4 RNA levels were minimal. There was a discrete methylation pattern of CpG island of CXCR4 (methylation-specific PCR) in both HEL and PMF CD34+ cells, while HL-60 cells that do not express detectable amount of CXCR4 had extensive methylation pattern of CXCR4 gene promoter. A partial methylation pattern of CXCR4 CpG island was observed also in CD34+ of PMF patients. *Conclusions.* In conclusion, these data indicate the possibility to up-regulate CXCR4 membrane expression in CD34+ cells of PMF patients using hypomethylating agents, through a mechanism that might act on specific methylated regions of gene promoter.

0668

INCREASED DOSE OF IMATINIB FOR SUBOPTIMAL RESPONSE IN CML IN CHRONIC PHASE. RESULTS OF THE SPANISH REGISTRY OF CHRONIC MYELOID LEUKEMIA (RELMC)

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Background. Imatinib (standard dose 400mg/day) is highly effective in patients with CML in early chronic phase (CP) or in Interferon resistant patients. Therapeutic options for CML resistant or with suboptimal response to imatinib 400 mg are limited. Escalating imatinib doses may overcome resistance and improve hematological and cytogenetic response. *Aims.* We analyze the outcome of those patients who increase imatinib dose in the Spanish Registry of CML. *Methods and Results.* Among the entire population of 330 patients with CML in CP, 39 (11,8%)(14 women and 25 men) received imatinib 600 mg after resistance or suboptimal response to imatinib 400 . Median age was 42 years. Sokal index at diagnostic was high in 8, Intermediate in 12, low in 17) . Eleven were interferon resistant. With median follow-up of 30 months, complete hematologic responses were observed 85% of patients and best cytogenetic response was complete in 17 (43,5%) and partial in 6 (15%).4 patients obtained molecular response 19 patients (6%) received Imatinib 800 (9 upfront, 10 after Imatinib 600). Sokal index at diagnostic was (high 2 Intermediate 4 low 12). With median follow-up of 26 months, complete hematologic response were observed in 76% of patients and best cytogenetic response was complete in 5 (26%) and partial in 4 (21%). 1 patient obtained molecular response. 5 patients reduced dose to 600 and 3 changed to dasatinib. In Table 1 we present toxicity more prevalent in each group of treatments (maximum grade per patient). Grade 3-4 non-hematologic toxicity was minimal. Superficial edema and fluid retention (36% and 41%) was more prevalent. Cytopenias were not more frequent with 800 mg. *Conclusions.* In our sample, one out of six patients with CML-CP receive increased doses of Imatinib.The use of Imatinib 600 or Imatinib 800 in our population appears to be safe

and effective therapy for CP-CML resistant to conventional imatinib doses, with improved cytogenetic and molecular response rates, and acceptable toxicity.

Table 1.

TOXICITY				
	Grade 1-2		Grade 3-4	
CTC	Imatinib 600 mg	Imatinib 800 mg	Imatinib 600 mg	Imatinib 800 mg
Astenia	20,5%	16%		
Edema	33%	31,5%	2,5%	10%
Hypophosphatemia	2,5%	26%	0%	5%
Pain	2,5%	5%		
Cramps	13%	10,5%		
Anemia	5%	5%		
Neutropenia	5%	5%	0%	5%
Thrombocytopenia	0%	5%		

0669

IMATINIB MESYLATE SIGNIFICANTLY DECREASES PLASMA LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND SOLUBLE ENDOGLIN BUT NOT BASIC FIBROBLAST GROWTH FACTOR IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Introduction. Angiogenesis is currently considered an important factor in biology of various hematological malignancies including chronic myeloid leukemia (CML). Several studies have recently reported elevated levels of key angiogenic activators such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in CML patients. However, there have been only few data on the influence of imatinib mesylate (IM) treatment on the levels of angiogenic cytokines in CML. *Aims.* To analyze peripheral blood levels of angiogenic activators in patients with newly diagnosed CML and during imatinib treatment. *Methods.* We measured plasma concentrations of VEGF, bFGF and soluble endoglin (sCD105) using sandwich enzyme-linked immunosorbent assay (ELISA) in 16 patients with chronic-phase CML and 80 healthy blood donors; furthemore, samples collected at the time of hematological remission and cytogenetic response (available in 14 patients) were analyzed.

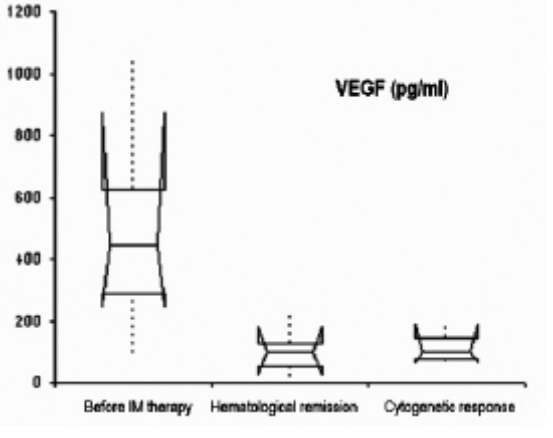

Figure 1. VEGF before and during imatinib mesylate therapy.

Results. We found a statistically significant increase in VEGF (mean±SD [standard deviation], 491.0±365.3 vs. 64.2±69.5 pg/mL, 95% CI [confidence interval] of mean, 296.4-685.7 vs. 51.0-77.5 pg/mL, $p<0.0001$) and sCD105 (7.0±1.95 vs. 4.57±1.51 ng/mL, 95% CI of mean, 5.83-8.18 vs. 4.20-4.93 ng/mL, $p<0.0001$) but not bFGF ($p=0.606$) in CML patients when compated to the control group. VEGF and sCD105 levels decreased significantly in patients who achieved hematological remission during therapy with IM (VEGF, 542.3±361.1 vs. 99.7±65.9 pg/mL, 95% CI, 333.8-750.8 vs. 61.7-137.8 pg/mL, $p=0.0001$; sCD105, 7.2±1.8 vs. 5.6±1.3 ng/mL, 95% CI, 6.1-8.3 vs. 4.8-6.4 ng/mL, $p=0.0034$). Interestingly, shift from hematological to cytogenetic remission did not cause further reduction in either cytokine (VEGF, $p=0.64$; sCD105, $p=0.94$). There was no significant decrease in bFGF levels during IM treatment ($p=0.76$). *Conclusions.* We found significantly elevated VEGF and sCD105 levels but not bFGF in CML patients. In addition, achievement of hematological response during IM treatment resulted in significant decrease of VEGF and sCD105 with no further change at cytogenetic remission. These data suggest that these angiogenic activators may play an important role in CML biology and that IM exhibits antiangiogenic properties.
Supported by research project MZO 00179906 from Ministry of Health of Czech Republic

0670

SUBMINIMAL LEVEL OF BCR-ABL TRANSCRIPT CAN BE DETECTED WITH HI-SENSITIVE REAL TIME PCR IN PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS SUBSEQUENTLY EVOLVED IN PH POSITIVE CML

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Background. Chronic Myeloid Leukemia (CML) diagnosis is actually done when the presence of t(9;22)(q34;q11) translocation is demonstrated with cytogenetic and PCR analysis. Usually cytogenic screening is applied to patients with clinical suspect of CML, followed by Reverse Transcriptase PCR (RT-PCR) in order to confirm cytogenetic data and to define the breakpoints producing the bcr/abl fusion gene. Actually no high sensitive analysis is required for diagnostic screening, due to the expansion of the leukemic clone, and a sensitivity around 10-3 is commonly accepted for RT-PCR. *Aims, methods and Results.* Ph negative (Ph) chronic myeloproliferative disorders was diagnosed in two male patients who presented clinical features of myeloproliferative disease with normal 46 XY caryotype on conventional cytogenetic analisys. RT-PCR (sensitivity 10-3) analysis confirmed the absence of bcr/abl hybrid transcript. On the basis of clinical features and marrow biopsy the first patient (aged 58) was diagnosed as *Chronic Idiopathic Myelofibrosis*, instead the second patient (aged 65) as *Chronic Myeloproliferative Disease unclassificable* according to WHO classification. After 18 and 42 months respectively of clinical observation, both of them suddenly presented a clinical evolution with more aggressive features. The first one presented a rapid increase in leucocytes and red blood cells count, the second one instead presented a mild anaemia associated to elevated leucocytosis and spleen enlargement. A restaging consisting in osteo-medullary biopsy, marrow aspirate and cytogenetic was compatible with chronic myeloid leukemia; 100% of analyzed nuclei was Ph positive (Ph^+). PCR analysis (sensitivity 10-3) confirmed the presence of t(9;22)(q34;q11). We also re-analysed with standard RT-PCR the criopreserved sample from the initially diagnosis of Ph^- mieloproliferative disorders and we confirmed the previous data. Instead, by analyzing with quantitative real time PCR (qrt-PCR) we found a minimum signal of bcr-abl positivity under the quantificable level. *Conclusions.* Our hypothesis is that a small Ph^+ clone was present in the marrow at the onset of the disease, also if the large majority of proliferant cells was Ph^-. This clone was so small to be not evaluable with conventional cytogenetic and molecular analysis. During the evolution of the disease the Ph^+ clone expanded and became dominant with a more aggressiveness, which justifies the clinical evolution. The dilemma of these situations is to explain the coexistence of two myeloproliferative clones, one Ph^+ and one Ph^-. In both of these cases we observed an initial prevalence of Ph- clones but subsequently they showed an evolution and a predominance of the Ph^+ clones. Further studies on these particular cases need to explain these events. Considering this experience, we actually retain that in presence of clinical features of myeloproliferative disorders Ph-, an hi-sensitive PCR needs to confirm the real negativity of bcr-abl hybrid transcript. We also retain that a re-evaluation of cytogenetic and PCR for bcr-abl is required every time that a change in clinical evolution occurs in this particular subset of patients.

Myeloma and other monoclonal gammopathies - Clinical II

0671

REDUCED INTENSITY CONDITIONING ALLOGENEIC TRANSPLANTATION IN MULTIPLE MYELOMA. A SINGLE CENTER EXPERIENCE

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The role of AT is still controversial in the management of MM patients. In order to identify factors influencing the outcome post graft we analyzed the results of RIC AT performed in 45 MM patients of our institution between June 1999 and August 2006. Median interval between diagnosis and AT was of 32 months (range: 7-172). At time of AT, median age was 57 years (range: 44-64), 37 patients were considered as complete (n=2) or partial (n=35) responders. Before AT 13 patients received only one line ot treatment as the 32 other received two or more lines of treatment. The number of autograft before AT was none or one in 25 cases and two or more in 19 cases. RIC regimen consisted of fludarabine associated with busulfan (n=32), total body irradiation (n=9), treosulfan (n=2), idarubicine and cytosine arabinoside (n=1) or melphalan (n=1). In addition 34 patients received ATG. The donor was an HLA-identical sibling in 30 cases or an unrelated donor in 15 cases (HLA-identical: 11 or HLA mis-match: 4). With a median follow-up of 27 months (range:4-84), 17 patients are alive and 28 are dead. The causes of death were: transplant-related complications (n=13), relapse or progression (n=14) and secondary malignancy (n=1). The 3-year event free survival (EFS) and overall survival (OS) from AT were respectively 20±6.9% (95%CI) and 34±38.4% (95%CI). The complete remission (CR) rate after AT was 40% for the 42 patients who engrafted. The median EFS duration from AT for patients achieving CR was 31 months (range:3-56). Univariate analysis showed that obtention of CR post-AT and occurrence of chronic GVHD were the factors who had a significant impact on EFS and OS (p<0.0001 and =0.007 for EFS and p=0.0004 and 0.036 for OS respectively). In conclusion the better OS and EFS observed in patients with chronic GVHD confirmed the importance of graft-versus-myeloma effect in RIC AT. Our results suggested that another way to improve the OS and EFS could be a better control of disease before and/or after AT by the use of novel agents as proteasome inhibitors or immunomodulatory drugs.

0672

QUALITY OF RESPONSE AND SURVIVAL IN MYELOMA PATIENTS UNDERGOING CONVENTIONAL CHEMOTHERAPY

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Background. Up to now, few data have been collected on the quality of life (QOL) of myeloma patients. Results of a recent study suggest that response to treatment is an important factor in this regard. *Aims.* Our aim was to further investigate the impact of response to treatment on the QOL of myeloma patients undergoing conventional chemotherapy. *Methods.* We prospectively collected QOL data using the EORTC QLQ-C30 in a study comparing continuous versus intermittent prednisone plus VMCP in 292 newly diagnosed myeloma patients. A QOL-questionnaire was distributed to each patient at each visit. Using the official EORTC scoring manual, we analyzed the relationship of response to global QOL, all functional scales and the symptom scale fatigue. *Results.* QOL data are available in 186 of 260 evaluable patients. Mean QOL scores during induction therapy were significantly associated with the quality of response achieved. This was still true for the following scales, if patients with progressive disease were excluded from the analysis: global QOL (p 0.02747), fatigue (p 0.00017), role functioning (p 0.04032, Figure 1), cognitive functioning (p 0.0224), and emotional functioning (p 0.01274). When QOL at the last visit of induction treatment was analyzed, global QOL (p 0.02273), physical functioning (p 0.01541) and fatigue (p 0.004616) were significantly associated with quality of response. In the case of global QOL and fatigue this was still true when patients with PD were excluded (p 0.04991 and p 0.009635, respectively). Mean physical functioning and cognitive functioning during induction were also inversely correlated with time to first response (p 0.04025 and p 0.04674, respectively). *Conclusions.* Quality of response during induction treatment significantly influences central parameters of QOL including cognitive function in myeloma patients undergoing conventional therapy. If patients with progressive disease are excluded, the magnitude of response is still associated with QOL. Even if survival cannot be prolonged by achieving a better response, myeloma patients might benefit from a better response by having a better quality of life.

Figure 1. Quality of response and overall survival.

0673

QUALITY OF LIFE OF MYELOMA PATIENTS UNDERGOING CONVENTIONAL INDUCTION THERAPY IS DEPENDENT ON QUALITY OF RESPONSE TO TREATMENT

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Background. Up to now, few data have been collected on the quality of life (QOL) of myeloma patients. Results of a recent study suggest that response to treatment is an important factor in this regard. *Aims.* Our aim was to further investigate the impact of response to treatment on the QOL of myeloma patients undergoing conventional chemotherapy. *Methods.* We prospectively collected QOL data using the EORTC QLQ-C30 in a study comparing continuous versus intermittent prednisone plus VMCP in 292 newly diagnosed myeloma patients. A QOL-questionnaire was distributed to each patient at each visit. Using the official EORTC scoring manual, we analyzed the relationship of response to global QOL, all functional scales and the symptom scale fatigue. *Results.* QOL data are available in 186 of 260 evaluable patients. Mean QOL scores during induction therapy were significantly associated with the quality of response achieved.

Figure 1. Mean role functioning and quality of response.

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0674

SERUM C-REACTIVE PROTEIN IS THE MORE POWERFUL FACTOR PREDICTING OUTCOME OF MM TREATED WITH ANTRACYCLIN-THALIDOMIDE BASED THERAPY

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Background. Few studies investigated factors affecting outcome in MM patients treated with thalidomide-chemotherapy based therapy when they could be helpful in selecting potentially benefiting patients thus allowing a risk-based therapy since previous prognostic score (i.e ISS and others), may not suit new drugs based-therapy. *Aims.* We investigated factors affecting response, time to progression (TTP) and overall survival (OS) in Multiple Myeloma patients (MM) treated with ThaDD regimen (Thalidomide 100 mg/day, Dexamethasone 40 mg day 1-4, 9-12, pegylated liposomal doxorubicin 40 mg/mq day 1 every 28 days) in selecting patients benefiting more from this therapy. *Patients and Methods.* One hundred and twenty seven MM patients were treated with ThaDD regimen. Median age was 71 years (range 41-83); 66 were newly diagnosed and 61 relapsed/refractory MM, respectively. We analysed the following variables to search for factors affecting response (≥ VGPR vs < VGPR), TTP and OS: age (≥ 70 vs < 70), sex, ECOG performance status (0-1 vs 2-4), MM isotype (IgA vs others), D-S stage I-II vs III), bone marrow plasmocytosis (″ 50% vs > 50%), haemoglobin (< 10 vs ≥ 10 g/dl), platelets (< 100000 vs ≥ 100000/μL), β2-microglobulin (″ 3.5 vs >3.5 μg/dL), serum albumin (″ 3.5 vs >3.5 mg/dL), ISS (1-2 vs 3), serum C-reactive protein (normal vs abnormal), serum creatinine (″ 2 vs > 2 mg/dl), FISH cytogenetics abnormalities [unfavourable: del13, t(4;14), t(14-16), ipodyploid vs normal, hyperdyploid and t(11;14)] disease status (newly diagnosed vs relapsed-refractory), stem cell transplantation at diagnosis and time to first progression (the last 2 parameters only for relapsed/refractory MM). *Results.* Overall, 69 patients (53%) showed response, ≥ VGPR median TTP and OS was 23.5 and 35.5 months, respectively. By univariate analysis, factors positively affecting response were normal sCRP (73% vs 37%; p<0.0001) and newly diagnosed disease (68% vs 36%; p=0.001). Multivariate analysis selected only sCRP as predictive factor for response (p<0.0001; OR= 1.42; CI95% OR= 1.2-1.7). By univariate Cox analysis factors predicting significantly longer TTP were favourable cytogenetics (p=0.06), response ≥ VGPR (p= 0.0008) and normal sCRP (p=0.0001). Multivariate Cox analysis identifies only normal sCRP as predictive factor for significantly longer TTP [median=40 months (95%CI=35-44) vs 29.5 months (95%CI=25-34); p=0.0001]. As per OS, univariate Cox analysis identified newly diagnosed disease (p= 0.0258), response ≥ VGPR (p= 0.0039), age <70 years (p=0.032), β2-microglobulin ″ 3.5 μg/dL (p= 0.0375), and normal sCRP (p=0.0027) as factors predicting significantly longer survival. Again multivariate Cox analysis pointed to normal sCRP as the only factors positively affecting OS (3 yrs OS 85% vs 52%; p=0.006). *Conclusions.* sCRP is the only factor predicting outcome in MM patients treated with anthracyclin-thalidomide based therapy. Patients with normal sCRP at enrolment showed very good outcome whereas patients with abnormal sCRP could improve it with the same therapy followed by transplant if possible or with alternative therapy.

0675

FLOW CYTOMETRIC EVALUATION OF THE BONE MARROW PLASMA CELL POPULATIONS IN PATIENTS WITH MULTIPLE MYELOMA, MONOCLONAL GAMMOPATHY OF UNCERTAIN SIGNIFICANCE AND POLYCLONAL PLASMACYTOSIS

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Background. The phenotype of myeloma PCs has long been considered similar to that of normal PCs. A differential expression of surface markers in malignant PCs has been recently described. Although neoplastic PCs retain some of the phenotypic characteristics of normal PCs, such as strong CD38 expression, several studies demonstrated that myeloma PCs lack CD19 and may express CD56. *Aim.* The rationale of this study was to detect the relevance of the malignant PC phenotype in non neoplastic polyPL and MGUS compared to MM. *Methods.* PCs from 26 polyPL, 41 MGUS and 77 MM were characterized based on their light scatter distribution and sequential gating strategy, and reactivity to CD19, CD45, CD38 and CD56 antibodies. Gene expression profiling (GEP) of PCs from an independent panel including 4 normal subjects, 11 MGUS and 102 MM was performed on U133A chips using the 7G scanner (Affymetrix) as previously described by us. *Results.* A significantly higher percentage of $CD19^+$ PCs was demonstrated in polyPL cases as compared to MGUS group, while the lowest proportion of PCs expressing CD19 was accounted in MM cases. In contrast, a progressive increase of CD56+ PCs was found from polyPL to MM with an intermediate value observed in the MGUS group. To dissect the best cut-off values of the two different immunophenotypic profiles as discriminating among the different entities of PC dyscrasias, ROC analyses were performed. We determined 47% (AUC=0.898, p<0.0001) as the best cut-off values of CD19 expression to discriminate between benign polyPL and the remaining cases. Conversely, analyzing the CD56 the best cut-off to distinguish between malignant MM and the other cases was 56% (AUC=0.722, p=0.005). Based on these results we were able to split cases in 3 groups with a different PC phenotype, namely neoplastic PCs (CD19low/CD56high), normal PC ($CD19^{high}$/$CD56^{low}$) and PC with an intermediate phenotype. None of the polyPL cases showed a neoplastic phenotype. Conversely, the neoplastic phenotype was detected in 29.3% and 62.7% of MGUS and MM, respectively. In order to further extend our investigation at a different level, we took advantage of a GEP database to evaluate the absolute expression levels of CD19 and CD56 in PCs purified from normal control, MGUS and MM. A significant higher expression of the CD19 gene transcript was observed in normal subjects (232±23, mean ±sem) as compared with MGUS (92±7) and MM (90±6). With regard to CD56, although its expression was higher in MM cases (378±33), it did not reach a statistical significance compared to MGUS (243±68) or normal subjects (60±25). This finding could be related to the low number of control as well as to the high variability of CD56 expression associated with MM patients. *Conclusions.* Our results indicate that flow cytometry analysis may clearly identify a bone marrow involvement by normal PCs. As expected, the worst phenotype is prominent in MM cases; GEP provides similar results and support these findings. Whether the presence of such a phenotype in MGUS may have an impact in predicting a different clinical outcome should be further evaluated.

0676

THE ROLE OF AUTOLOGOUS STEM CELL TRANSPLANTATION AS FRONT-LINE THERAPY IN POEMS PATIENTS

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POEMS is a rare multisystemic paraneoplastic syndrome, the acronym refers to Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal protein, Skin changes. Major diagnostic criteria include: polyneuropathy and monoclonal plasmacell proliferative disorder, and minor criteria such as organomegaly, endocrinopathy, skin changes, edema, papilledema, Castleman disease, sclerotic bone lesions. POEMS patients must have all major criteria and at least 1 of minor criteria . Autologous peripheral blood stem cell transplantation (APBSCT) seem to increase the quality of life of POEMS patients. The aims of this study are to confirm that

the therapy of choice for POEMS is APBSCT and that it should be performed as front-line therapy in order to reduce risks of APBSC due to the progressive organ damage. In this study 4 patients affected by POEMS syndrome were treated with high dose chemotherapy and autologous peripheral stem cell transplantation (aPBSCT). Three patients were male and one female, median age was 53 yrs (44-62). At diagnosis all patients had a severe, rapidly progressive sensory-motor peripheral neuropathy, involving extremities, with inability to walk. All patients had melanosis, monoclonal component IgA-lambda and 1 had also monoclonal component IgG-lambda. Bone marrow biopsy documented in all patients mild plasmacytosis (8-10%) endocrinopathy as thyropaty was present in all patients and two patients experienced respectively hypogonadotropic hypogonadism and hypophysary adenoma also. Two patient had splenomegaly, and 2 hepatomegaly. One patient had sclerotic bone lesion. Two patients were previously treated with high dose of intravenous immunoglobulin. and steroids in the neurologic unit. One patient had significantly low pulmonary function before aPBSCT. As induction/mobilization therapy all patients received intermediate dose of cyclophosphamide (1500 mg/m^2 on day 1,3) and Methylprednisolone (250 mg from day 1-4) for 2 cycles. G-CSF was added after the 2nd cycle in order to mobilizing peripheral stem cell. Time from diagnosis to aPBSCT was 5 months. Conditioning regimen was HDMel (Melphalan 100 mg/m^2 for 2 consecutive days). The median number of CD34$^+$ cells infused was 4.47 (range 3.08-5.63)×10^6/kg. Engraftment was rapid and sustained. After a median follow-up of 25.5 months (range 4-37), all patients are alive with slow but progressive improvement in neurological disease, skin changes, performance status and without evidence of plasmacytosis. Organomegaly was resolved in both cases. Negativization of monoclonal component was observed in a patient. Patient with sclerotic bone lesion received radiotherapy (dose 5000 cGy) as consolidation. Our experience confirms that HD-Mel and aPBSCT is feasible and efficacious and should be the treatment of choice for POEMS, arresting and even reversing the disease course. Early diagnosis is important to led APBSCT that is able to obtain the best response and improve clinical outcome.

0677

THE USE OF BORTEZOMIB AS FIRST LINE TREATMENT FOR PRIMARY PLASMA CELL LEUKEMIA

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Background. We have recently shown that bortezomib is an effective agent for the treatment of plasma cell leukemia (PCL), an aggressive, rare variant of multiple myeloma (MM) (Musto et al, Cancer 2007, in press). PCL represents about 2-4% of all MM and exists in two forms: 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 leukemic transformation occurring in about 1% of patients with a previously diagnosed MM. *Aims.* Our previous study included both primary and secondary PCL, the majority of whom were heavily pre-treated before of receiving bortezomib. In the present study we focused on the effects of bortezomib as first line therapy in primary PCL. *Methods.* Four patients (two male, two female; 61 to 76 years old) are so far evaluable. Circulating plasma cells ranged from 6 to 40×10^9/L. Median WBC count was 40×10^9/L (range 19-81). Two patients had concomitant extramedullary disease (muscle and pleural effusion). Del 13 was observed in 2 out of 3 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, two 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. *Results.* According to the international uniform response criteria, three partial remissions (reduction of M-component > 50%) and one 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 8 months, without circulating plasma cells in peripheral blood. Three out of them remain in remission phase, one developed extramedullary progressive disease after 6 months. Grade 3-4 hematological toxicity and infections occurred in 2 patients. No other significant adverse effects were observed. *Summary.* Global response rate to standard chemotherapy in primary PCL 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.

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PRESENTATION AND SURVIVAL OF MULTIPLE MYELOMA PATIENTS: SIX YEAR SURVEY IN A DISTRICT GENERAL HOSPITAL

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Background. Multiple Myeloma has an annual incidence of 5 in 100,000 populations. It has a wide spectrum of clinical features ranging from asymptomatic paraproteinaemia to a rapidly progressive disease with multiple end organ damage. *Aims.* To assess Multiple Myeloma presentation, survival and therapy in a district general hospital. *Methods.* Retrospective analysis of our databases for patients diagnosed with Multiple Myeloma between 2000 and 2006. We were able to retrieve 60 out of 69 patient records. Out of these 60 patients, 37 were males (61.7%) and 23 were females (38.3%). Median age at presentation was 71 years (36 to 89). *Results.* 24 patients (40%)were asymptmatic and were referred because of incidental finding of paraproteinaemia. 3 patients (5%) progressed from previous MGUS. The remaining 33 patients (55%) who were symptomatic presented with: bone pain in 13 patients (21.7%), Anaemia in 9 patients (15%), Renal failure in 3 patients (5%), weight loss in 3 patients (5%), pancytopenia in 2 patients (3.3%), hypercalcaemia in 1 patient (1.7%), we also encountered a rare presentation as amyloidosis in 1 patient (1.7%), and cord compression in another (1.7%). 48 patients (80%) were treated at presentation. The remaining 12 patients (20%) were stable and did not require treatment. Of those who required treatment; 27 patients (56.3%) were over 70 years of age and received Melphalan with or without Prednisolone as a first line therapy. Patients who were less than 70 years old received treatment in the form of: VAD regimen in 4 (8.3%), Z-DEX in 10 (20.8%), Dexamethasone in 3 (6.3%), Cyclophosphamide in 1 (2.1%), ABCM in 1 (2.1%), Prednisolone in 1 (2.1%) and Thalidomide in 1 (2.1%).Some patients received additional treatment in the form of: Radiotherapy in 14 patients (29.2%) and Bisphosphonates in 22 patients (45.8%). 8 patients (13.4%) were referred for stem cell transplant, of which 5 patients (8.4%) had successful stem cell transplant done and are still under regular follow-up.During this 6 year period, 24 out of 60 patients diagnosed with Multiple Meyloma have died (40%). Survival range for these patients was between 1 month and 45 months (Mean survival of 37.5 months). 60% of the patients diagnosed are still under regular follow-up. *Conclusions.* Our retrospective study of Myeloma patients in our hospital showed that a significant percentage of patients (20%) had stable Myeloma with no organ damage and did not require treatment. Usual presenting symptoms were less pronounced than in other studies, reflecting possibly early diagnosis. Only 13.8% of patients were referred for assessment for autologous stem cell transplant, however only 8.4% had successful stem cell transplant.

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MYELOMA-EPIDEMIOLOGY: FARMERS ARE AT HIGHER RISK OF MYELOMA IN THE UK

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Background. There are several publications which have shown an epidemiological relationship between plasma cell dyscrasias or myeloma and farming in general[1,2] or only some specific kinds of farming (e.g. cultivating potatoes or sheep farming) in several different countries like the U.S., France or Norway and Sweden.[3,4,5,6,7] There are however no data so far published for the UK. *Aims.* We wanted to demonstrate for the U.K. that farmers are more prone to develop myeloma than other occupational groups as has been shown for other countries. *Methods.* A survey was conducted amongst the patients with myeloma or MGUS in the years 2005-2006 regarding their occupation and patients were grouped into patients from city areas versus county areas according to their postcode. The Leicester Royal Infirmary Haematology Department is the single referral centre for the whole of Leicester city and rural Leicestershire ensuring that both patient groups are represented without any geographical selection bias. *Results.* A total of 255 patients with myeloma or MGUS were surveyed, out of these 8 were farmers. Assuming a total

working population of 419961 for Leicester (city and county)[8] this gives a prevalence of 6.07×10^{-4}. The number of farmers in Leicester is 4175[9] which gives a proportion of farmers with myeloma of 1.91×10^{-3}. This increase is significant with a p-value of 0.009 on the one sample proportion test. The overall age range was 37-97 yrs (city 37-92 years, county 44-92 years) with a median age of 69.5 years (city) versus 70.5 years (county) which is not significantly different. However the prevalence was highly significantly different between patients from city (49/100000) versus county (19/100000) with a p-value of 1.47×10^{-14} indicating that living in a rural environment per se does not predispose to myeloma. *Conclusions.* As a central referral centre for patients from Leicester (city and county) we can confirm for the population of Leicestershire that farmers have a higher risk of developing myeloma, this is not related to just living in a rural environment.

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MIP-1 ALPHA AND ITS INFLUENCE ON SURVIVAL IN MYELOMA MULTIPLE PATIENTS

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Background. Macrophage Inflammatory Protein-1alpha (MIP-1α) is a member of CC chemokine family inducing osteoclastic activity in Multiple Myeloma (MM), by a mechanism independent of the classic OPG/sRANKL, that has shown its correlations with the bone disease extension and bone resorption markers. *Aims.* It has been previously suggested the MIP-1α prognostic value on survival. Our group communicated a similar observation in a small group with little follow-up. This is an update of this data with more cases and more follow-up. *Material and Methods.* 104 MM (84 with serum MIP-1 α determination) patients diagnosed in Hospitals of Castilla-Leon Community-Spain, whose serum were collected at diagnosis and stored at -80°C. The median follow-up of the series was 100.4 months. The serum MIP-1 α was measured by double-sandwich enzimoimmunoassay (EIA) (R&D System). We have also analyzed bone resorption markers (CTX (β-crosslaps), Crosslinks and bone formation markers (Osteocalcin (OC), bone Alkaline Phosphatase (bAP)) and serum cytokines (IL6, TNF-α, IL-1β, srIL6, HGF, VEGF, OPG and sRANKL) by EIA. Statistical *Methods.* Non-parametric test (U de Man Whitney, Spearman correlation); Survival curves of Kaplan-Meier were compared by long-rank, Breslow and Tarone test; Multivariate analysis was realized by Regression Cox. *Results.* The survival curves of our series were calculated at several follow-up times (2, 3, 4, 5 and 9 years). We evaluated the impact on survival of serum MIP-1 α to separate the patients in two subgroups (lower and high) by several cut-off points (ten percentiles). Patients with serum MIP-1 α concentrations higher than 26.6 pg/mL (percentile 40) showed worse survival at 4 and 5 years of follow-up (log-rank < 0.05; Breslow< 0.05; Tarone <0.05). However this impact on survival was lost at 9 years of follow-up. Both groups (higher and lower 26.6) were homogeneous for the main clinical-biological characteristics with value on prognosis, except the radiological bone affectation that was significantly more advanced in the higher group. We made multivariate analyses with the most important clinical-biological prognostic parameters, as well as with other clinical parameters at diagnosis related with bone disease (serum calcium, Rx bone scale,). Age, Hemoglobin (Hb) and serum MIP-1 α as categorical variable, were the parameters that shown independent influence on survival at 4 years of follow-up. When we included in the multivariate analyses the anterior clinical-biological parameters plus bone resorption markers and cytokines with influence on survival in univariate set, serum MIP-1 α keep the prognostic role together with age, Hb and β2-microglobulin. *Conclusions.* Serum MIP-1-α has independent prognostic value on MM patient's survival at 4 and 5 years of follow-ups, but not at 9 years, probably because of the natural history of this disease, which shows that almost all patients were deceased at this moment. On behalf of Castellano-Leones Cooperative Group for the study of Monoclonal Gammopathies. Supported by the FIS-Spain Grant 98/0206 and Spanish FIS Thematic Network Grant G03/136

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HAEMATOLOGICAL AND ONCO-HAEMATOLOGICAL ASPECTS OF GAUCHER DISEASE: CONSENSUS RECOMMENDATIONS FOR TREATMENT AND DISEASE MANAGEMENT

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Background. Gaucher type 1 (non neuronopathic) disease is a rare multisystem disorder, with greatly differing rates of progression and severity. Haematological complications include anaemia, thrombocytopenia, bleeding, platelet and coagulation abnormalities, and increased risk of multiple myeloma (de Fost *et al.,* 2006). Imiglucerase enzyme replacement is considered the gold standard treatment for type 1 and 3 Gaucher disease [Cox, *et al.*, 2003]. Substrate reduction therapy is also available for adults with mild /moderate disease for whom imiglucerase is not a therapeutic option (EMEA, 2006). *Aims.* To review knowledge of the haematological / onco-haematological aspects of Gaucher disease and identify best clinical practice in monitoring, treatment and disease management. *Methods.* In October 2006, a panel of European haematologists and experts in Gaucher disease convened to review evidence from peer-reviewed literature, the International Collaborative Gaucher Group (ICGG) database, and their own clinical experience to support recommendations. *Results.* Gaucher disease is a complex disorder that does not preclude co-morbidities requiring specific treatments. Cytopenia is an almost universal finding in untreated Gaucher patients. Awareness of typical patterns of cytopenia in asplenic and spleen-intact Gaucher patients can help clinicians distinguish haematological co-morbidities. The panel recommended thorough red blood cell studies and complete iron metabolism evaluation at baseline for all Gaucher patients. They agreed that haemoglobin levels defining anaemia should be raised (proposed by Beutler and Waalen, 2006) and used in Gaucher disease treatment / monitoring. Surgeons should be aware that Gaucher patients are at risk of bleeding complications and require careful assessment and measures to reduce risk before, during, and after surgery. Multiple myeloma incidence is elevated in Gaucher disease. MGUS is considered a pre-malignant condition in multiple myeloma. The prevalence of MGUS in Gaucher disease suggests a relationship between the diseases. However, there is insufficient evidence linking MGUS, or any pattern of hyperimmunoglobulinaemia, with increased risk of multiple myeloma in Gaucher disease. Similarly, no consistent pattern of cytokine production implicates specific mediators in Gaucher-related malignancy. Since MGUS carries risk of progression to malignancy in non-Gaucher patients, it is advisable to check for monoclonal abnormalities in Gaucher patients at baseline and every two years (<50 years) or every year (>50 years). If MGUS is found, general MGUS guidelines should be followed and bone marrow biopsy and aspirate performed with full cytogenetic profile. If a patient with MGUS has symptomatic Gaucher disease Gaucher-specific therapy should be used to treat symptoms and prevent disease progression. MGUS in an otherwise asymptomatic Gauch-

er patient does not indicate such treatment. Where multiple myeloma is coincident with Gaucher disease, chemotherapy may aggravate cytopenia. Gaucher-specific therapy before chemotherapy may improve the patient's tolerability to chemotherapy. Each case must be evaluated individually. *Conclusions.* While evidence is insufficient to conclude that multiple myeloma represents disease progression in Gaucher disease, certain pathophysiological consequences of Gaucher disease may influence the aetiology of haematological malignancy in Gaucher patients. Clinicians should be vigilant to higher risk of multiple myeloma in Gaucher disease. Future studies should focus on the utility of early treatment to prevent immunoglobulin abnormalities and multiple myeloma.

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THE INFECTIONS COMPLICATIONS IN THE EVOLUTION OF MULTIPLE MYELOMA

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Introduction. Recurrent bacterial infections are a major cause of illness and are the most frequent cause of death in-patients with advanced myeloma. *Material and method*. The study included 94 patients (58 men and 36 women) with multiple myeloma followed up between October 1983 and June 2005. The age range was 45 to 83 years (mean age, 65,9 years). *Results.* 58,82% patients had a monoclonal IgG, (23,52%) had IgA, 14,7% had only light chain in urine, 1,47% had IgD and 1,47% was nonsecretory myeloma. Over a median follow-up of 27,8 months, the study group presented 164 febrile episodes longer than 3 days and in 116 of them the etiology was identified. Only 29,41% of patients did not present infectious episodes in their course of disease, most of them being alive, 85% (17 from 20) versus 52,08% (25 from 48) but don't reach the level of significance. 58,1% of the infection episodes occurred in the first 3 months after diagnosis and chemotherapy initiation, 33.72% occurred in the relapsing phase and 8,13% in the plateau phase. Streptococcus pneumonia and Hemophilus influenzae, are the most common pathogens in previously untreated, non-neutropenic myeloma patients 76% versus 24% in neutropenic patients with refractory disease $p<0,046$ However, in neutropenic patients and in those with refractory disease, Staphylococcus aureus and gram-negative bacteria are the predominant organisms 63% versus 18% $p<0,049$ Repetitive infections with the same localization occurred in 3 patients, 2 of them with a favorable local cause. 13,95% of patients had infection at first presentation, but only 6,95% are systemic. Polyclonal hypogammaglobulimemia less than 1 gr/dL occurred in 50% of cases, 75% of these presenting infectious manifestations versus 50% of the rest of patients (Yates corrected = 3,54; $p<0,059$); granulocytopenia $<1000/mm^3$ was present in 26,47% of the infectious episodes. 82,35% of the infectious episodes had bacterial etiology, Gram positive/negative germs being involved in 71,43% of cases. 16,17% of episodes had viral and 1,47% had fungal etiology. Respiratory tract infections represent 76,47%; cutaneous infections 8,82%, genito-urinary tract infections 14,7% and 2,68% other localization. Infection associated other complication in 26,47% of cases and was involved in 52,63% of decease. *Conclusions.* Infection is frequent in MM patients, occurring mostly in the first three months after the initiation of therapy and it has prevalently bacterial etiology. Polyclonal hypogammaglobulimemia correlates to the risk of infection. Infection is the cause of death in more than 50% of cases. The prophylaxis and rapid therapy of the infectious episodes may ameliorate both the quality of life and the duration of survival of the patients with MM.

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THE RELATION BETWEEN PROLIFERATION INDEX AND ANGIOGENESIS IN MULTIPLE MYELOMA

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Intensified angiogenesis correlates with disease progression in various haematological malignancies. The monoclonal antibody to Ki-67, a nuclear antigen present during G1, S, G2 and M phase of the cell cycle, is a marker strictly associated with cell proliferation. The aim of study was to analyze possible relation and prognostic significance of proliferation index and angiogenesis in the bone marrow (BM) of MM patients (pts) by their immunohistochemical markers. *Patients and Methods.* Sixty newly diagnosed MM pts (33 male/27 female pts, mean age 60 years, range 35-75) were distributed according to the clinical stage (CS, Salmon&Durie) as: I 8pts, II 22pts, III 30pts. IgG myeloma was diagnosed in 35pts; IgA in 12pts; light chains in 12pts. Regarding ISS score, the group included: ISS1 18pts, ISS2 13pts, ISS3 29pts. All patients were treated with conventional chemotherapy. All samples of BM biopsies were analyzed for the immunohistochemical expression of FGFR-3 and Ki-67. In order to analyze the microvessel density (MVD), BM vessels were visualized by immunohistochemical staining for CD34. The number of vessels per 400× high power field (HPF) was counted in the area of the most dense vascularization. *Results.* MVD was significantly higher in MM pts in III CS vs. I CS (15 vs.7,5/×400 field, $p<0,001$); and in pts with ISS3 vs. ISS1 (17,5 vs. 9,7/×400 field, $p<0,05$). The expression of FGFR-3 was found significantly higher in III CS vs. I CS (47,5 vs. 25%, $p<0,05$); and in pts with ISS3 vs. ISS1 (60 vs. 22,5%, $p< 0,001$). The proportion of Ki-67 positive plasma cells was significantly higher in III CS vs. I and II CS (10 vs. 5%, $p<0,01$), and in pts with ISS3 vs. ISS1 (13 vs. 5%, $p<0,01$). High levels of proliferation index were associated with strong activity of angiogenesis in III CS and indicated significantly shorter overall survival of those pts vs. I CS (26 vs. 43,5 m, log rank, $p<0,05$). Similarly, the overall survival of pts with ISS3 was significantly shorter vs. ISS1 (19,5 vs. 36m, log rank, $p<0,001$). In conclusion, the assessment of the activity of angiogenesis and proliferation index represents important indicators of disease activity, significant predictive factors and also, possible targets of novel therapeutic strategies in MM.

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OSTEONECROSIS OF THE JAW IN MULTIPLE MYELOMA PATIENTS RECEIVING BISPHOSPHONATES

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Background. The last decade bisphosphonate therapy has been included in the standard management of patients with MM-related bone disease. Although its efficacy in reducing skeletal events is important, a potential side effect has been raised and this is the ONJ. *Aim.* To assess the frequency of ONJ in patients receiving bisphosphonates as well as to identify the major risk factors. *Methods*. Our study includes 102 patients who have received bisphosphonates, for at least 4 cycles, from January 1998 until January 2007. From 1998 to 2000 all patients received only pamidronate 90 mg per month, 2 hours infusion and then they continued with zoledronic acid 4 mg per month, 15 minutes infusion. Since January 2006 the patients who had completed 2 years of bisphosphonate therapy they continued bisphosphonate infusions every 3 months. The diagnosis of ONJ was based on clinical evaluation by the maxillofacial surgeon and on X-ray of the jaws. In one case biopsy performed in order to exclude MM involvement. *Results*. The major characteristics of the 102 patients are the following: a) median age at MM diagnosis 60years (30-79), b) 57 men and 45 women, c) the median time of exposure to any bisphosphonate therapy was 30 months (4-106), d) median number of bisphosphonate cycles received were 22 (4-90), e) median time of exposure to pamidronate was 19 months (1-66), f) median time of exposure to zoledronic acid was 28 months (1-66). In this period (approximately 9 years) 6 patients (5,88%) developed ONJ. Three were women and three men. Median age at the time of ONJ diagnosis was 56 years. There was a wide range of symptoms, from a slight toothache and sudden tooth loss to the formation of a cutaneous fistula in one patient. Three patients had received pamidronate therapy followed by zoledronic acid and three had received only zoledronic acid. The median time of exposure to bisphosphonates was 37 months (20-68), to pamidronate was 20 months (8-27) and to zoledronic acid 30 months (20-60). None of the patients who had received only pamidronate therapy developed ONJ. *Conclusions*. In our study the incidence rate of ONJ is comparable with that reported from other investigators. To our experience the development of ONJ seems to be related with the time of exposure to bisphosphonate therapy and the use of zoledronic acid.

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NERVE CONDUCTION STUDY IN MULTIPLE MYELOMA PATIENTS PRESENTING BORTEZOMIB INDUCED PERIPHERAL NEUROPATHY

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Introduction. Bortezomib produces high response rates in patients with relapsed or refractory MM. However, Bortezomib induced neuropathy influence negatively patients' quality of life. Although there is an increasing experience on the use of Bortezomib, its associated neuropathy has not been studied extensively. *Aims.* To evaluate the neurotoxicity of bortezomib in relapsed/ refractory MM patients by physical examination and nerve conduction velocities. *Patients and Methods.* 75 refractory/relapsed MM patients (21 F, 54 M) were treated with Bortezomib at standard doses and schedule, in our Department. Patients' median age was 70 years (32-83). 59% had IgG MM., 26% IgA, 4% IgD, 11% BJ. 38 patients received Bortezomib as second line treatment while the others had received multiple prior therapies (median 3 lines). 81% of patients responded (12% CR, 20% nCR, 32% PR,16% MR). 32% had prior treatment with thalidomide. No patient had neuropathy greater than grade 1 at bortezomib initiation. 45 (60%) patients presented neuropathy (1 grade 4, 17 grade 3, 17 grade 2, 9 grade 1). Of these patients, 20 underwent complete neurological examination including nerve conduction study. 8 were evaluated between third and fourth cycle of Bortezomib administration (at presentation of neuropathy), 6 immediately after the end of therapy and 6, more than 5 months from the end of therapy. 2 patients were reevaluated when neuropathy improved. *Results.* The most common symptoms revealed by physical neurological examination were numbness, tingling, paresthesias, dysaesthesias, pain and weakness. Eight out of 20 patients (40%) had neuropathy of grade 3, 9/20 (45%) grade 2 and 3/20 (15%) grade 1. Nerve conduction velocities (NCV) findings are shown in Table 1. Four out of 6 patients (66%) that were far from Bortezomib treatment still had abnormal findings in NCV Although 7 patients had resolution of pain they still had impaired velocities in NCV. The pain assessment by the neurologist and the hematologist were in agreement but the sensory neuropathy was underestimated by the hematologist. The differing evaluation was more pronounced for patients evaluated far from Bortezomib treatment for who the hematologist considered that the neuropathy resolved. *Conclusions.* Bortezomib induces either axonal or demyelinated type of neuropathy. Lower extremities are more frequently affected. Resolution of pain does not necessarily mean normal nerve conduction velocities - this should be taken in account in case other neurotoxic modalities are going to be used. Further studies are needed for a better understanding of the pathogenesis of bortezomib neurotoxicity and of its management

Table 1.

NCV FINDINGS	# Patients
Sensorymotor axonal polyneuropathy	6
Sensorymotor demyelinating polyneuropathy	6
Motor demyelinated neuropathy	2
Motor axonal polyneuropathy prominent in lower extremities	1
Sensory neuropathy prominent in lower extremities	1
Sensorymotor neuropathy prominent in lower extremities	1
None	3

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SECOND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA IN FIRST RELAPSE

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Background. Induction chemotherapy to maximum response followed by autologous stem cell transplantation (ASCT) has become standard practice for eligible, newly diagnosed patients with multiple myeloma (MM). This approach is well supported by evidence obtained from large randomised trials. However, ASCT is not curative and virtually all patients relapse. Re-induction and a second ASCT is usually offered, although there are limited data on the outcome of this approach. *Aims.* We retrospectively analysed the results of second ASCT after re-induction chemotherapy for MM patients in first relapse and identified parameters of prognostic value. *Methods.* From July 1994 to September 2005, 228 patients with MM aged 26.7 to 72.5 years (median 56.2 years) received ASCT using melphalan (200 patients) or melphalan/TBI (28 patients). Thirty-five (15.4%) patients achieved complete response (CR) and 153 patients (67.1%) partial response (PR). The transplant related mortality (TRM) of the first ASCT was 1.3%. Patients treated by elective tandem autologous or a subsequent allogeneic SCT and patients who died in remission were excluded from the current study. Relapse post-transplant occurred in 141 (61.8%) patients. Re-induction chemotherapy and a second ASCT with melphalan 100-200 mg/m^2 conditioning was performed in 42 (29.8%) patients, while 99 (70.2%) patients had other salvage treatments. Response was defined by EBMT criteria. *Results.* Twenty-nine patients (69%) responded to second ASCT. Three patients (7.1%) achieved CR and 26 (61.9%) PR. Eight patients (19%) had minimal or no response to the second transplant procedure. The median time to neutrophil recovery (>0.5×10^9/L) was 13.5 days (range 7-19) and for platelets (>50×10^9/L) was 17 days (range 13-56). Four patients had primary graft failure (9.5%). Five patients died of TRM (11.9%) 11 to 70 days post transplant. The median event-free survival (EFS) after the second transplant was 10.8 months. The patients with a first remission period of <18 months had significantly lower EFS after the second autograft (median EFS 4.9 months) compared to those with a long first remission ″18 months (median EFS 14.1 months, p=0.03). Also, there was a trend towards lower OS after first relapse for patients with <18 months first remission period (median 12.5 vs 29.8 months, p=0.09). The median OS after the first relapse for those patients treated with a second ASCT was 52.9 months and for the patients treated otherwise 13.1 months (p<0.001). *Conclusions.* A second ASCT after re-induction is an effective salvage approach for MM patients in first relapse. The majority of patients will achieve response and despite higher TRM and graft failure rates, a second ASCT may result in longer OS when compared to other salvage treatments. The duration of remission after the first autologous SCT may predict the EFS after the second autograft.

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EFFICACY OF DOUBLE AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA - A PROSPECTIVE SINGLE CENTER EXPERIENCE IN 71 PATIENTS

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Background. Clinical trials so far have shown that HDT and ASCT improves response rates, DSF, OS in symptomatic patients with MM younger than 65 years. We conducted a prospective trial of two successive ASCT in patients with advanced stage MM. The aim of the study was to evaluate the feasibility, efficacy, and toxicity of this treatment approach. *Patients*. From December 1994 to November 2005, 71 consecutive, previously untreated patients with MM stage II or III were included in the program of double ASCT. Median age of patients was 53 years (range 30-65 years), M/F ratio 39/32. *Methods.* Patients received induction with 3 to 10 cycles of VAD, few of them also other salvage regimen due to refractoriness to VAD. After achieving PR they proceed to mobilization procedure with CTX 4 g/m^2 and G-CSF. Conditioning regimen for majority of patients consisted of MEL 200 mg/m^2, 24 patients received MEL 140 mg/m^2 and fractionated TBI 800 cGy prior to second transplant. *Results.* 63 patients (89%) actually received double transplant. Eight patients (11%) received only one transplant due to progression of MM (1 pt), no enough stem cells (3 pts), low PS (3 pts) or refusal (1 pt). A CR was achieved in 47 patients (66%). With the median follow up of 45 months (range 10 to 150 months) 36 (51%) patients were alive in CR or VGPR, 19 (26%) were alive with disease, and 14 (20%) patients died from relapse. Only two patients died from TR complications (3%). Survival was calculated from the time of diagnosis. Probability of EFS at 7 years after the diagnosis was 35%. Median PFS was 60 months. Probability of OS at 7 years was 56% and the median survival was not reached. Multivariate analysis identified age, disease stage at dg II vs III, preASCT response status CR/PR vs other, hgb <80 g/L, β2 M >2,5 mg/L, and albumin <normal value as factors associated with OS and EFS. *Conclusions.* Double ASCT is feasible in majority of patients, achieve a favorable EFS and OS and is associated with low TRM rate. The toxicity is low even in older patient population, not higher than with single transplant. For all patients with stage II or III MM double ASCT should be planned from the time of diagnosis.

Myeloma and other monoclonal gammopathies - Molecular biology and cytogenetics

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GENE EXPRESSION PROFILES AS PROGNOSTIC FACTORS FOR HIGH-DOSE THERAPY AND BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA

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Background. The standard treatment of newly diagnosed multiple myeloma (MM) is based on induction treatment followed by high-dose melphalan. CR/nCR percentages range from 20-50% with event free survival (EFS) ranging from 18 months to 28 months. The 5-year survival rates are 25% to 50%, however all patients eventually relapse and succumb to the disease. Classical unfavourable prognostic factors include high serum β2-microglobulin and chromosome aberrations such as-13/13q-, t(4;14) and t(14;16). Bortezomib, a proteasome inhibitor, and Thalidomide, an anti-angiogenic and immunomodulatory drug, have recently shown a remarkable effect in patients with relapsed or refractory MM with 30-40% response rates. In combination with Dexamethasone and/ or other conventional agents overall response rates of 50-70% can be achieved. In newly diagnosed patients the response rates vary from 70-85%. Moreover, Bortezomib was found to overcome poor prognostic factors like a high β2-microglobulin and/ or deletion of chromosome 13. However, 15-30% of newly diagnosed patients do not respond to Bortezomib or Thalidomide. Secondly, 30% of the patients treated with these novel agents have to stop prematurely because of intolerable side effects, such as polyneuropathy, thrombocytopenia, thrombosis and gastro-intestinal symptoms. *Aims.* In order to develop new, genetic prognostic factors for clinical response and toxicity associated with Bortezomib and Thalidomide, we have started to analyze gene expression profiles of myeloma specific genes in plasma cells purified from bone marrow from myeloma patients at diagnosis who have been treated in a prospective randomized trial, HOVON 65. This large multicenter, prospective, randomized phase III trial compares Bortezomib in combination with Adriamycin, Dexamethasone (PAD, arm A) followed by HDM followed by maintenance with Bortezomib vs. VAD (arm B) followed by HDM and maintenance with Thalidomide (HOVON65/GMMG-HD4). This cooperative trial in the Netherlands, Belgium and Germany has recruited over 400 patients since April 2005 and will include 800 patients. Methods. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells were performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. Data obtained from micro-array studies were submitted to Cox regression analysis and multifactorial analysis with the clinical data set from these patients. *Results.* We will present an unsupervised cluster (SAM) analysis based on the array results from the first cohort of 130 patients. The analysis shows that the majority of cases can be identified according to the TC classification. The initial results of clinical outcome of these cases will be presented.

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C-JUN AND C-ABL IN HUMAN MULTIPLE MYELOMA CELL DEATH

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Background. The tyrophostin adaphostin achieves remarkable responses in patients with chronic myelocytic leukemia, including Bcr/Abl-positive, Bcr/Abl-negative, and Bcr-Abl T315I mutant tumor cells resistant to both imatinib mesylate and second-generation BMS354825 and AMN107. In addition, it demonstrates cytotoxicity against chronic lymphocytic leukemia and acute myelocytic leukemia cells. Several mechanisms have been proposed as a basis for its robust anti-tumor activity including generation and release of reactive oxygen species (ROS), cytochrome-c and apoptosis- inhibiting factor (AIF), caspase cleavage, JNK activation, as well as inactivation of Raf-1, Stat3, and Stat5. *Aims.* To determine the potential molecular sequelae and therapeutic promise of adaphostin in MM and to delineate the role of c-Jun and c-Abl in MM cell death. *Methods.* Studies were performed in MM, erythroleukemia, and CML cell lines. Microarray and western blot analysis of MM cells were used to demonstrate adaphostin- induced c-Jun upregulation and c-Abl cleavage. The effect of specific knockdowns of c-Jun or c-Abl using siRNA, as well as transient overexpression of wild-type c-Jun, c-Abl, as well as c-Abl cleavage mutants and c-Abl- fragments, was assessed in proliferation and survival assays. *Results.* After demonstrating the anti-MM cytotoxicity of adaphostin, we carried out expression profiling of adaphostin- treated MM cells to identify its molecular targets. Surprisingly, c-Jun was the most upregulated gene, even at the earliest point of analysis (2 hours). We also observed adaphostin- induced c-Abl cleavage in immunoblot analysis. Proteasome inhibitor bortezomib, but not melphalan or dexamethasone, induced similar effects, indicating agent-dependent mechanisms. Using caspase inhibitors as well as caspase-resistant mutants of c-Abl (TM-c-Abl and D565A-Abl), we confirmed that c-Abl cleavage in MM cells requires caspase activity. Importantly, knockdown of c-Jun and c-Abl expression by siRNA confirms that adaphostin- induced c-Jun upregulation triggers downstream caspase-mediated c-Abl cleavage, inhibition of MM cell growth, and induction of apoptosis. Finally, our data suggest that this mechanism may not be restricted to MM, but may also be important in a broad range of malignancies, including erythroleukemia and solid tumors. *Summary and Conclusions*. These data demonstrate a new mechanism of drug- induced growth- inhibition and apoptosis involving c-Jun upregulation and fragmentation of c-Abl.

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PLASMA CELL PROLIFERATION USING KI67 ANTIGEN EXPRESSION DEFINES SUB-GROUPS RELATED TO SHORT SURVIVAL IN MULTIPLE MYELOMA ESPECIALLY WITH LOW B-2 MICROGLOBULIN

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Background. The current most powerful prognosis model in Multiple Myeloma (MM) combines β-2 microglobulin (β2M) with albumin, corresponding to the International Staging System (ISS). However, the prognosis of patients within the group I of ISS (high albumin and low β2 microglobulin) may vary. Proliferative activity of plasma cells has been previously related to prognosis in MM, but methods proposed so far are difficult to apply in routine practice. Ki-67 is a nuclear protein associated with cell proliferation, and its expression is reported as a powerful prognosis marker in solid tumours and several hematological malignancies. We retrospectively evaluated the% of bone marrow plasma cells (BMPC) expressing Ki-67 antigen (Ki67 index) in a series of 174 untreated patients with MM at diagnosis and we looked for its prognostic value on survival in MM. *Method*. Ki-67 index was determined after double immunocytochemistry on PC from BM cytospins (ABC peroxidase to identify cells expressing Ki-67, and alkaline phosphatase to identify PC expressing either kappa or Lambda light chain). Conventional cytogenetic study and interphase FISH (research of Rb1 gene deletion) were performed in 114 and in 128 pts respectively. *Results.* Median survival (±se; months) for pts with stage III, II, and stage I of ISS score were 20 (±3), 41 (±3), 51 (±3) months, respectively (p<0.001). Median Ki-67 index (±se) was of 3.0% (±1.2), 6.1% (±1.2), and 6.5% (±1.4) in ISS stage I, stage II, and stage III patients, respectively (p<0.004). Independently of the ISS staging system, Ki-67 index ≥4% was highly predictive of adverse prognosis, with a median survival of 26 ±4 months and of 49±10 months over and under that value, respectively (p<0.0001).β2 M (threshold at 3 mg/L) gave identical results than Ki-67 index (p<0.001), whereas chromosome 13 deletion (del 13) was less powerful (p<0.02). Ki-67 index correlated well with several markers of intrinsic malignancy, with markers of tumour burden, but it was unrelated to age, serum creatinine and β2 M. There was a strong relationship between hypodiploidy and BMPC proliferation: within the group of pts displaying Ki-67 index ≥4%, 93% pts were found hypodiploid (p<0.0001). Within ISS stage I, median survival [±se; RR of death (95%CI)] was of 31±4 months [2.65 (1.5-4.6)] and of 67±6 months in patients with Ki-67 index ≥4% and <4%, respectively (p<0.001). Chromosome 13 deletion also delineated two groups within ISS stage I pts, but the difference did not reach statistical significance (p=0.243). Finally, the combination of Ki-67 to β2 M produced an efficient prognostic model that appeared the most effective in our series

compared to known models such as β2 M/chr 13 deletion and ISS. The -2Log (likelihood) scores calculated on 155 patients were 1107.885, 1113.256 and 1116.829 for Ki-67/β2 M model, ISS model and β2 M/del13 model, respectively. *Conclusions.* Ki-67 index is easy to perform in routine practice, and is a good prognostic marker, which provides additional survival prognostic information to β2 M into the ISS model. (TG and XL are fisrt author)

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COMPARISON OF CLASSIC CYTOGENETICS AND METAPHASE FISH WITH INTERPHASE FISH ANALYSIS ON UNSELECTED AND SELECTED PLASMA CELLS IN MULTIPLE MYELOMA. THE NEED FOR A UNIFORMLY ACCEPTED METHOD

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Multiple myeloma (MM) is an incurable disease with heterogeneity in survival and prognosis. The establishment of prognostic factors is very important in handling the disease and so far cytogenetic status has been proved as the most important one. However, conventional cytogenetics has a limited extent because of the low proliferation of plasma cells (PC), the reduced extent of marrow involvement and the difficulty to detect cryptic translocations. Recently, classical cytogenetics has been replaced by the fluorescence *in situ* hybridization (FISH) technique, which allows the identification in a short time of specific target regions at diagnosis and the evolution of the disease. A number of diagnostic centers have proposed in the literature different FISH procedures in order to ameliorate FISH results and to overcome the problem of PC low infiltration of the marrow. In addition, there is an argument on the cut off levels for the identification of aberrations. We studied 185 patients with MM using G-banding, M-FISH, metaphase FISH on bone marrow aspirates and interphase FISH on cytospin centrifuged cells either by unseparated bone marrow cells or PCs selected by Magnetic cell separation (MACS) using the CD 138 monoclonal antibody. The aim of the study was to compare these methods and to find the more accurate, less expensive and time consuming technique. For FISH we used commercial probes for the detection of the deletion of 13q14 and 17p13 (p53) regions, and for the detection of rearrangements of IGH locus with the FGFR3, the cyclinD1 and the MAF genes. Our results were in accordance with previously published studies. In most cases, excluding the detection of aneuploidy, cytogenetics failed to reveal specific chromosome rearrangements. M-FISH in the abnormal cases helped us to identify the complex chromosome abnormalities. Metaphase FISH was very informative in detecting cryptic abnormalities in metaphases. Interphase FISH in unseparated cells proved to be of equal reliability for the detection of cryptic abnormalities in comparison with interphase FISH in selected PCs. However, the cut off levels in unseparated cells was 5-8% in accordance to the level of bone marrow infiltration, and significantly lower than that proposed for selected PCs. Also, FISH proved to be a useful method in detecting aneuploidy for the chomosomes examined. *Conclusions.* Convetional cytogenetics is without a doubt the only method that can give information on the aneyploidy status in MM. We need to ameliorate our classic cytogenetic methods in order to obtain better chromosome quality. Metaphase FISH can serve in the detection of cryptic rearrangements and in this method can be used on already existed cytogenetics material. Interphase FISH on cytospins using unseparated bone marrow cells is a reliable, easy and rapid technique, less expensive and could be performed in cytogenetic labs, where elegant techniques such as magnetic cell separation are difficult to be established. From the study arises the need for a uniform procedure for the detection of chromosome abnormalities in MM taking in consideration simplicity, time and cost effectiveness.

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MOLECULAR TARGETING OF THE PKC-β INHIBITOR ENZASTAURIN (LY317615) IN MULTIPLE MYELOMA

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Background. Over the last few years, great efforts have been made searching for novel agents that specifically target signalling pathways which regulate multiple myeloma (MM) cells growth and survival. The deregulation of phosphatidylinositol 3-kinase (PI 3-K)/Akt pathway, a prominent regulatory pathway governing the apoptotic response, may play an oncogenic role in MM. Constitutive activation of the PI 3-K/Akt pathway has been shown to frequently occur in MM *in vivo* and has been also observed in factor-independent MM cell lines. Although the biological mechanisms that lead to PI 3-K deregulated activation are still unknown in MM, recently a link between protein kinase C (PKC) activity and the activity of the PI 3-K/Akt pathway has been reported. *Aims.* The purpose of the present study was to test the oral PKCβ inhibitor, Enzastaurin (LY317615 - Eli Lilly) for its effect on proliferation and survival of a wide panel of human myeloma cell lines (HMCLs). The possible mechanisms by which enzastaurin exerts its effects were also investigated by analysing the transcriptional pattern altered following enzastaurin treatment. *Methods.* IC_{50} values in 20 HMCLs cultured in 10% FCS containing medium, were calculated from curves based on enzastaurin concentrations ranging from 2.5 to 12.5 μM using both WST-1 assay and cell viability assessment by Trypan Blue exclusion. Cell apoptosis, as suggested by an increase in the proportion of cells with a sub G0/G1 DNA content and by membrane permeability by PI versus FSC, was assessed by flow cytometry. The effect of enzastaurin on caspases activation as well as on AKT and GSK3β phosphorylation was evaluated by Western blotting. The gene expression profiling (GEP) data generated by means of high-density oligonucleotide arrays (Affymetrix U133A arrays) were analysed by the Significant Analysis of Microarrays software for the supervised analyses. *Results.* Enzastaurin showed a clear growth inhibition effect in 19 out of 20 HMCLs. Based on the sensitivity to enzastaurin and PKCβ expression, five cell lines were then selected for further studies: AMO1, KMS-26 (both expressing the two PKCβ isoforms) and MM1.S (expressing PKCβ) among the most sensitive cell lines; the intermediate sensitive KMS-18, expressing PKβ and the less sensitive RPM showing a weak PKCβ expression Enzastaurin induced apoptosis in sensitive cell lines was partially caspase dependent. Phosphorylation status analysis of AKT and of GSK3β, a downstream AKT substrate, up to 48 h of treatment showed the inhibition of AKT phosphorylation and a marked decrease of phosphorylated GSK3β levels in all sensitive cell lines. GEP data analysis of KMS-26 cell line treated or not with enzastaurin for 24 hours, showed that 62 genes were upregulated and 32 were downregulated in the treated samples. *Conclusions*. Our data suggest that, in HMCLs, enzastaurin elicits its antitumor effect through the AKT signaling pathway. A functional analysis of deregulated genes following enzastaurin treatment revealed a significant fraction of genes involved in signal transduction, in the immune and inflammatory responses, in transcription regulation, in cellular adhesion and apoptosis processes.

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CHROMOSOMAL ABNORMALITIES OF PLASMA CELLS AND CORRELATION WITH IMMUNOPHENOTYPE IN MULTIPLE MYELOMA

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Background and Aims. Multiple Myeloma (MM) is characterized by a marked heterogeneity of genetic lesions, including numerical and structural chromosomal abnormalities. In the present study we have investigated the relationship between the immunophenotypic profile of myelomatous plasma cells and their specific genetic features by FISH. *Materials and Methods.* Between August 2002 and January 2007, 840 con-

secutive patients with MM, referred to our Hematology Department, entered our study: 639 at diagnosis and 201 at relapse. FISH analysis were performed on bone marrow plasma cells (BMPC) purified using anti-CD138-coated magnetic beads (Miltenyi Biotech GmbH, Germany). Nuclei from fixed PC were prepared for interphase FISH using standard methods. DNA probes (Vysis, Downers Grove, IL) were used to detect chromosome 13 anomalies, t(4;14)(p16;q32), t(11;14)(q13;q32), t(14;16)(q32;q23), del17(p13.1) and gain of 11q23 (MLL). The immunological phenotype of BMPC was assessed using triple or quadruple combinations of MoAbs for the detection of the following antigens: CD56, CD45, CD40, CD19, CD20, CD52, CD117, kappa/lambda. *Results.* Overall, 89.5% of the patients showed at least one chromosomal abnormality and del13 was identified in 49.7%. No difference in del13 prevalence was observed according to age, serum β-2 microglobulin, clinical stage and immunoglobulin isotype. A significant correlation between del13 and poor prognosis (p=0.02) was observed in a group of 201 patients. p53 gene deletion was detected in 12.1% of 320 patients, t(11;14) in 20.9% of 220 patients, t(4;14) in 22.5% of 485 patients, t(14;16) in 3.7% of 140 patients and gain of 11q23 in 56% of 100 patients. PC carrying del13 showed a significant lower expression of CD45 than those without (p<0.0001). Moreover, they less frequently expressed CD19 and CD20 (p<0.0001 and p=0.003). We also observed that patients with PC carrying del13 had a significantly higher BMPC infiltration (p=0.001), were more frequently female (p=0.001) and lambda subtype (p=0.03). PC carrying delp53 were more frequently del13 (p=0.04) and less frequently $CD52^+$ (p=0.04) and CD19+ (p=0.04). The presence of t(11;14) has been associated with CD20 expression (p<0.0001) and with a lower expression of CD56 (p<0.0001) and CD117 (p=0.003). Moreover, the frequence of del13 was significantly lower in patients carrying t(11;14), (p=0.02). A correlation was also observed between t(4;14) and del13 (p=0.002) and the absence of expression of surface monoclonal immunoglobulins (p=0.03). Patients with del13 showed less frequently 11q23 amplification (p=0.02). At least 45% of the patients carried almost one IgH traslocation and had a significantly higher frequency of delp53 (p=0.02). They showed more frequently a CD45 negative (p=0.001), CD19 negative (p<0.001), CD117 negative (p<0.0001) and CD20 positive (p<0.001) phenotype. PC carrying both del13 and CD45 negative phenotype were also associated with the expression of surface monoclonal immunoglobulins (p=0.003). *Conclusions*. The molecular classification of myeloma subtypes and a longer patient follow up are mandatory to better identify the exact prognostic value of chromosomal changes and it may be useful to define more accurately patients into prospective therapeutic trials.

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CORRELATION OF QUANTITATIVE GENE EXPRESSION, VDJ STRUCTURE AND GENOMIC ABERRATIONS IN MULTIPLE MYELOMA

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Introduction. Clinical course and pathogenesis of multiple myeloma (MM) are highly heterogeneous. Some chromosomal abnormalities, such as deletion of chromosome band 17p13 (17p'), impact prognosis while their biological effects are poorly understood. Dysregulation of critical genes resulting in plasma cell (PC) immortalization can be set off by primary IgH translocations (e.g. affecting CCND1, CCND3, FGFR3, MMSET, c-MAF, or MAFB) or chromosomal extra copies (hyperdiploidy). Additionally, a restricted usage of specific VH genes has been reported implicating a role for specific B-cell receptor rearrangements. *Aims.* To elucidate potential pathomechanisms in MM, we investigated the interrelation between genomic abnormalities, deregulated gene expression, and VDJ configuration in a large cohort of patients. *Methods.* Prior to analyses, purification of plasma cells to a median percentage of 85% was performed using immunomagnetic separation. FISH screening for abnormalities involving chromosome bands 1q21.2, 9q34, 11q25, 13q14, 14q32, and 17p13 was performed in all patients. 115 cases were investigated by real-time RT-PCR (RQ-PCR) for the following gene transcripts: CCND1 (coding region and 3`UTR, for the determination of 1.7 kb and 4.5 kb CCND1 variant expression), CCND2, CCND3, FGFR3, MAF, MMSET, MUM1, TACC, RB1, p16, E2F1, p27, p21, CDK4, TP53, ATM, MDM2, MCL-1, BCL-XL, BCL-2, c-MYC, BTBD3, ITGB7, CX3CR1, TYMS, FNTA, EIF3S12, and CXCL12. VDJ analysis was performed in 100 cases. *Results.* Most of the cases exhibited heavily mutated VH genes (median VH homology 92.7%), only 14 showed a sequence homology of more than 96%, thereof 6 with more than 98%. Most frequently used VH genes were V3-30 (21 cases), V3-23 (8), V3-33 (7), V3-21 (6), and V4-59 (6). Only 2 tumors used V4-34, which is frequently rearranged in normal B-cells. The level of VH mutations was equal between the genomic subgroups under study. Distribution of the frequently used VH genes was balanced within the genomic subgroups except for V3-21 gene usage, which was exclusively observed in cases with deletion 14q32 (14q') and 13q14 (13q'). The most characteristic gene expression differences were observed in relation to alterations at 1q21.2 followed by 13q14, and 14q32. For example, cases with +1q showed a highly significant downregulation of CCND1 and BCL-2, whereas CCND2 was upregulated in these cases. 13q' cases showed a lower expression of CCND1 while CCND2 and p16 were overexpressed. Cases with 14q' showed an overexpression of CCND2, CCND3, p16, and p27. Expression of the 1.7 kb CCND1 variant, which confers an aggressive phenotype in mantle cell lymphoma, was observed in only one case in our series and is therefore an infrequent event. *Conclusions.* Abnormalities at 1q21.2, 13q14, and 14q32 were associated with highly characteristic gene expression patterns arguing for a major pathogenic role of these abnormalities. Most of the differentially expressed genes were related to the CCND1 pathway pointing to a central role of the G1/S-phase checkpoint in the pathogenesis of myeloma. Detailed analyses of genome-transcriptome interrelations are ongoing and additional data will be presented at the meeting.

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PROSPECTIVE EVALUATION OF BONE HISTOMORPHOMETRIC CHANGES ASSOCIATED TO BORTEZOMIB IN MULTIPLE MYELOMA PATIENTS

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Background. The loss of bone observed in multiple myeloma (MM) is the result of an uncoupling of bone formation and bone reabsorption. We have previously reported the association between increased alkaline phosphatase (ALP) and response to Bortezomib (B), suggesting a bone anabolic effect of bortezomib. We now report a prospective analysis of bone histomorphometric changes during B treatment in myeloma patients. *Methods.* Single agent B (1.3 mg/m^2 on days 1, 4, 8 and 11, 21 day intervals x 3 cycles) was administered to patients with relapsed/refractory MM. Transiliac bone biopsies were obtained at baseline and after 3 cycles of treatment and examined by high-resolution microcomputed tomography (microCT). To visualize the trabecular architecture of each specimen, a total of 600 microtomographic sections were acquired at 70 KV and 145 ȝA at a nominal isotropic resolution of 37 ȝm^3. To compare samples of varying size, standardized regions of interest (ROI) were analyzed in the trabecular bone between the two intact cortices of the bone biopsy. Bone Volume/Total Volume (BVTV) and trabecular thickness (TbTh), were measured. Bone formation was also determined by tetracycline labeled bone histomorphometry. *Results.* 10 patients were evaluated; adequate baseline biopsy samples were obtained in 7. After 3 cycles of B treatment, follow-up biopsies were available for analysis in 5 patients. Within the 12 weeks of the study period, an increase in BV/TV of > 40% from baseline was observed in 4/5 patients (Table 1).

Table 1.

BVTV			Tb.Th			Tetracycline		Myeloma Response
PRE	POST	Δ %	PRE	POST	Δ %	PRE	POST	
36.4	50.16	38%↑	0.3584	0.3031	16% ↓	-	-	PD
12.85	90	600%↑	0.1338	0.7452	456%↑	-	+	CR
60	66	10% ↓	0.2052	0.2952	44%↑	-	-	PD
33.39	50.06	50%↑	0.2673	0.3371	26%↑	+	+++	CR
52.6	74.03	40%↑	0.346	0.4137	20%↑	-	P	SD

Δ %: % changes. P: Pending

A parallel increase in TbTh. was also observed, defined as a positive skeletal response to B therapy. In addition to the microCT analyses, dynamic histomorphometry measures of the tetracycline labeled bone biopsies were obtained from the same baseline and follow-up samples were in agreement with the microCT *Results.* Myeloma response to B is also shown in Table 1. *Conclusions.* This the first prospective study by micro-CT analysis confirming that even a short course (12 weeks) of bortezomib treatment has a potent bone anabolic effect in patients with multiple myeloma.

0696

ANALYSIS OF DNA REPAIR GENES VARIANTS IN MULTIPLE MYELOMA PATIENTS

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Introduction and Aim. A malfunction of the network responsible for genome stability e.g. DNA repair and high accuracy of DNA synthesis during DNA replication may be related to the pathogenesis of Multiple Myeloma (MM), whose tumoral clone is characterized by a remarkable high genetic instability. Among all the chromosomal alterations so far described, translocations involving chromosome 14 and several chromosomal partners are the most frequent and the most importantly correlated to MM prognosis. In particular, it has been shown that the presence of t(4;14)(p16;q32) at diagnosis has a unfavourable impact on prognosis. In this study, a group of 82 MM patients and a group of 259 healthy donors were genotyped for common SNPs in DNA repair genes. The aim was to evaluate the overall frequency of these variants in MM patients and in particular in those patients carrying t(4;14)(p16q32). *Methods*. PCR-RFLP assays were used to detect five polymorphisms in the DNA repair genes APE1, XRCC1, NBS1, XRCC3, and XPD. An RT-PCR assay was adopted to identify the IgH/MMSET fusion gene, as t(4;14)(p16q32) surrogate. *Results.* Allele frequencies in XRCC1, XRCC3, XPD23 and NBS1 SNPs genes were similar in MM patients and healthy donors. On the contrary, the APE1 variant genotype was significantly associated to a MM increased risk (p=0,04). Moreover, genotype combination of polymorphic-XRCC3 and normal-NBS1 had a marginally significant lower frequency in MM patients, when compared to healthy donors (2,4% vs. 8,5%, p=0,05). Overall, t(4;14)(p16q32) frequency was 26%. Considering MM patients with NBS1 variant allele, the incidence of the t(4;14)(p16q32) positive patients was higher, compared to t(4;14)(p16q32) negative patients (19,1% vs. 9,8%), however this difference was not statistically significant. *Conclusions.* The preliminary results presented here support the hypothesis that common polymorphisms in DNA repair genes may be an important modifier of individual susceptibility to MM. In particular, APE1 polymorphisms may have a role in MM onset. Moreover, the contribution of the NBS1 variant allele to MM onset or to t(4;14)(p14q32) insurgence cannot be excluded. Further studies are ongoing in a larger sample-size population.

0697

GENOME-WIDE ANALYSIS OF DNA COPY NUMBER CHANGES IN MULTIPLE MYELOMA USING HIGH-DENSITY SNP ARRAYS

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Background. Multiple myeloma (MM) is characterized by a profound genomic instability that involves both ploidy and structural rearrangements. Nearly half of MM tumors are non-hyperdiploid and frequently show deletion of chromosome 13 and constitutive activation of CCND1(11q13), CCND3(6q), MAF(16q24), MAFB (20q), or FGFR3/MMSET (4p16.3) genes as a result of chromosomal translocations involving the immunoglobulin heavy chain (IGH) locus on chromosome 14q32. The remaining tumors are hyperdiploid and show a low prevalence of IGH translocations and chromosome 13 deletions. Despite the remarkable recent advances, the spectrum of genetic lesions leading to the biological and clinical variability of MM has not been defined yet. Novel high-throughput approaches may help to progress into the definition of the profound genomic instability generating the bio-clinical heterogeneity of plasma cell dyscrasias. *Aims*. The purpose of the present study was a genome wide analysis of genetic lesions in a representative and stratified panel of MM patients, to provide more insights into the genomic heterogeneity associated with plasma cell neoplasms. *Methods.* Genome wide profiling data have been generated on high-density SNP arrays (50K). After pre-processing, the piecewise constant estimates of the underlying local DNA copy number variation was calculated using the DNAcopy Bioconductor package, which looks for optimal breakpoints using circular binary segmentation (CBS). The median of the estimated profiles was scaled back to a nominal multiplicity of two. Inferred copy number of more than 2.3 or less than 1.7 corresponded to gain or loss of DNA, respectively. The regions presenting a copy number of more than 4 were referred to as gains and subsequently analyzed to investigate copy number alterations. Hierarchical clustering was performed by using of dChip software. *Results*. Hierarchical clustering analysis was used to investigate altered copy number in 41 MM patients, 7 plasma cell leukaemia (PCL) and 7 normal samples. By considering only SNPs with copy number alteration in almost 20% of patients, our analysis showed that chromosome 1q gain (p=1×10^{-4}) and chromosome 13 deletion (p=2.3×10^{-5}) are the main genetic aberrations driving samples grouping. Highly increased copy number DNA levels were found in six different regions, specifically from chromosome 1q and 19, and those chromosomes involved in hyperdiploidy (7, 9, 11, and 15). With regard to regions with decreased copy number, we found the involvement of the two chromosomal regions 18q and 4p not previously reported that warrant further investigations. *Conclusions*. 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.

0698

CYTOGENETIC PATTERNS IN MULTIPLE MYELOMA AFTER A PRECEDING MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MM POST-MGUS) HAVE DIFFERENT PROGNOSTIC IMPLICATIONS THAN IN MULTIPLE MYELOMA WITH *UNKNOWN* PRIOR HISTORY

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Background. Patients with monoclonal gammopathy of undetermined significance (MGUS) may progress to multiple myeloma (MM) or a related disorder with a probability of 1% per year. However, it is at present unclear whether or not MM post-MGUS is biologically and clinically different from MM developing without a durable MGUS-phase (referred to as MM with *unknown* prior history, MM-U). *Aims.* We studied 41 patients with MM post-MGUS using interphase FISH to determine the cytogenetic pattern and clinical outcome. Results were compared with cytogenetic abnormalities found in a reference population of 287 patients with MM-U. *Methods.* Interphase FISH analysis of any 14q-translocation, t(11;14)(q13;q32), t(4;14)(p16.3;q32), 13q-deletions, 17p-deletions. *Results.* In MM post-MGUS, a t(11;14) was found to be more frequent than in MM-U (24% versus 14%) and it was associated with significantly shortened survival (24 months versus 70 months in MM-U; p=0.01). MM post-MGUS was further characterized by a higher frequency of 13q-deletions only (absence of all other specific abnormalities; 28% versus 12% in MM-U; p=0.02). A 13q-deletion only was an indicator of long survival in MM post-MGUS (median not yet reached) as opposed to MM-U (median survival, 29 months; p=0.001). 17p-deletions were infrequent in MM post-MGUS (3% versus 16% in MM-U; p=0.04). Survival times for patients with t(4;14) and/or 17p-deletions and other abnormalities were similar in both MM patient cohorts. *Conclusions.* Our data suggest that t(11;14) and 13q-deletions have distinct prognostic implications in the context of MM post-MGUS.

0699

REGULATORS OF G1 CYCLIN-DEPENDENT KINASES AND MULTIPLE MYELOMA

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The current view of the MM pathogenesis presumes that Cyclin D dysregulation is the early and unifying pathogenic event in this disease. This would modify the cell cycle activity, which would enhance tumor development. Cyclins D form complex and activate cyclin dependent kinases 4 and/or 6 (CDK4/6). The active complexes help cells to pass from early to late G1 phase. CDK4/6 is negatively regulated by the INK4 family of cell cycle inhibitors by preventing cyclin D binding. Inhibitors from the Cip/Kip family can also inhibit CDKs. Accordingly, the balance between D cyclins and CDK inhibitors should play a key role in the behavior of the tumor cell clone from which MM emerges. *Aims.* 1.To evaluate the expression of cyclins D (D1, D2 and D3) and CDK4/6 inhibitors (p15, p16, p18, p19 and p21) by quantitative PCR in tumor plasma cells 2. To determine

whether gene expression values may influence plasma cell proliferative activity and patient outcome. *Patients and methods.* Sample from 75 cases were included in the analysis, accounting for: 51 untreated MM patients, 6 with smoldering MM (SMM) and 10 MM cell lines (MMCL). Gene expression was analyzed in RNA from purified plasma cells (PC) obtained with magnetic separation based on the CD138 expression. RQ-PCR was carried out using the assays-on-demand gene expression mixes specific for these genes (Applied Biosystems), using ABL as control gene. The relative quantification was estimated through the cycle threshold increment method. *Results.* Gene expression was different between the three groups analyzed. The expression of most inhibitors was lower in MMCL than in the other groups, while CD2 was significantly over expressed. By contrast, SMM showed the highest gene expression values for all inhibitors expression of CD2. Finally, MM patients displayed lower values for p16 and p15 than SMM patients. In addition, MM patients with an S-Phase PC <1.8% displayed a gene expression profile similar to SMM, as well as lower values for CD1 and CD2. No significant correlations were found between gene expression and age, performance status, hemoglobin, LDH, creatinine, β2microglobulin or C-reactive protein. Moreover, they were not either correlated with the treatment response or survival, with the exception of the previously observed relationship between a high p15 and p16 expression and good prognosis. Finally, although cyclin expression was not statistically different between the three defined groups, we could confirm a significant correlation between the expression of the different cyclins and the presence specific translocations. *Conclusions.* Although cyclin D seems to play a central role in the pathogenesis of MM, its effect could be regulated through the expression of CDK inhibitors that exert its function downstream in the pathway. The expression of CDK inhibitors is related with the type of monoclonal gammopathy and correlates with several clinical and biological characteristics of the disease. Cyclin D expression is correlated with the MM subtype based on the presence of chromosomal translocations.

0700

ZHX2, CHC1L AND RAN GENE EXPRESSION LEVELS DETERMINE DIFFERENT PROGNOSIS GROUPS IN MM PATIENTS

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Gene Expression Profiling through RNA expression arrays has provided new clues on Multiple Myeloma (MM) pathogenesis and prognostic evaluation. Recently, ZHX2, CHC1L & RAN expression has been remarked as an important key in this disease. In the present paper, we have evaluated these genes by RT-PCR in purified plasma cells from 85 patients with plasma cell discrasias. *Material and Methods.* RNA was obtained from purified plasma cells obtained from the following patients: six MGUS, seven smoldering MM, 52 untreated MM patients, six relapsing MM patient, four Plasma cell leukemia (PCL) and 10 MM cell lines. RQ-PCR of CHC1L, RAN and ZHX2 genes was carried out using the standard protocol from TaqMan® gene expression assays (Aplied Byosistems, CA). ABL gene was used as control gene and the expression value was obtained with the CT increment method. *Results.* CHC1L (C), ZHX2 (Z), and RAN (R) gene expression was heterogeneous. Z gene was slighly underexpressed in aggressive forms of plasma cell discrasias (PCL and MM cell lines) while R gene was highly expressed in all cell lines. This result has been confirmed by our own by group by Expression Arrays in 13 MM patients and 3 MM cell lines. Within newly diagnosed symptomatic MM patients, C underexpression in hyperdiploid compared to diploid MM cases suggesting that the participation of this gene in the chromosomal condensation during the mitosis is crucial for the generation of this two main MM subtypes. In addition increased expressions of C were associated with favorable prognostics: β2 microglobulin <4 mg/L, LDH <460 UI/mL, Creatinine <2 mg/mL, good performance status (ECOG<2), and stage I of International scoring system (ISS). However, the differences did not reach statistical significance. High levels of R transcripts were related to monosomy of chromosome 13 (p=0.03), but no other association with clinical and biological characteristic could be observed. Finally, patients with high Z expression showed good prognostic features (low B2M, High Hb levels and good performance status, p<0.05) as well as better overall survival at 3 years (71% vs.45%, p=0.033).*Conclusions.* In this study we confirm that CHC1L and especially ZHX2 genes may play an important role in the pathogenesis of MM, which could influence the clinical behaviour and survival of the patients.

Non-Hodgkin lymphoma - Clinical

0701

DOES MALT-LYMPHOMA OF THE LUNG REQUIRE IMMEDIATE TREATMENT? AN ANALYSIS OF 11 UNTREATED CASES WITH LONG TERM FOLLOW-UP

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Background. Mucosa-associated lymphoid tissue lymphoma (MALT lymphoma) of the lung is a relatively rare disease with minor growth for a prolonged period of time and the potential for late dissemination, as generally known for MALT lymphoma. *Aims.* As little is known about the natural clinical course of MALT lymphoma if left untreated, we have analysed all patients undergoing a watch-and-wait policy at our institution. *Patients and Methods.* A retrospective analysis identified a total of 11 patients with MALT lymphoma of the lung who did not undergo treatment following initial diagnosis. All patients had undergone extensive staging and were closely observed with restaging every three months. *Results.* Five patients (48%) had MALT lymphoma restricted to the lung, while the remaining six patients had additional extrapulmonary sites detected during staging. The median time of observation without therapy was 26.3 months (Inter-quartile range: 5 to 60 months); within this time all 11 patients showed at least stable disease. Six of these 11 patients (52%), however, had spontaneous regressions and wax-and-wane phenomena of the pulmonary lesions, but not of extrapulmonary manifestations. Three of these patients had evidence of t(11;18)(q21;q21), while the remaining three had no evidence of genetic aberrations. One patient was referred to treatment after progression in the lung, while two patients progressed outside the lung. Currently, all patients are alive, with 8 patients still being only watched. *Discussions.* Our findings suggest MALT lymphoma of the lung as a very indolent disease with the potential for spontaneous regression. In view of this, patients diagnosed with pulmonary MALT lymphoma might not need require immediate treatment in the absence of symptoms, and a watch and wait policy could be adopted.

Table 1. Patient characteristics.

Gender	Age at diagnosis	Manifestation outside the lung	Bilateral involvement of the lung	Time of observation (months)	Regressions during the observation period	Referred to treatment	Autoimmune disease	Genetic aberrations
Female	70	No	No	5	No	No	No	None
Male	40	Parotid, stomach, lacrimal gland	Yes	20	No	Yes	No	t(11;18)(q21;q21)
Female	66	No	Yes	56	Yes	No	No	t(11;18)(q21;q21)
Male	51	No	No	23	Yes	No	No	t(11;18)(q21;q21)
Female	54	Breast, parotid, orbita	No	15	Yes	No	SS*	Trisomia 3, 18
Female	68	No	No	5	No	No	No	None
Male	73	Colon	No	25	No	Yes	No	t(11;18)(q21;q21)
Male	62	Stomach, bone marrow	No	34	No	No	Hepatitis (B+C)	t(11;18)(q21;q21)
Female	60	No	No	60	Yes	Yes	No	None
Female	74	Stomach	No	13	Yes	No	No	t(11;18)(q21;q21)
Female	42	Parotid, conjunctiva	Yes	24	Yes	no	SS	None

*sjögren syndrom

0702

THE IMPACT OF SELECTED CELL SURVIVAL REGULATORS (OF AKT-2, BCL-2, CD29, PKC-DELTA AND SURVIVIN) ON PREDICTION OF CLINICAL OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Background. Apoptosis plays an important role in normal and malignant lymphopoiesis. Therefore signaling pathways participating in this process are intensively investigated to find their crucial molecules suitable for development and application of new antineoplastic agents. *Aims.* Based on results of our previous studies and literature search, we selected 9 molecules which play an important role in survival of malignant cells to validate their impact for prediction of outcome in DLBCL. *Methods and Materials.* We studied 46 patients with primary DLBCL.The group consisted of 29 men and 27 women with a median age of 59 years (range, 24-79). Advanced stage (III/IV) was observed in 24 cases (52%) and the distribution according to the IPI was as follows: low risk, 20 cases (44%);

low/intermediate risk, 12 (26%); high/intermediate risk 9 (19%); and high risk, 5 (11%). All patients were treated with curative intent and, except for one case, an initial treatment started with a CHOP chemotherapy regimen with a median of 6 administrated cycles. Overall response rate was 69% with a complete response (CR) in 31 patients (67%). For subsequent analyses patients were divided into two subgroups: 1. with cured disease (31 cases, 54%; median follow-up 4,3 years), and 2. with fatal course of disease (21 cases, 46%; median follow-up 9 months). Only patients, who died of disease progression were included. Imunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections with monoclonal antibodies against: Akt-1, Akt-2, phospho-Akt-Ser-473 and phospho-Akt-Thr-307, Bcl-2, integrin-βI (CD29), protein kinase C β I and II (PKC-βI, PKC-βII), gama (PKC-γ), delta (PKC-δ), and Survivin. The expression was considered positive if 5% or more of the tumor cells were stained with the antibody, except for the cases of CD29, Survivin and Bcl-2, where the limits were: 10%, 20% and 40%, respectively. In addition, the expression of PKC isoforms was analyzed by Real-Time-PCR and obtained C_T-values were compared. *p*-value was based on Gehan-Wilcoxon test or chi-square test and *p*-values 0,05 were considered significant. The Kaplan-Meier method was used to estimate overall (OS) and progression-free survival (PFS). *Results.* Tumor cells'expression of phospho-Akt-2-Ser-473, Bcl-2, CD29 and Survivin correlated with an inferior OS and PFS and predicted an adverse outcome in patients with DLBCL. Thus, patients with positive expression of phospho-Akt-2-Ser-473 had a median of OS and PFS lower compared to those with negative expression (OS: 45 vs 11 months, $p<0,008$; PFS: 37 vs 10 months, $p<0,004$). Similar data were obtained for: Survivin (OS: 40 vs 29 months, $p=0,1$; PFS: 40 vs 15 months, $p<0,035$), Bcl-2 (OS: 45 vs 12 months, $p<0,015$; PFS: 30vs6 months, $p<0,02$), CD29 (OS: 45vs20 months, $p<0,04$; PFS: 40 vs 10 months, $p<0,025$). In the case of PKC-δ higher mRNA expression correlated with favourable outcomes (OS: 45 vs 10 months, $p<0,015$; PFS: 35 vs 9 months, $p<0,075$). The other results were not statistically significant. *Conclusions.* Our results have shown that expression of Akt-2, Bcl-2, CD29 and Survivin was significantly associated with a poorer clinical outcome in DLBCL patients. Those molecules could be potential targets for therapeutic interventions in DLBCL.

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Figure 1. Kaplan-Meier curves of overall survival: p-Akt-2.

0703

CHARACTERISTICS OF 29 PRIMARY ORAL CAVITY LYMPHOMAS, INMMUNOCOMPETENT AND HIV+ , ASSISTED AT THE ONCOLOGY HOSPITAL MARIE CURIE, BUENOS AIRES, ARGENTINA

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Primary oral cavity lymphomas (POCV) are uncommon and must be distinguish from widespread involvement of systemic lymphomas. The primary intraoral lesions are frequently miss out, and the diagnosis delayed. Countries with high incidence of oral malignancies report cancer predominance. Alcohol and tobacco have been mentioned like risk factors. HIV positive population have higher prevalence than immunocompetent patientes. The International Prognostic Index has been taken in consideration. Patients attended Hospital on referal from medical or odontology centers and others on their own. A total of 29 patientes were studied. Aged 21 to 70, media 47,3 years. Male predominance: 69%. Histopathology: NHL high grade B: 19 cases, NHL low grade B : 4, NHL high grade T: 3 and plasmoblastic 3. Sites: superior maxilla: 12, inferior maxilla 11, tongue 3, palate 3. No predominance for right or left locations. HIV positive 9 patients (31%). The prevalence among inmmunocompetent was markedly lower: in 662 diagnosed with NHL 20 cases=3,02%, in 32 NHL HIV positive 9 cases=28,12%. More frequent complaints were: painless swell of the maxilla, movable teeth, dysphagia, facial edemaThe stages were I and II, 28% with locoregional extension: adenopathies or bone infiltration. The IPI was 0 in17, 1 in 10 and 2 in 2 patients. All patients received chemotherapy (CVP, CHOP), with initial good response and early relapse in HIV+. *Conclusions.* In correspondence with others reports,we found predominance in male and middle age, B cell lineage and connection with HIV infection. POCL may lead to the diagnosis of an unknown HIV infection. The little specificity of the initial symptons tends to delay the diagnosis and the referal to a specialized center. IPI did'nt prove useful as a prognostic assessment.

0704

CLINICAL FEATURES AND TREATMENT OUTCOMES OF PRIMARY TESTICULAR DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction. This study was designed to determine response, outcome and patterns of failure of patients with testicular diffuse large B-cell lymphoma (TDLBCL). *Methods.* Consecutive patients (pts) with TDLBCL under observation at the Russian Cancer Research Center beetween 1995 and 2005 that received treatement with doxorubicin-based regimens ± radiotherapy and intrathecal therapy were considered for this study. We identified 25 pts whose median age was 60 years (range 34-79), 16 (64%) pts presented with CS IE-IIE, 3 (12%) had B symptoms, 21 pts (84%) had IPI score < 3. All 25 pts treated with orchidectomy (3 pts after that loss to follow-up), 16 pts received CHOP and 6 pts R-CHOP with additional scrotal radiation (5 pts) and intrathecal methotrexate (9 pts). *Results.* Seventeen pts (77%) achieved a complete response and 5 failed initial therapy, 9 responding pts had a relapse. 12 pts (55%) experienced a relapse in multiple extranodal sites, 4 of them had CNS and 6 contralateral testis involvment. Three-years progression free survival (PFS) was 36% with median 14 months, overall survival (OS) - 56% with median 48 months. Among the 9 pts, who received intrathecal methotrexate, 2 had CNS relapse. No testicular relapse was observed in patients receiving scrotal radiation. *Conclusions.* Our data confirms that most patients with TDLBCL had unfavourable chances for long-term survival. CNS was found to be the principal site of relapse and no progression to contralateral testis was observed in those patients, which had additional scrotal radiation. Poor prognosis of diffuse large B-cell lymphoma of the testis calls more effective treatment strategies of this patients with the purpose to provide control of both sistemic disease and disease of the CNS. The contralateral testicular irradiation has to be taken into the treatment planning.

0705

THE SHIFT OF TREATMENT FOR NASAL AND NASAL-TYPE NK/T CELL LYMPHOMA,TEN YEARS EXPERIENCE AND PROGNOSTIFICATION ANALYSIS IN A SINGLE INSTITUTION

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Background. For nasal and nasal-type NK/T cell lymphoma the prognosis is poor,the two-year survival ranges from 20% to 40%,and there is no standard modality of treatment. Even patients presented with limited stages of disease undergoing radiotherapy with or without chemotherapy,there are substantial percentage of patients died of local tissue destruction complicated with infection and/or distant relapse of disease. *Aims.* We are looking for the best modality of treatment in the past 10 years (1996~2006) at our hospital to achieve better survival for these patients. We are also in search of the impact of immunophenotypes and treatment modalities upon survival. *Methods.* This is a retrospective observation study to treat 27 patients of nasal and nasal-type NK/T cell lymphoma at out hospital from 1996 to 2006 in three periods of time. In the first period, from 1996 to 2002,we treated 16 patients with radiation alone or CHOP chemotherapy followed by radiotherapy. In the second period,from 2002 to 2004,we treated 5 patients with more intensive induction chemotherapy BFM-90 protocol followed by radiotherapy. In the third period, from 2004 to 2006, we treated 6 patients with concurrent chemoradiotherapy (CCRT) with ICE. *Results.* For total 27 patients, the 2-,3-,and 5-year overall survival are 45%,33%,and 33%, respectively, and it seems to be in plateau until 84 months. For 16 patients, 5 patients, and 6 patients treated in the 1st, 2nd,and 3rd periods of times, the overall survival are 25%,40%, and 66.6%,respectively. For $CD3^-$/ $CD56^+$ compared with $CD3^+/CD56^+$ patients, the 5-year overall survival are 80% and 19%,respectively (p value 0.10). For $CD4^-/CD8^-$ compared with other patients, the 5-year overall survival are 67% and 21%,respectively (p value 0.037). For patients undergoing CCRT compared with sequential treatment, the 3-year overall survival are 57% and 33%,respectively (p value 0.27).For patients underwent more intensive chemotherapy as compared with CHOP, the 5-year overall survival are 64% and 25%, respectively (p value 0.08). *Conclusions.* Concurrent chemoradiotherapy is better than sequential chemoradiotherapy and more intensive chemotherapy has better overall survival than CHOP chemotherapy. $CD3^-/CD56^+$ and $CD4^-/CD8^-$ NK/T cell lymphomas have better prognosis.

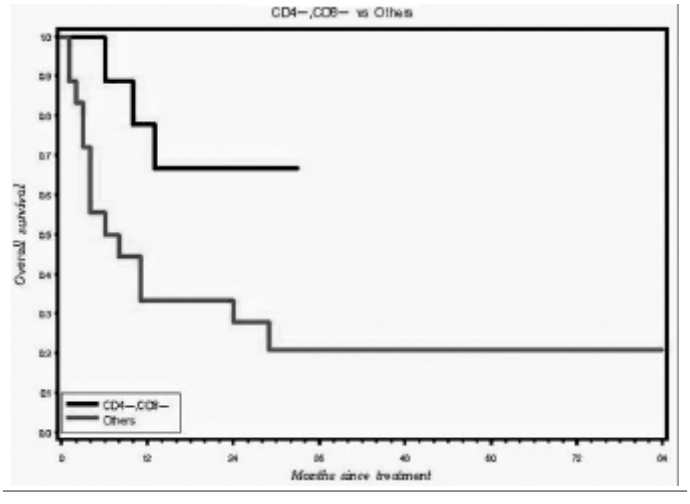

Figure 1.

0706

PRIMARY EXTRANODAL FOLLICULAR LYMPHOMA: CLINICOBIOLOGICAL FEATURES AND OUTCOME

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Background. Follicular lymphoma (FL) is typically a nodal disease. Primary extranodal FLs, that represent less than 10% of the cases, might have differentiated clinicobiological features. *Aims.* To analyze the main clinicobiological characteristics, response to therapy and outcome of a series of patients with FL primarily extranodal in origin, and compare them with nodal FLs. *Methods.* Seventeen patients (10M/7F; median age, 61 years) with FL and primary origin in extranodal location, diagnosed at a single institution during a 25-year period, were the subject of the study. Skin FL was excluded from the study. The control group was constituted by 212 patients with nodal FL diagnosed during the same period of time. Main clinicobiological features were recorded and analyzed. *Results.* The sites of the primary disease were: Waldeyer's ring, 4; GI tract, 3; bone marrow, CNS and parotid (two cases each); and pancreas, thyroid, kidney and orbit (one case each). Main histological and clinical features are listed in the table. Treatment was given without considering the nodal or extranodal origin of the disease and consisted of: monotherapy with alkylating agents (35 cases), polychemotherapy (122), and fludarabine alone or with other drugs (14) and others, including surgery and observation (58). CR rate was higher in extranodal than in nodal FL (85% vs. 53%, respectively; p=0.02), but no differences were found in overall survival. FLIPI score was the most significant variable predicting overall survival in the global series as well as in either in nodal or extranodal FL. *Summary.* Extranodal FL have some peculiar clinicobiological features with respect to nodal cases. Regarding the outcome, although patients with extranodal FL showed a higher CR rate, the overall survival was similar in both groups.

Table 1.

	Extranodal FL	Nodal FL	P
Age (median, range)	61 (28-82)y	55 (24-93) y	NS
Sex (M/F)	10/7	100/112	NS
Histological grade 3 (%)	7	10	NS
CD10+ (%)	90	90	NS
Bcl2+ (%)	75	91	NS
Bcl2/JH (%)*	40	75	0.03
Stage IV (%)	47	64	NS
Bone marrow + (%)	35	62	0.02
LDH > 450 IU/L (%)	12	24	NS
ß2-m > 2.3 mg/L (%)	6	41	0.05
High-risk FLIPI (%)	23	35	NS
CR rate (%)	85	53	0.02
5-year OS (%)	79	74	NS
5-year FFS (%)	79	74	NS

*In 5 and 80 patients with extranodal and nodal FL, respectively

0707

ANALYSIS OF 136 REPORTED HAEMATOLOGICAL AUTOIMMUNE CASES IN NHL

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Background. Haematological autoimmune complications are relatively common in CLL but are much less common in NHL. In large studies the prevalence of autoimmune hemolytic anemia (AIHA) is 1,57%, of autoimmune thrombocytopenia (AITP) is 0,76% and of Evans-syndrome 0,18%. *Methods.* We have analysed 136 individual cases of NHL (excluding CLL) associated AIHA, AITP and Evans-syndrome reported in the literature (88 cases of AIHA, 34 cases with AITP and 14 with Evans-syndrome) with regard to demographic factors, prevalence in subtypes and treatment response. *Results.* The median age of all patients was higher than in idiopathic cases. In contrast to idiopathic AIHA and AITP there was no female sex prevalence. In AIHA the sex prevalence was different in subtypes. AIHA and AITP occurred in all subtypes in NHL with the exception of AIHA in mantle cell-lymphoma (no case). There was of high prevalence of localized of extranodal early stage lymphomas (in particular in DLCL). In both AIHA and AITP sustained responses to steroids and HD-IgG were uncommon. Splenectomy had high efficacy only in SLVL. Surgical removal of extranodal lymphomas and/or chemotherapy was effective in about half of the cases. The mortality was higher in AIHA than in AITP. Rituximab was effective in some highly refractory cases of AITP and AIHA. *Conclusions.* This compilation of data indicates a complex relation of lymphoma and AIHA/AITP and warrants more attention and specific studies.

0708

RAPID INFUSION RITUXIMAB IS AS EFFECTIVE AND SAFE AS CONVENTIONAL INFUSION REGIMES IN THE TREATMENT OF DIFFUSE LARGE B CELL LYMPHOMA: A 2 YEAR PROSPECTIVE STUDY

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Background. Rituximab is increasingly used as standard therapy for B-cell lymphomas and refractory benign haematological disorders. This has strained capacity of chemotherapy units, especially given recommended long infusion times (3-4 hours) to avoid infusion-related toxicity. Several studies suggest rituximab may be tolerated by rapid infusion (90 minutes) if combined with steroids (Sehn *et al.*, 2007; Salar *et al.*, 2006), but no study has examined whether rapid infusions impact on the efficacy of rituximab. *Aims*. To determine whether rapid rituximab infusions are as effective and safe as standard infusions in treatment of diffuse large B cell lymphoma (DLBCL). *Methods*. This study was conducted prospectively in the Haematology Unit, Norfolk and Norwich University Hospital, UK, between October 2004 and September 2006. Patients diagnosed with DLBCL (WHO Code 9680/3) were treated with 6-8 cycles of CHOP chemotherapy in combination with 375 mg/m^2 rituximab each cycle. All received 100 mg prednisolone, 8 mg chlorphenamine, 1 g paracetamol and 5 mg tropistetron 1 hour before rituximab. Patients received their first rituximab at the standard rate. If this was tolerated (NCI toxicity grade <1), patients received subsequent rituximab by rapid (90 minute) infusions. Response to treatment and overall survival were analysed. *Results*. Our study comprised 61 patients with DLBCL (see Table 1).

Table 1. Responses to rapid rituximab infusions.

93% (57/61) patients received 3 weekly CHOP with rituximab for 6 - 8 courses. Three patients received either 3 cycles of R-PMITECBO or R-CODOX-M as well as 3 or 4 cycles of R-CHOP as their treatment. One DLBCL patient received fortnightly R-CHOP. One patient experienced Grade 1 toxicity during the initial standard-rate rituximab infusion; therefore, all patients received subsequent rituximab by rapid infusion. 250 rapid infusions were administered to 61 patients with no toxicity recorded. The efficacy of rapid rituximab infusions was assessed after median follow-up of 16 months (range 6-27 months). CR and OS rates were 69% and 77% respectively. In the 60-80 year old group (34 patients), the CR and OS rates were both 79%. In the 18-60 year old group with IPI scores 0 or 1 (13 patients), the CR and OS rates were both 92%. *Summary and conclusions*. Ours is the first study to demonstrate that rapid rituximab infusions have similar efficacy to standard infusions. In the French GELA trial, R-CHOP treatment of patients between 60-80 years with DLBCL resulted in a CR rate of 75% and OS of 70% after 2 years (Coiffier et al, 2002). In our matched patient cohort, CR and OS rates were both 79% (p=0.28, comparing OS), confirming non-inferiority of rapid rituximab. In the MinT trial, R-CHOP for DLBCL patients aged 18-60 years with IPI 0 - 1 had a CR rate of 86% after 15 months (Pfreundschuh et al, 2006), similar to our 13 matched patients with CR and OS rates both 92% (p=0.27, comparing CR). This study also confirmed rapid rituximab infusions are safe from the second infusion onward. No adverse events were experienced by any of the 61 patients over 250 rapid infusions. In conclusion, rapid infusion rituximab should be the standard of care for DLBCL if the first standard-rate infusion is well tolerated.

0709

SECOND MALIGNANCIES AFTER TREATMENT FOR INDOLENT LYMPHOMA: A 16 YEARS FOLLOW-UP STUDY

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Background. Second malignancies have been associated with Non Hodgkin Lymphoma treatment. Most studies report a high risk of second cancers (2 to 8 fold increase), mainly due to high incidence of MDS/AML and solid tumors, such as lung, bladder and gastro-intestinal cancers. Nevertheless few analyses have addressed this issue focusing on indolent lymphoma. *Aims.* Aims of this study are to determine the incidence and the risk factors for the development of second cancers during long term follow up of patients treated for indolent lymphoma. *Methods.* The Gruppo Italiano Studio Linfomi (GISL) maintains a database on clinical characteristics, treatment and follow up of all patients who entered clinical trials. To address a uniform patients population in this study, we identified 563 previously untreated patients with histologically confirmed diagnosis of indolent lymphoma, enrolled in GISL trials between 1988 and 2003. The incidence (numbers of second neoplasia by person-years under analysis) of second cancers in the study population was compared to the incidence of solid cancers in the Italian population, utilizing age-, sex- and calendar period-specific incidence rates, derived from ISTAT database. Standardized incidence ratio (SIR, the observed/expected ratio) and absolute excess risk (AER, observed cases in our cohort minus numbers expected, divided by person-years at risk) were calculated after stratifying for age cohorts. Time Free to second Tumor (TF2T) was measured from the end of the first treatment to last follow-up or date of diagnosis of second tumor. TF2T was calculated with a Kaplan-Meier estimate. Effects of potential risk factors on second cancer rates were examined in a Cox proportional-hazard model. *Results.* After a median follow-up of 62 months , we observed 33 cases (6%) of second cancers, including hematologic malignancies (2%). Ten out of 33 patients developed MDS/AML and 23 solid tumors, including 6 lung cancer, 6 gastro-intestinal cancer and 11 other type of cancers. Overall, incidence rate (1000 person-years) was 11.5. Excess of risk for second solid cancer was detected in the cohort age 50-54 (SIR =4,2; 95% CI 1.9-9.40; AER =1.27). Median TF2T was 28 months for MDS/AML, 44 for lung cancer and 47 for gastro-intestinal cancer. By univariate and Cox regression analysis, after stratifying for histology, we observed a significant negative impact (all p<.05) on TF2T for age at first treatment , male sex and fludarabine-containing therapy. Further, we divided the log(HR), estimated by Cox regression analysis based on age, male sex and fludarabine-containing therapy, at the 33° and the 66° percentiles. Based on this analysis, we observed three groups with significant difference in the risk of developing second cancers (p<0.0001). *Conclusions.* Patients treated for indolent lymphoma are at elevated risk of developing second primary malignancies. The SIR and AER are increased in patients who were younger at first treatment. Age, male sex and fludarabine-containing chemotherapy have a negative impact on TF2T. Finally, utilizing these parameters in a Cox regression model, we were able to identify groups with an increased risk of second malignancies.

Acknowledgement. We would like to thank the GISL Trial Office for data management.

0710

CLINICO-PATHOLOGICAL FEATURES, RESPONSE TO FIRST-LINE THERAPY AND SURVIVAL OF A LARGE SERIES OF PATIENTS WITH PRIMARY CUTANEOUS LYMPHOMA FROM AN ACADEMIC REGIONAL HOSPITAL IN ITALY

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Background/Aims. 2005-WHO/EORTC classification for primary cutaneous lymphomas (PCL) has provided clear-cut diagnostic criteria, relative frequency and patients' survival for each entity. We tested the utility of this classification and calculated relative frequency and survival for each entity in our series of PCL patients. *Methods.* Of all our PCL patients diagnosed and treated from 1990 to 2006, diagnoses were reviewed according to the WHO/EORTC scheme and the clinical data retrieved. Overall and major/minor-event-free survival (OS, major/minor-EFS) were calculated by Kaplan-Meier method. *Results.* Clinico-pathological features: Our patients were 259, 170 M/89 F (median age, 62 yrs): 191 Mycosis Fungoides (MF: 185 classic/6 folliculotropic type; 168 stage IA-B, 14 IIA-B, 6 IIIA-B, 3 IVA); 43 cutaneous B-cell lymphomas (CBCL: 25 follicular; 14 marginal zone; 3 diffuse large B-cell lymphoma-leg type; 1 lymphoblastic lymphoma); 10 Sézary Syndrome (SS); 7 CD30+ lymphoproliferative disorders (7 Lymphomatoid Papulosis-LyP); 8 rare cutaneous T-cell lymphomas (rare-CTCL: 5 peripheral T-cell lymphoma unspecified, 1 g/d lymphoma, 1 CD4+/CD56+ Hematodermic Neoplasia, 1 T-cell lymphoblastic lymphoma; all stage IV). First lesions appeared at a mean age of 54 yrs, with a mean interval to the diagnosis of 57 mo.s. Patients had patches in 57%, nodules in 20%, plaques/papules in 17% and erythroderma in 6%. Lesions were disseminated in 57%, multiple with regional distribution in 30% and single in 13%. Pruritus was the most frequent symptom (46%). First-line therapies consisted of phototherapy and interferon in 54%; mono-/poli-chemotherapy in 14%; heliotherapy in 13%; radiotherapy in 8%, surgery in 6%; other therapies in 3%; no therapy in 2%. Response to therapy: The overall response rate was 100% in LyP (all complete remissions-CR), 93% in CBCL (88% CR), 92% in MF (81% CR), 50% in rare-CTCL (38% CR) and in SS patients (20% CR). Median time to CR was 1 month in CBCL, 2 mo.s in rare-CTCLs, 5 in MF, 7 in SS and 13 in LyP. Follow-up: 30% MF, 16% CBCL and 14% LyP patients experienced a minor event. Median minor-EFS rate was 71 mo.s. A major event occurred in 75% rare-CTCL, 30% SS, 14% CBCLs and 7% MF patients. Major-EFS was significantly worse in rare-CTCL patients (median 6 mo.s). In MF the 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 35 patients (13%) were dead. Median OS was 156 mo.s, significantly influenced by the lymphoma type: 10 mo.s in rare-CTCLs, 135 in SS and 115 in CBCLs; patients with LyP (100% rate at 180 mo.s) and MF (83% rate at 140 mo.s) behaved much better. *Summary and Conlusions*. Our series confirms the clinical utility of the WHO/EORTC classification which segregates patients with different outcome. MF at initial stage and CBCLs are indolent disorders with a risk of minor events, whereas advanced MF, SS and particularly rare-CTCLs behave aggressively. PCL subtype relative frequences in our series differs from WHO/EORTC data maybe due to a peculiar case-selection in a Hematological setting.

0711

PATHOLOGY AND CLINICAL COURSE OF MALT LYMPHOMA WITH PLASMACYTIC DIFFERENTIATION

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Background. The feature of plasmacytic differentiation (PD) is present in up to 30% of patients diagnosed with MALT lymphoma. To date, the influence of PD on the clinical course of MALT lymphoma has not been assessed. *Aims and Methods.* Therefore, we have retrospectively analysed the clinical characteristics and the course of the disease in 34 (25%) patients with PD as compared to 101 (75%) MALT lymphoma-patients without this histological feature. *Results.* Patients with PD had significantly more extragastic lymphomas (28/34 (83%) vs. 54/101 (53%), p=0.003) and a significantly lower rate of t(11;18) (2/26 (8%) vs. 22/72 (31%), p=0.02). There was no significant difference of age at diagnosis (62 years vs. 64 years, p=0.64), relapse rate (48% vs. 37%, p=0.27), median time to relapse (26.5 months vs. 29 months, p=0.84), monoclonal gammopathy (50% vs. 44%, p=0.63), t(14;18) involving IGH/MALT1 (11% vs. 8%, p=0.68), trismomy 3 (31% vs. 27%, p=0.69), trismomy 8 (8% vs. 10%, p=0.74) and the presence of autoimmune diseases between both groups (53% vs. 37%, p=0.09). *Conclusion.* In conclusion, we found that PD is predominantly found in extragastric MALT lymphoma but has no significant impact on clinical course and prognosis.

0712

A VALIDITY STUDY OF CYTOMORPHOLOGY AND FLOW CYTOMETRY IN LYMPH NODE BIOPSIES FROM PATIENTS WITH ADENOPATHIES

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Background. Cytomorphology and flow cytometry may be an useful diagnostic test in tissues from patients with adenopathies. *Objective.* The aim of this study was to assess the efficacy of flow cytometry for diagnosis of lymphoma in patients with lymphadenopathies with or without suspected haematological disorders. *Material and Methods.* The study was conducted from January 2004 through February 2007. The study was designed with 90% power at a significance level of 0.05 to detect a sensitivity of 70% or more, assuming an overall incidence of lymphoma of 38% (EPIDAT 3.1 program).We prospectively evaluated the cytomorphology and the immunophenotype of 268 consecutive biopsies. Cytomorphology of touch imprints (May Grünwald Giemsa) and flow cytometry were performed on fresh material. All biopsies underwent histologic and immunohistochemistry study (*Gold Standard Study*). The following combinations of monoclonal antibodies were systematically used in the immunophenotype study: CD3/CD4/CD8/CD45, CD19/CD5/CD20/CD45, CD19/CD10/CD20/CD45, CD19/sIgK/sIgL. In certain cases, the study was expanded with other B, T, NK or myeloid monoclonal antibodies in accordance with the initial immunophenotype *Results.* Cytomorphology and flow cytometry studies and histologic and immunohistochemistry studies were interpreted independently by hematologists and pathologists respectively. The samples were categorized positive if an aberrant phenotype was detected by flow cytometry or if Hodgkin cells were detected in the touch imprint. *Results.* Both studies were completed in 239 biopsies. 29/268 (10.8%) samples were not analyzed using flow cytometry because of necrosis or insufficient number of cells. The biopsies were performed in 142 males (53%) and 126 females (48%). The mean age was 51 years (1-93). In 24/239 (10%) cases, there was a previous history of lymphoma. The most common locations of lymphadenopathies were abdominal 41 (15%), inguinal 32 (12%), otolaryngologic 30 (11%), supraclavicular 26 (9%), axillary 18 (7%), mediastinal 13 (5%), splenic 2 (0.7%), and other locations 10 (4%). The cytomorphology and flow cytometry were significantly faster in recognizing lymphomas than classical pathological examination (1.8 days versus 8.7 days respectively p<0.01). Overall sensitivity, specificity, positive predictive value and negative predictive value were: 90.83% (85.25-96.41), 98.33% (95.63-100), 98.20% (95.27-100), 91.47% (86.27-96.68) respectively. There were 11 false negative *Results.* 6 Hodgkin Disease, 2 T cell rich B cell lymphoma, 1 diffuse large B cell lymphoma, 1 marginal zone lymphoma, 1 lymphoplasmacytoid lymphoma. There was only one false positive result: flow cytometry showed incorrectly a lymphoblastic T lymphoma. The histologic and immunohistochemistry study cofirmed a granulocytic sarcoma. *Conclusions.* Cytomorphology-Flow Cytometry is an useful diagnostic test in tissues from patients with adenopathies. It is an excellent method for identifying lymphomas

0713

TREATMENT OF ANTI-MAG-ASSOCIATED POLYNEUROPATHY IN PATIENTS AFFECTED BY MONOCLONAL GAMMOPATHIES AND LYMPHOPROLIFERATIVE DISORDERS

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Background. The anti-myelin-associated glycoprotein (MAG) neuropa-

thy is an antibody-mediated demyelinating neuropathy caused by anti-MAG monoclonal IgM or rarely IgG in the context of a monoclonal gammopathy or lymphoproliferative disorder. The clinical picture is characterized by a distal, symmetric and sensori-motor neuropathy associated with a reduction or lack of deep reflexes and a decrease of superficial and deep sensitivity. The diagnosis relies mainly on blood tests demonstrating, by ELISA or Western blot, the Ig reactivity against MAG. Anti-MAG neuropathy is frequently treatment-resistant and most of the therapies currently used (high-dose IV immunoglobulins, chlorambucil, INF-α and plasma exchange) cause toxicity or patient's disconfort. Since it has been demonstrated that the lowering of the MAG-antibody titer correlates with an improvement in neuropathic symptoms, the main task of treatment for anti-MAG neuropathy is to reduce the antibodies concentration. Rituximab, a monoclonal anti-CD20 antibody suppressing B cell clones, has been recently introduced in the treatment of this neuropathy. *Aims.* The aim of this study was to review clinical characters and therapy in a series of patients treated in our Institution for anti-MAG neuropathy. The feasibility and effectiveness of rituximab in patients resistant to first-line treatments was also evaluated. *Patients and Methods.* Eleven patients (three women and eight men), with a median age of 63 years (range 52-71) were included. Five patients were affected by a monoclonal gammopathy of unknown significance (MGUS), five by a low-grade B-cell non-Hodgkin lymphoma, one by AL amyloidosis with MGUS. All patients received, at diagnosis and after each treatment, a complete haematological staging including bone marrow biopsy and molecular analysis of BCL-2/IgH or IgH VDJ rearrangements when required. A neurological assessment was also conducted through clinical evaluation and motor and sensory nerve conduction velocity (MCV/SCV) test. Serum anti-MAG antibodies were determined by ELISA or by Western Blot analysis. All patients received at least one previous line of therapy and rituximab treatment was delivered in case of absence of neurological clinical and instrumental response according to the following schedule: 375 mg/mq I.V. for 4 weekly doses then repeated every 3-6 months. *Results.* Patients characteristics and treatment are summarized in the table. Among the eleven evaluable patients, only two (n°3 and 4) had a clinical and instrumental improvement following first-line treatments (chlorambucil + steroids and melphalan respectively) while the remaining were resistant to more than one line of therapy. Overall, seven patients of our series received rituximab because of a worsening of the polineuropathy: two of them obtained a transient clinical improvement, three a positive neurological response with a reduction of monoclonal paraproteinemia and anti-MAG antibodies. Two patients maintained a stable disease. No side effects were registered. *Conclusions.* In our experience rituximab appears to be a safe and well-tolerated treatment for anti-MAG neuropathy, causing a positive, sometimes transient, clinical response even in patients resistant to other therapies. Since we observed better results in patients with a mild neurological disability as compared to those with advanced disease, a prompt introduction of rituximab should be considered in subjects resistant to first-line treatment.

Table 1.

Pts n°	sex	Age (yr)	Haemathological disease	Time from onset (yr)	MC (gr/L)	Previous Treatments	Mob-anti CD20
1	M	56	CIDP\NHL low grade	6 months	IgMk 6,7	IVIG,chlorambucil, plasmapheresis	no
2	M	71	CIDP\Amiloidosis	8	IgGk 5,0	Interferon-α, alkeran,steroids	no
3	M	52	CIDP\NHL low grade	4	IgMλ 2,0	Chlorambucil,steroids	no
4	M	60	CIDP\NHL low grade	1	IgMk 10	Chlorambucil	no
5	M	68	CIDP\NHL low grade	5	IgMk 3,2	Plasmapheresis, chlorambucil	Yes
6	M	69	CIDP\MGUS	3	IgMk 2,0	Chlorambucil, steroids	Yes
7	M	58	CIDP\MGUS	3	IgGk 8,0	Chlorambucil, steroids, IVIG	Yes
8	M	70	CIDP\MGUS	3	IgMk 1,0	Chlorambucil, steroids	Yes
9	F	68	CIDP\MGUS	2	IgMλ 1,0	Cyclophosphamide, steroids, IVIG	Yes
10	F	63	CIDP\MGUS	9	IgMk 1,0	Cyclophosphamide,steroids, Azathioprine, IVIG	Yes
11	F	64	CIDP\NHL low grade	5	IgG k 7,0	Chlorambucil, steroids	Yes

0714

THE FOLLICULAR LYMPHOMA INTERNATIONAL PROGNOSTIC INDEX IS APPLICAPLE IN LYMPHOPLASMACYTIC LYMPHOMA / WALDENSTROMS MACROGLOBULINEMIA

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Introduction. FLIPI is a simple and effective model for the evaluation of prognosis in patients with follicular lymphoma. Recently, it has been applied and validated in primary nodal marginal zone lymphoma (MZL) and non-gastric MZL. Consequently, the assessment of its prognostic significanse in other low grade B-cell lymphomas seems reasonable, especially in LPL/WM, a disease presenting a degree of overlap with MZL. *Aims.* To assess FLIPI score validity for prognosis in LPL/WM. *Patients and Methods.* 122 patients were studied. Fourty-two (25 women, 17 men) presented LPL (not secreting IgM), their median age was 61 years, 7 were in Ann-Arbor stage I, 2 in II, 2 in III and 31 in stage IV. Lymphadenopathy was present in 31 and splenomegaly in 6. Their median follow-up was 96.9 months. Eighty patients presented WM, 31 women and 49 men with a median age of 64 years. All patients were in Ann-Arbor stage IV, by definition, due to bone marrow involvement, 15 presented lymphadenopathy and 10 splenomegaly; their median follow up was 139 months. FLIPI score at diagnosis was calculated in all patients who were accordingly classified in three groups (low, intermediate and high risk). Survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. *Results.* In the LPL group, 13 patients were classified as low risk, 16 as intermediate and 13 as high risk; median survival was 65.1, 96.9 and 34.5 months respectively. The corresponding 5- and 10-year survival was 70% vs 94% vs 45% and 35% vs 47% vs 17% in the low-, intermediate- and high-risk group respectively (p=0.09). When low and intermediate risk group together were compared to the high risk one, the corresponding 5- and 10-year survival was 84% vs 70% and 42% vs 35% (p=0.03). In the WM group, 7 were in the low risk, 23 in the intermediate and 50 in the high risk group. Median survival for these groups was 132.2 , 155.2 and 62.9 months respectively. The corresponding 5- and 10- year survival was 100% vs 94% vs 54% and 100% vs 69% vs 29% in the low-, intermediate- and high-risk group respectively (p=0.03). 5- and 10-year survival was 96% vs 54% and 76% vs 29% (p=0.008), when the low and intermediate groups were compared together to the high risk group. *Conclusions.* FLIPI seems applicable in LPL/WM.

0715

THERAPEUTIC ROLE OF RITUXIMAB IN THE TREATMENT OF INTRAVASCULAR LYMPHOMA

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Background. Intravascular lymphoma (IVL) is a rare form of non Hodgkin lymphoma, characterized by the growth of large neoplastic lymphocytes within the blood vessel. Diagnosis is often difficult and outcome generally poor. The standard therapy remains to be defined, but anthracycline-based chemotherapy is the most commonly used treatment. A few recent case reports seem to suggest a further improvement deriving from the addition of rituximab to chemotherapy. *Aims.* to explore activity and efficacy of rituximab (375 mg/m^2 on day 1) plus CHOP or CHOP-like chemotherapy (R-CT) in comparison to the same chemotherapy alone (CT) in IVL patients selected from the largest series collected in western countries. *Methods.* 25 IVL patients eligible for anthracycline-containing regimen were evaluated. Clinical features and outcome of 5 patients treated with R-CT strategy were compared to a series of 20 patients treated with CT. *Results.* Median age of the 25 patients was 67 yrs (range 39-86 yrs). Twelve patients were males. The most common involved sites were: skin (12 patients), CNS (7), bone marrow (7), spleen (5), and liver (4). B symptoms were complained by 19 (76%) patients and an elevation of serum LDH was present in 20 (80%). Anaemia, leucopoenia and thrombocytopenia were observed in 8 (32%), 19 (76%), and 8 (32%) cases respectively. Eighteen (72%)

patients presented a stage IV disease. No significant differences in patients' characteristics between R-CT and CT groups were observed. After a median follow-up of 26 months, 15 patients achieved complete remission (CR), and 3 partial response (PR), while 6 patients experimented progressive disease (PD), and one patient died of toxicity (CT group). Five R-CT patients (100%) and 10 (50%) CT patients achieved CR (p=0.06). The only variable related to CR rate was the addition of rituximab. At a median follow-up of 14 months, all 5 R-CT patients are alive and relapse free; at a median follow-up of 71 months only 6 CT patients are alive and disease-free. The 2-year EFS was 35+/-10% in CT group and 100% in R-CT one (p<0.0001). The 2-year OS was 45% and 100%, respectively for CT and R-CT group (p<0.0001). *Conclusions.* In this rare malignancy, where prospective trials are hardly performed, this international effort suggests that, like for other lymphomas the addition of rituximab to anthracycline-based chemotherapy could significantly improve outcome in IVL patients. These results deserve to be better studied in larger series with a longer follow-up.

0716

CLINICAL FEATURES OF BONE MARROW INVOLVEMENT IN MARGINAL ZONE LYMPHOMAS: ANALYSIS OF 120 PATIENTS

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Background. WHO recognized three subtypes of marginal zone lymphomas (MZL): splenic MZL, nodal MZL, and the extranodal MZL of MALT. In splenic MZL bone marrow involvement is detected virtually in all patients; in this type of lymphoma a peculiar sinusoidal infiltration was described. Series of MALT lymphoma report heterogeneous rates of bone marrow localization at diagnosis while few data are available on bone marrow features in primary nodal MZL, due to the rarity of this subtype. *Methods.* we analyse the incidence and patterns of histologic bone marrow involvement in a series of 120 pts with MZL diagnosed at Division of Hematology of Pavia (48 splenic MZL, 59 extranodal MZL of MALT, 13 primary nodal MZL). *Results.* median age was 59 years for splenic MZL, 64 for MALT MZL and 61 for nodal MZL. Leukemic disease was present in 54% of splenic MZL patients, 42% for nodal MZL patients and 2% for MALT MZL patients. Hemoglobin was <12 g/dL in 36% in splenic, 8% in nodal and 25% in MALT type, Platelets were < 100,000/mcl in 18% for splenic MZL and 4% for MALT MZL. HCV serology was positive in 30% of splenic MZL, 42% of MALT MZL and 8% of nodal MZL. A serum monoclonal component was detected in 45% of splenic MZL, in 30% of MALT and in 27% of nodal MZL. Histologic bone marrow involvement was detected at diagnosis in 43 patients with splenic MZL (90%), in 13 of 59 patients with MALT lymphoma (22%) and in 7 patients with nodal MZL (54%) and. Median extent of bone marrow localization was 40% in splenic MZL, 20% in MALT MZL and 30% in nodal MZL. The patterns were nodular (inter- and paratrabecular: 38 splenic, 2 nodal, 13 MALT), and interstitial (1 splenic, 4 nodal); diffuse areas were present in 2 patients with splenic MZL. Sinusoidal localization was seen in 32 cases of splenic lymphoma, in 3 cases of nodal MZL and in 1 MALT lymphoma. Hemoglobin <12 g/dL, elevated β2-microglobulin and presence of serum monoclonal component were associated to bone marrow involvement at diagnosis while HCV serology did not have any influence. In 42 patients with additional bone marrow biopsies during the course of the disease, bone marrow involvement was detected in 23 patients (12 splenic MZL, 3 nodal MZL and 8 MALT lymphoma), 8 of whom (2 nodal MZL, 6 MALT lymphoma) with negative bone marrow biopsy at diagnosis. *Conclusions.* amount of bone marrow involvement is different among the three subtypes of MZL, reflecting the heterogenous pattern of disease dissemination at diagnosis. In our series the most common patterns of bone marrow involvement are nodular and interstitial. Sinusoidal localization is more frequently but not mandatory in splenic MZL and is rare in nodal and extranodal MZL. During the course of disease, bone marrow re-evaluation seems clinically important mainly in extranodal MZL of MALT which more frequently do not localize in bone marrow at diagnosis.

0717

PATTERNS OF OUTCOME AND PROGNOSTIC FACTORS IN PRIMARY BONE LYMPHOMA (OSTEOLYMPHOMA): A SURVEY OF 499 CASES BY THE INTERNATIONAL EXTRANODAL LYMPHOMA STUDY GROUP

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Background. Primary bone lymphoma is an extranodal form of non-Hodgkin's lymphoma arising primarily in bone. Few large series are reported. *Aims.* Our aim was to collect data on a large series of patients so that treatment outcomes and prognostic factors could be accurately determined. *Methods.* Data were received and analysed regarding 499 cases from 35 institutions in 13 countries, treated between 1978 and 2003. *Results.* Of the 499 patients, 354 (71%) were DLBCL, the remainder were a wide variety of pathological types. There were 283 (57%) that were stage I/II. The mean age was 56yrs and the 84% presented with pain. The mean tumour size was 8.3 cm and 51% had extraosseous extension. Amputation was performed in 1%, internal fixation in 8%, radiotherapy in 75% (median dose 36 Gy), chemotherapy in 88% (most commonly CHOP, median 6 cycles). The median survival time was 13.9 years, the 5yr survival rate was 71%. Survival was significantly worse for age >60yrs, female sex, PS 2-4, Raised LDH, single modality treatment, <4 cycles of CT, RT dose <30Gy. *Summary and Conclusions.* The results of treatment were more favourable than for DLBCL in most other extranodal sites. The best results were achieved by combined modality treatment. Prospective studies could be considered, incorporating PET scanning and monoclonal antibody treatment.

0718

ASIAN VARIANT OF INTRAVASCULAR LARGE B-CELL LYMPHOMA: IS HEMOPHAGOCYTOSIS MANDATORY FOR ITS DIAGNOSIS?

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Background. The Asian variant of intravascular large B-cell lymphoma (AIVL) is unique in that it presents with hemophagocytic syndrome (HPS) and few neurological abnormalities or skin lesions, and it is almost exclusively reported in Japanese patients. In our recent analysis, a high prevalence (61%) of hemophagocytosis (HPC), a key pathologic sign of AIVL, was observed in 89 patients with intravascular large B-cell lymphoma (IVL). However, it was simultaneously noted that the majority of our series shared bone marrow involvement, pancytopenia, hepatosplenomegaly, and an aggressive clinical course within three years, with or without HPC. *Aims.* The aim of the present study was to re-evaluate the clinicopathologic and prognostic significance of HPC among Japanese IVL patients. *Methods.* Medical records of 125 Japanese patients with IVL (64 males, median age 67 years, range 41-89) were retrospectively analyzed. Informed consent was obtained from each patient. The clinical and laboratory criteria for AIVL were fulfilled by the

presence of at least two of the following: 1) anemia (hemoglobin <110 g/L or red blood cell count < 3.5×10^{12}/L) and/or thrombocytopenia (platelet count <100×10^{9}/L), 2) hepatomagaly and/or splenomegaly, and 3) absence of tumor formation (Murase *et al.* (2000) Brit J Haematol 111: 826). One hundred eight (86%) of the IVL patients fulfilled these clinical and laboratory diagnostic criteria for AIVL and were regarded as AIVL candidates. The patients were subsequently grouped according to the presence of HPC (i.e., definite AIVL, designated Group A, n=71 (57%)) or the absence of HPC (i.e., probable AIVL, designated Group B, n=37 (30%)) at presentation. The remaining patients fulfilled only one of the three criteria for AIVL and did not present with HPC (i.e., classical IVL, designated Group C, n=17 (14%)). *Results.* In addition to the listed criteria (anemia/thrombocytopenia, hepatosplenomegaly, absence of tumor formation), patients in Groups A and B had a higher prevalence of tumor cells in bone marrow and/or peripheral blood (p<0.001), B symptoms (p=0.048), advanced stage (>2; p=0.001), high or high-intermediate International Prognostic Index (p=0.002), and fewer neurological abnormalities (p=0.002) compared to patients in Group C (Table 1). Those parameters did not significantly differ in Groups A and B, except for HPC by definition and B symptoms. Notably, there was no significant difference in survival among the three IVL groups. *Summary and conclusions.* Our data revealed that the clinical and laboratory criteria for AIVL precede HPC, a key pathologic sign on morphologic grounds for AIVL diagnosis. AIVL is nearly equivalent to B-cell lymphoma with HPS in Japanese patients. Although HPS is generally regarded as an unfavorable prognostic factor for malignant lymphomas, this may not be the case for IVL patients. Further studies of IVL, including cooperative international studies, are warranted to further our understanding of the broad clinical spectrum and biologic properties of IVL.

Table 1.

Non-Hodgkin lymphoma - Clinical: Transplant; virus associated lymphomas

0719

MULTICENTER PHASE II CLINICAL TRIAL OF 90Y-IBRITUMOMAB TIUXETAN WITH HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN RELAPSED, REFRACTORY, OR HIGH-RISK B-CELL NON-HODGKINS LYMPHOMA, PRELIMINARY REPORT

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Background. The need of new effective regimen for high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) in aggressive B-cell non-Hodgkin's lymphoma (NHL) patients and promising results observed so far in trials with 90Y-Ibritumomab tiuxetan containing regimens in ASCT strongly warrants the investigation of 90Y-Ibritumomab tiuxetan combined busulfan/cyclophosphamide/etoposide (Z-BuCyE) high-dose chemotherapy with ASCT for relapsed, refractory, or high-risk B-cell NHL. *Aims.* We evaluated efficacy and safety of the combination of Z-BuCyE and ASCT in patients with relapsed, refractory, or high-risk B-cell NHL. *Methods.* This study was an open label, non-randomized, multicenter, phase II study. Treatment consisted of two doses of Rituximab (250 mg/m^2 , IV, day -21, -14) and a single dose of 90Y-Ibritumomab (0.4 mCi/kg, IV, day -14). All patients received conditioning regimen: busulfan (3.2 mg/kg, IV, day -7, -6, -5), etoposide (200 mg/m^2, IV, day -5, -4), and cytoxan (50 mg/kg, IV, day -3, -2) followed by ASCT (day 0). Response was evaluated at three month after transplantation administration by International Workshop Criteria. *Results.* Thirteen patients were entered the trial. The median age was 46.1 years (range: 25-60), and 6 (46%) were male. Histology was diffuse large B-cell (n=10), follicular (n=1), Burkitt (n=1), and mantle cell lymphoma (n=1). Before ASCT, 38.5% (5/13) patients had a complete response (CR), 53.9% (7/13) patients had a partial response (PR), and 7.7% patients had a progressive disease (PD). The objective overall response rate (ORR) was 76.9% (10/13): continued CR, 38.5% (5/13); induced CR, 23.1% (3/13); continued or induce PR, 15.4% (2/13). Three patients (23.1%) had a PD after transplantation and two of these patients died of progression. Median follow-up duration was 6.0 months. Median progression-free survival (PFS) and median overall survival (OS) has not yet been reached. Toxicity was principally non-hematologic. Grade 2 toxicity included mucositis (53.8%), nausea (61.5%), vomiting (15.4%), diarrhea (23.1%), and elevation of liver enzyme (7.7%). Grade 3 toxicity included mucositis (15.4%), nausea (23.1%), and diarrhea (23.1%). There was no grade 4 toxicity. Infection occurred in ten patients, bleeding in one patient, and there was no treatment related mortality. *Conclusions*. This preliminary analysis shows that the combination of Z-BuCyE and ASCT has excellent efficacy and is well-tolerated treatments for relapsed, refractory or high-risk B-cell NHL. This study will be continued upto 20 patients enrollment.

0720

A MULTICENTER PHASE II TRIAL OF ETOPOSIDE, METHYLPREDNISOLONE, HIGH-DOSE CYTARABINE, AND OXALIPLATIN FOR PATIENTS WITH REFRACTORY/RELAPSED AGGRESSIVE NON-HODGKINS LYMPHOMA: PRELIMINARY RESULTS

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Background. Etoposide (E), methylprednisolone (S), high-dose cytarabine (HA), and cisplatin (P) (ESHAP) combination is commonly used salvage regimen for non-Hodgkin's lymphoma (NHL). Oxaliplatin (Ox), a new platinum derivative, showed substantially different cytotoxic activity and adverse effects from both cisplatin and carboplatin. In addition, single-agent oxaliplatin was reportedly active in patients with NHL. *Aims.* We conducted to investigate the efficacy and toxicity of ESHAOx combination, substituting oxaliplatin for cisplatin in ESHAP combination, for relapsed/refractory aggressive NHL patients. *Methods.* Main eligibility criteria included aggressive NHL and failure to achieve a com-

plete remission or recurrent disease after previous chemotherapy. ESHAOx consisted of E, 40 mg/m^2 on days 1 to 4; S, 500 mg on days 1 to 5; HA, 2 g/m^2 on day 5; and Ox, 130 mg/m^2 on day 1, every 3 weeks. Eligible patients were scheduled to receive a maximum of 6 cycles, and high dose chemotherapy and hematopoietic stem cell rescue allowed. Responses were evaluated every 3 cycles. All patients gave written informed consent before study entry. *Results.* Between May 2006 and January 2007, 27 patients were enrolled. Nineteen (70%) patients with relapsed, 8 patients with refractory, and 10 (37%) patients with IPI 3-5 were included in this study. Twenty patients were evaluable for response with 7 ongoing and 26 for toxicity. A total of 61 cycles were administered for a median number of 2 (range 1-5) per patient. There were 4 (20%) complete responses and 6 (30%) partial responses, producing an overall response rate of 50% (95% CI, 28-72%). Most common grade 3/4 toxicity of the courses was myelosuppression with including neutropenia (39%) and thrombocytopenia (20%). There was one therapy-related death due to neutropenic sepsis. Non-hematologic toxicity was very favorable. *Conclusions.* The preliminary results of ESHAOx combination showed antitumor activity and favorable toxicity profile, suggesting it can be used as salvage regimen for relapsed/refractory aggressive NHL patients.

0721

LONG-TERM SURVIVAL OF A BROAD AGE POPULATION OF PATIENTS WITH MANTLE CELL LYMPHOMA AFTER FRONTLINE HIGH DOSE SEQUENTIAL CHEMOTHERAPY WITH RITUXIMAB AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Intensive therapeutic programs improve the clinical outcome of MCL, but are toxic in elderly patients and did not prevent the continuous relapse rate. The aim of the present study was to evaluate if an up-front Rituximab supplemented and age-adapted high-dose sequential chemotherapy (R-HDS) supported by ASCT can improve the survival of a broad age population with disseminated MCL. From. 1992 to 2005, 54 patients (pts) <61 year old (group 1) and 19 aged >60 years (group 2) were enrolled in the study. The majority of both groups had an advanced stage, bone marrow infiltration and >1 IPI risk factors. Group 1 received standard R-HDS including: HD-cyclophosphamide (CTX) 7 gr/sqm and HD-Ara-C (2 g/sqm every 12 hours for 6 days), followed by HDS HD-melphalan (180 mg/sqm) and/or HD-mitoxantrone plus melphalan (60 and 180 mg/sqm) and ASCT. Rituximab (375 mg /sqm) was given for a total of 6 doses, twice after HD-CTX and HD-Ara-C, as *in vivo* purging before CD34$^+$ cells harvest and twice after ASCT. Elderly patients received an age-adapted R-HDS: HD-CTX (3-4 gr/sqm) and HD-Ara-C (1-1.5 g/sqm every 12 hours for 3-5 days), followed by HD-melphalan (120 mg/sqm) and HD-mitoxantrone plus melphalan (40 and 120 mg/sqm). 35 pts (65%) in group 1 and 9 (47%) in group 2 completed the planned program. After ASCT the CR rate was 89% in young and 95% in elderly patients and the treatment-related mortality was 4%. With a median follow-up of 43 months (range 6-101) in group 1 and 30 months (range 9-68) in group 2, the 5-year estimated OS, EFS and DFS were respectively 77%, 71% and 72%, in group 1 and 58%, 56% and not yet achieved in group 2. We conclude that R-HDS produces long-term remissions with a manageable toxicity also in elderly pts with advanced stage MCL.

0722

MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Background. In Human Immunodeficiency Virus (HIV)-infected patients, the risk of non-Hodgkin lymphoma is significantly increased compared to the general population. However, mucosa-associated lymphoid tissue (MALT) lymphomas have been only occasionally reported during the setting of HIV infection. *Aims.* To describe the features of a retrospective series of 8 HIV-infected patients affected with MALT lymphoma. *Methods.* Morphological and immunohistochemical analyses including BCL10 immunostaining, were performed, as well as *in situ* hybridization for Epstein-Barr virus-encoded RNA (EBER) detection, fluorescence *in situ* hybridization (FISH) analyses with MALT1, immunoglobulin heavy chain (IGH) and BCL10 probes, PCR detection of monoclonal IGH gene rearrangements and RT-PCR detection of API2-MALT1 chimeric transcript resulting from the t(11;18)(q21;q21) translocation. *Results.* Four men and four women with a median age of 42 years (range, 35-67) were included. Six patients were infected with HIV-1, one with HIV-2 and one with HIV-O. Upon lymphoma diagnosis, 5 patients were receiving antiretroviral treatment, consisting of a highly active antiretroviral therapy (HAART) in 2 cases. The median CD4$^+$ cell count was 191×10^6/L (range, 44-640), and the plasma HIV RNA load, available in 7 cases, ranged from undetectable to 351,516 copies/mL (median, 8,813 copies/mL). One patient had a salivary gland lymphoma, and 7 had a gastric lymphoma, concomitant in one case with a pulmonary involvement. Disease stage was IE in 4 cases, IIE in 2 cases, and IV in the 2 others. Histological analyses of tumour samples displayed typical features of MALT lymphoma in all cases, with focal transformation into diffuse large B-cell lymphoma (DLBCL) in 2 cases. Concomitant Helicobacter pylori (Hp) infection of the stomach was found in 6 cases, including 5 patients with a gastric lymphoma. Immunohistochemistry showed a nuclear expression of BCL10 in the 5 studied cases. Three cases were found to be EBER-negative, whereas less than 1% EBER$^+$ cells were detected in 4 cases. A monoclonal rearrangement of IGH gene was detected in 4 cases out of 6 analyzed. RT-PCR and/or FISH analysis performed in 7 cases, displayed the presence of t(11;18) translocation in one case. Treatment for lymphoma consisted of Hp eradication therapy in the 5 patients with a Hp-associated gastric MALT lymphoma. Four of them achieved a complete remission (CR) whereas the disease remained stable in one patient, due to Hp resistance to 4 lines of antibiotic treatment. Partial gastrectomy followed by CHOP-derived chemotherapy was performed in one case with focal DLBCL. Surgical resection of a pulmonary lobe was performed in one case. One patient was treated with rituximab alone. All the patients received HAART from 0 to 30 months after lymphoma diagnosis. Five patients achieved a CR after the first-line treatment. Subsequent transformation into DLBCL occured in the lung of one patient. To date, 7 patients are alive, from 12 to 111 months after diagnosis, including 5 patients in CR and 2 with stable disease. One patient in CR died at 66 months, with cerebrovascular accident. *Conclusions.* In HIV-infected patients, MALT lymphoma seems to have an indolent clinical course similar to MALT lymphomas occuring in immunocompetent patients.

Table 1.

N°	Involved sites	Histology	Stage	Hp	B-cell clonality	Nuclear BCL10	t(11;18) RT-PCR	t(11;18) FISH
1	Stomach	ML	IIE	+	ND	+	ND	-
2	Lung	ML	IV		ND	+	ND	+
	Stomach	ML		-	+	+	+	ND
3	Stomach	ML	IE	+	+	ND	-	ND
4	Salivary glands	ML	IV	+	ND	+	ND	-
	Lung	DLBCL	IV		-	ND	ND	ND
5	Stomach	ML/focal DLBCL	IIE	-	+	+	ND	-
6	Stomach	ML	IE	+	+	+	-	-
7	Stomach	ML	IE	+	-	ND	-	ND
8	Stomach	ML/focal DLBCL	IE	+	ND	ND	ND	ND

0723

DIFFUSE B-LARGE CELL LYMPHOMAS WITH HEPATITIS C VIRUS (HCV) INFECTION: AN INTERIM REPORT OF A COMPARATIVE STUDY WITH OR WITHOUT ANTIVIRAL TREATMENT AFTER CHEMOTHERAPY

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Background. Antiviral therapy (AVT) with interferon ± ribavirin has shown to be effective in inducing neoplastic regression without chemotherapy (CT) in low-grade non-Hodgkin's lymphomas (mainly immunocytomas and nodal/extranodal marginal lymphomas) with associated HCV infection (HCV+ve). We have previously shown that high grade, Diffuse B-Large Cell Lymphomas (DBLCL) are HCV+ve in about 12% of cases in Italian population (ASH 2006, abs. 2242). 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 negative (HCV-ve) DBLCL, when treated with standard or even high dose CT and if significant signs of liver dysfunction are absent. *Aims.* In the present study we aimed to determine the possible role of AVT, performed after a standard CT treatment, in high grade, HCV+ve DBLCL. *Methods.* We evaluated the clinical outcome of 28 HCV+ve DBLCL patients who received AVT (α or pegylated interferon ± ribavirin, given at recommended doses and therapy duration for specific HCV genotypes and according to viral response) after first complete or partial remission was achieved by frontline standard CT. Classic or modified CHOP ± rituximab or PROMACE-CytaBOM regimens were generally employed. For comparison, a historical cohort of 24 patients with HCV+ve 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. *Results.* 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 54% of patients. An interim evaluation showed a not statistically significant trend (67 vs 54%) in favour of AVT-treated patients in terms of PFS at two years. A weak correlation between viral clearance and longer PFS duration was also observed. Two-year OS, however, was not different between AVT-treated or not treated patients (71 vs 68%, p n.s.). *Conclusions.* Our currently available data indicate that a sequential treatment with CT followed by AVT is feasible in HCV+ve DBLCL, may induce complete virus clearance and could have a positive impact on remission duration. A larger number of patients and a longer follow-up are required to establish the exact role (if any) of AVT in HCV+ve DLBCL patients.

0724

REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR RELAPSED LYMPHOMAS: CLINICAL AND MOLECULAR OUTCOME IS DIFFERENT BETWEEN FOLLICULAR LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. The long-term safety and efficacy of reduced-intensity conditioning (RIC) followed by allogeneic stem cell transplantation (SCT) for relapsed lymphomas is still unclear. In a prospective phase II multicenter trial 184 pts with relapsed/refractory lymphomas received a RIC regimen followed by SCT from sibling donors. *Aims.* Primary endpoint was non-relapse mortality (NRM). Secondary endpoints were overall survival and progression-free survival. *Methods.* Histologies were as follows (non-Hodgkin's lymphomas (NHL) [indolent (LG-NHL), n=67 (n= 35 CLL, n=28 FL, n=4= others); aggressive (HG-NHL), n=67 (n= 27 T-NHL, n=32 B-NHL, n=8 transformed); mantle cell lymphoma (MCL), n=16] and Hodgkin's disease (HD n=34). *Results.* The median follow-up was 33 months (range 12-82). The cumulative NRM at 3 years was 14%. Acute and chronic graft-versus-host disease were 35% and 52%, respectively. The 3-year overall survival (OS) was 69% for LG-NHL, 69% for HG-NHL, 45% for MCL and 32% for HD (p=0.058); the 3-year relapse incidence was 29%, 31%, 35% and 81%, respectively (p<0.001). Pts affected by FL and CLL/SLL had a significant difference in relapse risk (14% versus 46% at 3 years, p=0.040) and in OS (88% versus 53% at 3 years, p=0.013). Forty-seven of them, achieving CR, have been also evaluated for minimal residual disease (MRD): the 3-year cumulative incidence of relapse was 40% (19%-83%) for PCR positive or mixed and 0% for the PCR negative patients (p=0.01). In addition, molecular remission was achieved in the majority of pts with FL (94%) as compared to CLL pts (40%) (p=0.002) which further supported the different risk of relapse in the two groups. Interestingly, patients with aggressive lymphoma of B or T origin had not a significant difference in relapse risk (24% versus 36% 3 years, p=0.32) and overall survival (73% versus 62% 3 years OS, p=0.596). The above mentioned subgroups (FL versus CLL and T and B aggressive lymphomas) have not different in pre-transplantation characteristics. At multivariable analysis, OS was influenced by chemorefractory disease (hazard ratio, HR=3.6), diagnosis of HD (HR=3.5) and occurrence of acute GVHD (HR= 5.9). *Conclusions.* RIC allogeneic SCT is a feasible and effective salvage strategy in both indolent and aggressive NHL. Molecular monitoring of bone marrow cells in indolent lymphomas can be used to predict relapse as in the autografting setting.

0725

IMMUNODEFICIENCY ASSOCIATED MALIGNANT HEMOPATHIES IN CHILDREN

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Background. Malignant hemopathies are the main associated diseases of immunodeficiency (ID) syndromes and one of their important cause of mortality. The underlying mechanism of this association is not fully understood: immunodeficiency itself, unregulated expression of cytokines, chronic antigen stimulation, aso. *Objectives.* We aimed at evaluating the frequency and assesing the features of ID associated malignant hemopathies (IDAMH) comparing the data obtained in persons with primary ID vs acquired ID. *Patients and Methods.* In this retrospective observational monocenter study we included 71 patients with primary ID and 710 with HIV infection, their mean age being 5,95±4,51 vs 7,29±3,72 years respectively; the potential onset of ID was quite similar, as HIV infection occured vertically in 4,74% and horizontally presumable in the first year of life in the rest of the cases. *Results.* IDAMH occurred in 11,26% of PID and in 6,26% of HIV infected patients. Malignancy of PID patients consisted of Non- Hodgkin's lymphoma (NHL) in 75% and acute leukemia (AL) in 25%. The AIDS group was dominated by its defining neoplasia - NHL, assessed in 59,45% of cases; 4,76% developed AL; Hodgkin's lymphoma, Neuroblastoma, Rhabdomyosarcoma, Histiocytosis each represented 2,7% of cases. The mean age at diagnosis of malignancy was 11,1 in PID, and 10,59 years in AIDS group. No significant differences were observed between patients with horizontal and vertical HIV infection. 62% of patients with and 67,85% of those without malignant hemopathy have been treated with HAART, but the impact of therapy on the occurence of malignancy has been hard to establish. *Conclusions.* The frequency of malignant hemopathies in immunodeficient children is alarming. Therefore, they should benefit of a stringent screening and early detection program for an efficient secondary prevention of cancer.

0726

IMPACT OF HCV CO-INFECTION ON THE OUTCOME AND CLINICAL FEATURES OF HIV-RELATED NON-HODGKIN LYMPHOMA

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Background. Epidemiologic studies show an association between HCV and B-NHL. Treatment and outcome of patients with NHL and HCV infection in the general population and in HIV-infected individuals are still debated. *Aims.* The aim of the study was to describe and compare clinical features and outcome of NHL in HIV patients with or without HCV co-infection. *Methods.* HIV-infected individuals with NHL diagnosed and treated at the National Cancer Institute of Aviano (Italy) from April 1991 to October 2006, whose serology for HCV infection was

known at NHL diagnosis, were included in the study. Co-infected and mono-infected patients were treated with the same therapeutical protocols. *Results.* 301 HIV patients with NHL were included in the study: 123 (40.2%) HCV co-infected and 178 mono-infected. As regards HIV disease's characteristics, co-infected patients had a significant higher percentage of intravenous drug users (IDU) in comparison with mono-infected patients, whereas no significant differences were seen in CD4 and CD8 count, HIV viral load, AIDS defining condition at NHL diagnosis and antiretroviral therapy. As regards NHL's characteristics at the onset, co-infected patients showed a significant lower percentage of Burkitt histotype, whereas no significant differences were seen in Performance Status, stage, International Prognostic Index. Co-infected patients showed a significantly higher NHL involvement of the spleen. No differences were observed in response rate (RR), complete response rate (CRR) and overall survival (OS) at 5 years. Co-infected patients had a significantly higher percentage of G3-G4 liver toxicity during chemotherapy (4.5% vs 0%; p=0.0083) although it was not life threatening. *Conclusions.* Co-infected patients had a higher rate of IDU, Burkitt histotype and NHL spleen involvement; no other differences were observed in the characteristics of HIV and NHL disease. No differences in RR CRR and OS were observed between co- and mono-infected patients, although a higher rate of liver toxicity was observed in co-infected patients.

0727

COMPARISON OF STANDARD CHOP TREATMENT WITH SHORT- INTENSIVE SPECIFIC CHEMOTHERAPY IN AIDS-RELATED BURKITTS LYMPHOMA OR LEUKEMIA (BL/ALL3)

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Background and Objective. The results of CHOP therapy for AIDS-related BL/ALL3 are poor. Specific intensive multiagent chemotherapy schedules have been also used, but there are scarce studies comparing their results with those of CHOP-based regimens. This retrospective study aimed to compare 31 patients (pts) with AIDS-related BL/ALL3 treated with CHOP±rituximab with 33 pts treated with two consecutive specific protocols (PETHEMA-LAL3/97 and PETHEMA-LAL3/LB-04). *Design and Methods.* Pts from Group A (n=31) received 6 standard CHOP cycles every 3 weeks±rituximab. Protocols from group B included a pre-phase with CPM and PDN followed by six cycles including HD-MTX and HD-ARA-C. Rituximab was added to each block of chemotherapy in the PETHEMA-LAL3/LB-04 trial. Since 1996, HAART was given to all pts from diagnosis if it was not already being received. Response to chemotherapy, OS and EFS were compared. *Results.* Both groups were comparable for the main clinical and biological parameters at diagnosis, exept for ECOG score (0-2 in 61% pts from Group A vs 85% pts from group B, p=0.03), B symptoms (61% vs 85%, p=0.04) and number of extranodal sites involved (≥3 0% vs 18%, p=0.01). Group A: CHOP (n=29) and R-CHOP (n=2). Group B: PETHEMA-LAL3/97 trial (n=19) and PETHEMA-LAL3/LB-04 trial (n=14). Median age was 38 (25-58) yr. in grup A and 40 (23-65) yr. in group B, being males 22 (71%) and 28 (85%), in each group, respectively. 16 pts (51%) in group A and 11 pts (33%) in group B were in leukemic phase. CD4 lymphocyte count at diagnosis was over 200/mL in 42% and 55% of pts and HAART was administered in 60% and 73% of evaluable pts in each group, respectively. CR was achieved in 10 out of 31 (32%) pts in group A and 22 out of 33 (67%) pts in group B (p=0.006). Resistance was more frequent in group A (15/21) than in group B (4/11) (p=0.06). 3-year (95% CI) EFS was 32% (15-49%) for group A and 50% [30%-70%] for group B (p=0.1), and 3-year (95% CI) OS was 32% (15-49%) for Group A and 49% (29-69%) (p=0.097) for group B. *Conclusions.* Short-intensive specific chemotherapy in AIDS-related BL/L3ALL is feasible, with higher remission rate than that obtained with CHOP-based regimes. A trend for an improved survival is observed in pts receiving short-intensive specific chemotherapies.

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0728

LONG REMISSION ARE POSSIBLE AFTER NON MYELOABLATIVE TRANSPLANT IN PATIENTS WITH FOLLICULAR NON-HODGKINS LYMPHOMA: RESULTS OF TWO PROSPECTIVES MULTICENTER TRIALS

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Myeloablative transplant has been investigated in poor prognosis indolent lymphoma; although recurrence rate is low it is associated with high mortality; the use of non-myeloablative conditioning regimens could reduce TRM maintaining the GVH effect. Up to May 2006, 35 patients with follicular NHL received a Non Myeloablative related allogeneic according to two prospective multicenter trials; conditioning regimen consisted of Fludarabine 150 mg and Melphalan 70-140 mg. GVHD prophylaxis consisted of CSA plus short-course MTX. All patients received filgrastim-stimulated peripheral blood stem cells from a HLA related identical donor. Median age at transplant was 50 years (34-62) and 16 (46%) had received a previous autologous transplant. At transplant, 5 patients (14%) were in CR1 (after several lines of chemotherapy), 9 (25%) in >CR1, 12 (34%) in PR, 1 (3%) had stable disease (after 3 chemotherapy lines) and 8 (23%) progressive disease. All patients engrafted. Acute GVHD developed in patients 19 (54%) (17patients (48%) grade II'IV). Chronic GVHD developed in 18 out of 27 patients at risk (67%), being extensive in 11 (41%). Disease was evaluated at day +100 and at that moment 23 patients were in CR, (85%) 1 (4%) in PR, two (7%) had stable disease and 9 patients (26%) have died. With a median follow up of 60 months (range: 32-80 months), 20 patients (57%) are alive disease free, and 14 (43%) have died, 12 of them (37%) due to transplant toxicity and 2 patients (6%) due to progression. Overall Survival (OS) and Event Free Survival (EFS) are 57 and 54% respectively. Analysing variables which influence on OS and EFS, patients 55 years have a OS significantly shorter than those <55 years old (22% vs 69%; p:0,007). Moreover, patients who develop chronic GVHD have an EFS significantly better than those which do not develop it (78% vs 44%; HR: 3,9 (1,0-14,6); p:0,03). In conclusion, our results demonstrates high efficacy of non-myeloablative transplant in follicular NHL with a very low relapse rate, indicating the important role of chronic GVHD in the control of the disease; however, mortality rate is still high, mainly in patients 55 years. Although follicular lymphoma is considered as an incurable disease, our results after a large follow-up suggest that allogeneic effect could change this concept.

0729

IN VIVO PURGING FOLLOWED BY AUTOTRANSPLANT INDUCES LONG CLINICAL AND MOLECULAR REMISSION IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA PATIENTS

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Background. Follicular lymphoma (FL) is an indolent disease whose eradication is difficult to achieve. Prior studies demonstrated that combination of Rituximab with high-dose chemotherapy and autotransplant produces high rates of clinical and molecular remission in relapsed/refractory patients. *Methods.* In a multicenter prospective study (Clinical Trials gov Identifier: NCT00366275) we treated 64 patients with refractory/relapsed follicular lymphoma with a program of *in vivo* purging and autotransplant: after debulking with an anthracycline-containing regi-

men. Phase 1 (immuno-chemotherapy), consisted of 2-4 courses with Rituximab day 1, vincristine day 2, and cyclophosphamide day 2 to 6. Phase 2 (*in vivo* purging and PBSC mobilization), coupled Rituximab on days 1 and 9 with HD-AraC (2 g/m^2 every 12 h on days 2 and 3). Phase 3 (high-dose chemotherapy with autotransplant) consisted of BEAM followed by infusion of *in vivo* purged PBSC plus 2 consolidation doses of Rituximab on days +14 and +21 posttransplant. *Results.* Median age 50. Histology: 29 grade I, 27 grade II, 8 grade III; 84% stage III-IV; 56% had bone marrow involvement; 59% were PCR$^+$ for bcl-2 rearrangement in blood and/or marrow. At enrollment, FLIPI was low in 44%, intermediate in 34%, high in 11%, NA in 11%. Of 64 patients, after debulking 7 reached CR, 52 PR, and 5 stable disease; of 38 PCR+ patients, 9 (24%) achieved molecular response. After immunochemotherapy, 40 patients obtained CR, 22 PR, and 2 progressed; of the 38 PCR$^+$ patients, additional 24 achieved molecular response. Overall, 33 patients (87%) were molecularly negative before mobilization. Sixty-one patients proceeded to PBSC mobilization with *in vivo* purging. Toxicity consisted of WHO grade 3-4 granulocytopenia in 72%. One patient who developed E. Coli sepsis during cytopenia did not harvest. Leukaphereses were started after a median of 12 days (range 5-16) after the first dose of AraC. The median number of CD34$^+$ cells collected was 16.6×10^6/kg (range 3.8'80.6). All the 31 harvests tested for Bcl-2 were PCR-negative. Autotransplant was performed in 58 patients. The median time to neutrophils recovery over 0.5×10^9/L was 10 days (8-14), and to platelets over 20×10^9/L was 10 days (6-13). One patient developed transient cerebral vasculitis during the cytopenic phase. The two Rituximab doses post-transplant did not affect hemopoietic recovery. Asymptomatic WHO grade 3-4 neutropenia developed in 10 patients after a median of 90 days after transplant. After a median F-U of 3.4 years (range 1-8), 42 patients are in clinical remission (24 for more than 4 years). One patient became PCR$^+$ 34 months after transplant without clinical relapse and was treated with 4 doses of Rituximab achieving PCR$^-$, seven had clinical and molecular recurrence after a median of 16 months after transplant (range 5-40). The 5-year event-free survival of the whole series is 67%. *Conclusions.* This study demonstrates the role of harvesting lymphoma-free PBSC for the attainment of long-lasting clinical and molecular responses in refractory/relapsed follicular lymphoma patients. Asymptomatic late onset neutropenia may occur after Rituximab consolidation of autologous transplant with no clinical influence on the outcome of the disease.

0730

HEPATITIS C VIRUS INFECTION AND LYMPHOPROLIFERATIVE DISORDERS: A BAYESIAN APPROACH

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Background. A recent meta-analysis of epidemiologic studies (Dal Maso 2006) confirmed association between hepatitis C virus (HCV) infection and lymphoid malignancies but did not identified specific HCV-related diseases. The Bayesian approach offers an appealing alternative to classical statistical inference in order to detect specific HCV-related type of lymphoid neoplasms. *Methods.* We analysed 2,621 patients with lymphoproliferative disorders diagnosed since 1992 to 2006; HCV serology was available for 1,434 patients. We compared the estimates of HCV prevalence in each type of disorder obtained with four different statistical models. Model A: the classical maximum likelihood (ML) estimator for the binomial distribution, with the corresponding 95% confidence intervals. Model B: a fully Bayesian fixed-effects model based on the assumption that the number of HCV+ patients are independent binary response variables with group-specific prevalence as failure probabilities. On each failure probability a non informative prior distribution was specified. Model C: a fully Bayesian hierarchical model, based on the assumption that all the (logistic transformed) prevalence rates are all drawn from the same normally distributed random variable. The mean of the random variable is an *overall prevalence* to be estimated (with a flat normal hyperprior distribution), and the standard deviation is a random parameter on which a non informative (uniform) hyperprior distribution has been specified. Model D: a fully Bayesian hierarchical model, in which the (logistic transformed) prevalence of each diagnosis group has been assigned to one of two normal distributions with different mean and standard deviation parameters, but with the same non informative hyperpriors as for model C. All Bayesian computations were carried out using WinBUGS 1.4.1 © Imperial College and MRC (UK) http://www.mrc-bsu.cam.ac.uk/bugs *Results.* The resulting estimates and the 95% credible intervals obtained from Model B were overlapping to the estimates and the 95% confidence intervals obtained with the classic ML approach (Model A) (see Figure 1). The estimated prevalence ranged from around 5% for Hodgkin's lymphoma and hairy cell leukemia to more than 35% for diffuse large B-cell lymphoma (DLBCL) (43%), MALT lymphoma (44%), splenic MZL (34%). The estimates of the group-specific prevalences obtained with Model C were slightly pulled towards the overall mean value, shown at the top of Figure 1. However, it was still possible to point out the histotypes with high HCV seroprevalence (DLBCL 43%, MALT lymphoma 43%, splenic MZL 33%). In those groups, the credible intervals had little overlapping (or no overlapping at all) with the credible interval of the overall prevalence. Model C allowed to further highlight the presence of high and low HCV-seroprevalence neoplasms. The estimated overall HCV seroprevalences of the two subsets of lymphoma (high vs. low) had non-overlapping credible intervals, and the estimates in each lymphoma subtype were pulled towards the overall prevalence of the corresponding subset. This is particularly evident for splenic MZL. *Conclusions.* Bayesian hierarchical models are a very flexible tool, allowing to build complex models in which our prior beliefs and acquisitions are formalized and integrated in the analysis. In our analysis, the Bayesian approach highlighted a group of histotypes with high HCV-seroprevalence: DLBCL, MALT lymphoma and splenic MZL.

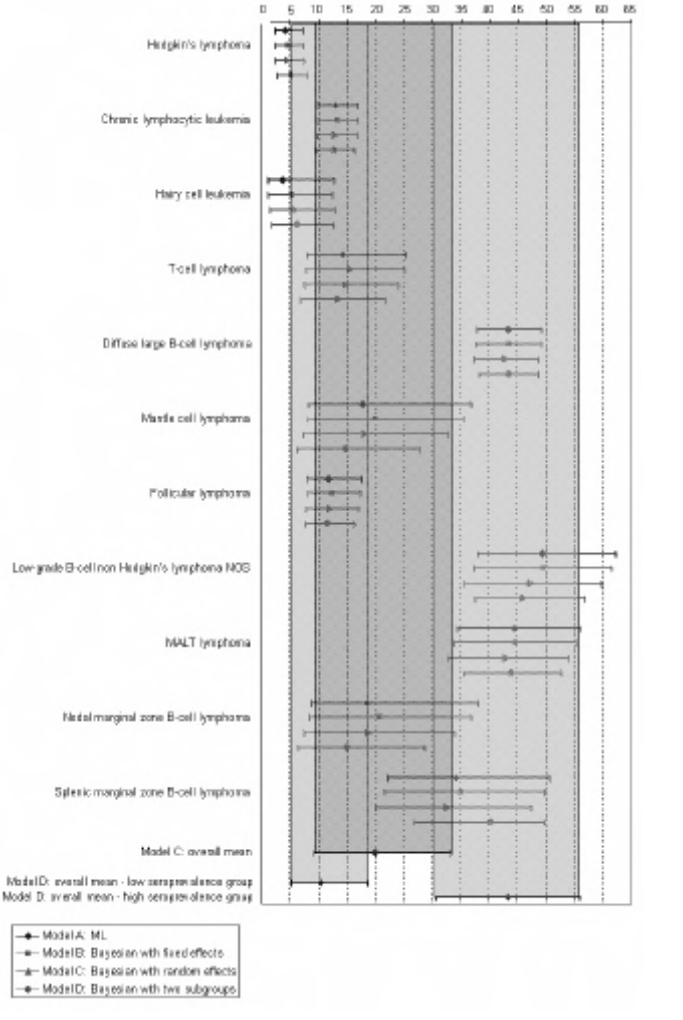

Figure 1. HCV and lymphoid malignancies: 4 different models.

0731

THE IMPACT OF HIV ON NON-HODGKIN'S LYMPHOMA AT CHRIS HANI BARAGWANATH HOSPITAL

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Background. in south africa, there are approximately 5.5 million people living with hiv/aids, and the number appears to be increasing. There is an increased prevalence of malignancy in hiv seropositive individuals. The risk of non-hodgkin's lymphoma (NHL) is approximately 60-200 fold compared to the general population. With the introduction of antiretroviral therapy (art), the incidence of primary cns lymphoma and opportunistic infections has decreased. However, the overall incidence of nhl appears to have increased, as individuals live longer in the art/haart era. *Aims.* to ascertain the impact of hiv/aids on the incidence of nhl and the clinical patterns of disease *Methods.* retrospective review of all histologically confirmed patients with nhl, seen at a single adult clinical haematology centre from january 1993 through to december 2005. *Results.* a total of 410 patients were seen. There were 236 males and 174 females, with a m:f ratio of 1.35:1. The mean age at presentation was 43 years (range 13-89). Of these individuals, 191 are seropositive (46.5%) and 219 are seronegative (53.5%). Moreover, the pattern of disease is different in seropositive compared to seronegative individuals. Seropositive individuals present at a younger age (36 vs. 48 years), have more frequent constitutional symptoms (91% vs. 84%), more advanced stage disease (83% vs. 68%), more aggressive histology (DLBCL=53%, BL/BLL=18% vs. Dlbl=24%, ALCL=11%, SLL=11%), more extranodal disease (71% vs. 49%), more infective complications, less favourable response to treatment, and a significantly shorter overall survival (11months vs. 33 months). *Conclusions.* compared to earlier studies done in the mid 1990's in south africa, which showed only a modest increase of nhl in hiv seropositive individuals - odds ratios of 4.8 and 5.5 (sitas *et al.,* 1997; sitas *et al.*, 2000), there has been a substantial increase both in the total number of patients and proportion of patients with seropositive disease. This has also impacted negatively and changed the demographic and clinical pattern of disease.

Novel therapeutics and targeted therapies II

0732

THE HSP32/HO-1-TARGETED DRUG SMA-ZNPP INHIBITS THE GROWTH AND VIABILITY OF NEOPLASTIC CELLS IN VARIOUS HUMAN LEUKEMIAS AND SOLID TUMORS

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Background. Heat shock protein 32 (Hsp32), also known as heme oxygenase-1 (HO-1), is a stress-related survival factor that is overexpressed in various neoplastic cells. Recently, specific Hsp32-targeting drugs such as styrene maleic acid encapsulated zinc protoporphyrin (SMA-ZnPP) have been developed. *Methods.* We examined the expression of Hsp32/HO-1 and the effects of SMA-ZnPP on proliferation and survival of various leukemic cell-lines, including KG1, U937, HL60 (acute myeloid leukemia), K562, KU812 (chronic myeloid leukemia) RAJI, NALM-6 (acute lymphatic leukemia), EOL-1 (chronic eosinophilic leukemia), HEL (erythroid leukemia), HMC-1 (mast cell leukemia), and RPMI 8226 and U266 (multiple myeloma). Moreover, we examined cell lines derived from solid tumors, such as U97MG (glioblastoma), A549 (lung cancer), MDA-MB-231 (breast cancer), BxPC-3 (pancreatic carcinoma), HepG2 (hepatocellular carcinoma), Colo201, Colo320DM, DLD-1 (colon carcinoma), and OvCar3 (ovarian carcinoma). Primary leukemic cells were obtained from the patients' bone marrow or peripheral blood. In case of AML and CML, malignant $CD34^+$ precursor cells were sorted. Normal white blood cells and normal lung fibroblasts served as controls. Moreover, Ba/F3 cells with doxycycline-inducible expression of oncoproteins (BCR/ABL, KIT-D816V, RAS-G12V) were analyzed. Expression of Hsp32 mRNA was examined by RT-PCR and Northern blotting, and expression of the Hsp32 protein by Western blotting. To silence Hsp32 in neoplastic cells, we used specific siRNA as well as SMA-ZnPP. Proliferation was analyzed by 3H-thymidine uptake and apoptosis by light microscopy and flow cytometry. *Results.* All neoplastic cell lines, primary neoplastic cells and malignant precursors tested were found to express Hsp32 mRNA and the Hsp32 protein in a constitutive manner. In Ba/F3 cells, induction of BCR/ABL, KIT D816V or RAS G12V enhanced the expression of Hsp32/HO-1. The Hsp32 siRNA was found to lead to a reduced viability and induction of apoptosis in all cell lines tested. Treatment of malignant cells with SMA-ZnPP also resulted in a significant decrease in proliferation and induction of apoptosis. The effects of SMA-ZnPP on primary neoplastic cells and cell lines were dose-dependent and occurred at pharmacologic concentrations (IC_{50} 1-30 μM). Moreover, SMA-ZnPP was found to synergize with various anti-neoplastic drugs such as cytarabine, fludarabine (AML), tyrosine kinase inhibitors (CML, systemic mastocytosis), bortezomib (multiple myeloma), and cisplatin (solid tumors) in counteracting the proliferation of neoplastic cells. *Conclusions.* Hsp32 is an important survival factor and an attractive therapeutic target in a broad spectrum of hemato-oncologic malignancies. The Hsp32-targeting drug SMA-ZnPP counteracts the proliferation and viability of malignant cells. Moreover, SMA-ZnPP sensitizes neoplastic cells against various other targeted or conventional antineoplastic drugs. Hsp32-targeting drugs may represent an interesting new approach to inhibit malignant cell growth in leukemias and solid tumors.

0733

REACTIVE OXYGEN SPECIES MEDIATE N-(4-HYDROXYPHENYL) RETINAMIDE-INDUCED CELL DEATH IN MALIGNANT T CELLS AND ARE INHIBITED BY THE HTLV-I ONCOPROTEIN TAX

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Background. N-(4-hydroxyphenyl) retinamide (HPR) is a synthetic retinoid that inhibits growth of many human tumor cells, including those resistant to natural retinoids. HPR is an effective chemopreventive agent for prostate, cervix, breast, bladder, skin, and lung cancers and has shown promise for the treatment of neuroblastomas. We have previously shown that HPR inhibits proliferation and induces apoptosis of HTLV-I associated adult T cell leukemia (ATL) and HTLV-I-negative malignant T cells, while no effect is observed on normal lymphocytes. *Results.* In

this report, we identified HPR-induced reactive oxygen species (ROS) generation as the key mediator of cell cycle arrest and apoptosis of malignant T cells. HPR treatment of HTLV-I negative malignant T cells was associated with a rapid and progressive ROS accumulation. Pre-treatment with the anti-oxidants vitamin C and dithiothreitol inhibited ROS generation, prevented HPR-induced ceramide accumulation, cell cycle arrest, cytochrome c release, caspase-activation, and apoptosis. Therefore, anti-oxidants protected malignant T cells from HPR-induced growth inhibition. The expression of the HTLV-I oncoprotein Tax abrogated HPR-induced ROS accumulation in HTLV-I infected cells, which explains their lower sensitivity to HPR. *Summary and Conclusions.* Defining the mechanism of free radical induction by HPR may support a potential therapeutic role for this synthetic retinoid in ATL and HTLV-I-negative T-cell lymphomas.

0734

ENZYME REPLACEMENT THERAPY DOSE-RESPONSE RELATIONSHIPS IN PATIENTS WITH TYPE 1 GAUCHER DISEASE

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Aims. To analyze if enzyme replacement therapy (ERT) with imiglucerase demonstrates dose-response relationships in patients with type 1 Gaucher disease (GD) within the range of doses used in routine clinical practice and across disease parameters. *Methods.* The analysis included all patients with type 1 GD enrolled in the ICGG Gaucher Registry, diagnosed in 1991 or later, received ERT with imiglucerase, and had an intact spleen. ERT dose was defined as the average dose over the first 3 years of treatment. Propensity score matching was used to control for differences in baseline disease severity between ERT dose groups categorized as 15U/kg (5″, <29 U/kg/2wk), 30 U/kg (29″, <48 U/kg/2wk), 60 U/kg (48″, <75 U/kg/2wk). Hemoglobin, platelet count, liver and spleen volumes were assessed during follow-up (0 to 60 months) through non-linear mixed effects models. The rate (T50) and extent (Emax) of ERT treatment effect for each parameter were compared across groups. *Results.* Propensity score matching resulted in three comparable groups of 122 patients each (ERT with imiglucerase at 15, 30, 60 U/kg/2wk). Statistically significant dose-response relationships were found across groups for each parameter analyzed: hemoglobin, platelet count, liver and spleen volumes, in regard to rate and extent of improvement over 60 months. *Summary and conclusions.* ERT with imiglucerase results in statistically significant dose-dependent improvement in hematological parameters and organomegaly in patients with type 1 GD. Propensity score matching and non-linear mixed effects models can be used to assess outcomes based on observational data from an international rare disease registry. Further analysis of clinically significant disease parameters is ongoing.

0735

A BENCHMARK ANALYSIS OF THE ACHIEVEMENT OF THERAPEUTIC GOALS FOR PATIENTS WITH TYPE 1 GAUCHER DISEASE

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Background. Gaucher disease (GD) is a clinically heterogeneous disorder requiring an individualized approach to therapy and disease management. Enzyme therapy with intravenous infusions of recombinant glucocerebrosidase (imiglucerase) is the current standard of care for patients with symptomatic GD. In 2004, an international panel of physicians proposed therapeutic goals for treatment of patients with type 1 (non-neuronopathic) GD. *Aims.* To report the frequency with which the therapeutic goals for several key clinical aspects of type 1 GD are met four years after starting imiglucerase and discuss possible determinants of treatment response. *Methods.* Data source: International Collaborative Gaucher Group (ICCG) Gaucher Registry. Inclusion criteria: Patients with type 1 GD treated with imiglucerase therapy at any dose for at least four years, with data available at 4 (±1) years for six parameters: haemoglobin concentration, platelet count, liver volume, spleen volume, bone pain, and bone crises. Data analysis: Patients were assessed for the total number of therapeutic goals met of the 6 parameters. The response groups were compared by geographic region and genotype group. The 6 parameters were also compared by the proportion of patients achieving the relevant therapeutic goal for each parameter. *Results.* 337 of a potential 1,473 patients met the inclusion criteria. Overall, 37.4% (126) had achieved all 6 therapeutic goals at the 4-year observation point. 72.1% (243) achieved at least 5 goals; 89.3% (301) achieved at least 4 goals; 98.2% (331) achieved at least 3 goals; 99.4% (335) achieved at least 2 goals. All 337 patients achieved at least one therapeutic goal. The achievement of goals varied among organ systems with 98.8% (bone crises), 91.0% (haemoglobin level), 90.0% (liver volume), 74.8% (bone pain), 72.7% (platelet count) and 69.4% (spleen volume) achieving the goal. Significant variation among geographic regions was found in the distribution of glucocerebrosidase locus genotypes and in attaining the therapeutic goals. However, genotype at the glucocerebrosidase locus was not a significant determinant of treatment goal attainment. *Summary and Conclusions.* The therapeutic goals for anaemia, thrombocytopenia, hepatomegaly, splenomegaly, bone pain and bone crises in type 1 GD are variably attained within the predicted intervals. The variability was not related to genotype, but did relate to geographic region, suggesting that further study is needed to determine how treatment response might also be influenced by pre-treatment disease severity, cumulative disease burden, co-morbid disorders, imiglucerase dose, and various clinical practice patterns. In the future, this benchmark analysis may also be used as a baseline to assess whether a structured disease management approach improves outcomes in patients with type 1 GD.

0736

EFFICACY AND SAFETY OF INTRATHECAL DEPOCYTE (LIPOSOMAL CYTARABINE) IN PATIENTS WITH PRIMARY CNS LYMPHOMA (PCNSL) OR LEUKAEMIC MENINGITIS ASSOCIATED WITH ACUTE LEUKAEMIAS: A SINGLE INSTITUTION EXPERIENCE IN FRANCE

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Background. Acute myeloid and lymphocytic leukaemias (AML, ALL) and PCNSL with cerebrospinal fluid (CSF) involvement have a poor prognosis. Standard treatment involves IT cytarabine or methotrexate (MTX), given 2-3 times per week to maintain effective IT concentrations. DepoCyte® is a sustained-release liposomal cytarabine with an extended CSF half-life, allowing administration by lumbar puncture once every 2 weeks for remission induction and every 4 weeks for maintenance. In a trial in patients with lymphomatous meningitis, response rates were significantly higher in patients randomised to IT liposomal cytarabine than in those receiving conventional IT cytarabine (71% vs 15%; p=0.006).[1] *Methods.* A retrospective analysis was conducted on patients (since 2004) treated for LM with liposomal cytarabine (50 mg fortnightly in CSF^+ and monthly in CSF remission) and surviving > 3 months. Dexamethasone 4 mg PO BID was given for 4 days as prophylaxis against arachnoiditis. *Results.* Among 11 evaluable patients (9 males; mean age 49 years, range 35-74), diagnoses were 5 AML CSF^+ at induction, 2 CSF relapse of ALL, 1 CSF relapse of AML, 2 CSF^+ PCNSL, and 1 CSF^+ CNS and pelvic NHL. Prior to treatment with liposomal cytarabine, patients received 1-12 (mean 3.8) IT injections of cytarabine or MTX; patients then received 1-4 (mean 2) cycles of liposomal cytarabine. Among 4 patients remaining CSF^+ after conventional IT therapy, CSF became negative after 1 cycle of liposomal cytarabine in 2 cases and after 2 cycles in 1 case. Adverse events (AEs) recorded before treatment with liposomal cytarabine included vertigo, blurred vision, diplopy, seizures, headaches and 1 case of subdural haematoma. AEs noted after treatment may be attributable to meningeal disease or treatment (headaches 2; TIA 1; subdural haematoma 1); transitory worsening of blurred vision in 1 case was probably associated with high-dose IV and IT cytarabine. With a median follow-up of 11 months (range 5-33), 4

patients had died of their underlying disease (1 PCNSL, 2 AML, 1 ALL), and 2 patients (1 ALL, 1 AML) treated with liposomal cytarabine for CSF relapse were alive in complete remission (CR) at 11 and 18 months following neuraxis irradiation (18 Gy). In the 3 remaining cases of AML, concomitant treatments were: chemotherapy, 1 (alive in CR at 22 months); chemotherapy + 2 allografts, 1 (alive in CR at 12 months); and chemotherapy + 18 Gy neuraxis irradiation, 1 (alive in CR at 12 months). Two patients with PCNSL were alive at 10 and 33 months following chemotherapy + autograft + whole brain irradiation (30 Gy, + 10 Gy in initial site in 1 patient). No long-term neurological sequellae were noted. *Conclusions.* Liposomal cytarabine was effective and well tolerated. IT injection once every 2-4 weeks is more convenient than the twice weekly conventional IT regimen. Prospective studies should be undertaken to assess the efficacy of IT DepoCyte® in patients with AML, ALL and PCNSL with CSF^+ histology at diagnosis.

Reference

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0737

LENALIDOMIDE AND DEXAMETHASONE SYNERGISE TO GREATLY ENHANCE DIRECT ANTI-PROLIFERATIVE EFFECTS ON NON-HODGKINS LYMPHOMA CELLS *IN VITRO*

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Lenalidomide is approved for the treatment of transfusion-dependent patients with anemia due to low- or intermediate-1-risk MDS associated with a del 5q cytogenetic abnormality with or without additional cytogenetic abnormalities, and in combination with dexamethasone for the treatment of multiple myeloma patients who have received at least one prior therapy. Preliminary clinical data also suggest a potential for clinical efficacy in B-cell non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Lenalidomide has multiple mechanisms of action including anti-angiogenic, anti-proliferative and immunomodulatory activities. Direct anti-proliferative effects on multiple myeloma cells, as a single agent and in combination with dexamethasone, have previously been demonstrated. However, there is little data to suggest direct anti-proliferative effects on NHL cells and no data assessing the potential for combination with dexamethasone. In this study we have assessed the ability of lenalidomide to directly induce growth arrest and apoptosis of NHL tumor cell lines either as a single agent or in combination with dexamethasone. NHL cells may to some extent rely on autocrine growth factors for proliferation and survival, e.g., VEGF production is prognostic in this disease. Because lenalidomide is an anti-angiogenic drug known to inhibit growth factor production and signaling we have also assessed the effect of lenalidomide on NHL-derived growth factors, such as VEGF and PDGF. NHL cell lines were treated with lenalidomide and/or dexamethasone using a variety of dosing, sequencing and kinetic regimens. We found that lenalidomide alone can inhibit the proliferation of Namalwa (Burkitt's lymphoma) cells as assessed by 3-day XTT assay and further supported by cell cycle analysis showing G0/G1 phase arrest. A clear synergistic interaction between lenalidomide and dexamethasone was observed when combined simultaneously or sequentially and was associated with hypo-phosphorylation of retinoblastoma (Rb) protein and activation of caspases 3 and 8, leading to extensive apoptosis. Lenalidomide alone had little effect on the proliferation of Jeko-1 and Rec-1 (Mantle cell lymphoma) cells. However, strong synergy was observed when combined with dexamethasone again associated with G0/G1 cell cycle arrest and apoptosis. Furthermore, in Jeko-1 and Namalwa cells, non anti-proliferative concentrations of lenalidomide (0.1 mM) potently inhibited production of VEGF and PDGF. Therefore, the lenalidomide/dexamethasone combination can inhibit NHL cell proliferation, initiating cell cycle arrest and apoptosis. Inhibition of secretion of VEGF and PDGF and associated autocrine signaling pathways by lenalidomide may play a role in this activity as well as its known anti-angiogenic effects. Overall, these results support the potential study of lenalidomide in patients with NHL, and in particular provide a rationale for combination with dexamethasone.

0738

CONTRIBUTIONS OF MK-0457 KINASE INHIBITORY ACTIVITY TO CLINICAL ACTIVITY

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Background. MK-0457 was designed as a small-molecule Aurora (AUR) kinase inhibitor with Ki,app values of 0.66, 18, and 4.2 nM against AUR A, B, and C, respectively. In enzymatic kinase assays, MK-0457 is highly selective over other serine/threonine kinases but exhibits potent cross-reactivity to a small subset of wild type and mutant tyrosine kinases, including BCR-ABL, JAK-2, and FLT-3. In line with its biochemical profile, MK-0457 has demonstrated activity in chronic myeloid leukemia and acute lymphocytic leukemia patients with the T315I BCR-ABL mutation and in patients with JAK-2 positive myeloproliferative diseases. *Aims.* The studies described here further characterize the observed *in vitro* kinase profile in cellular pharmacodynamic and phenotypic assays in cell lines expressing wild type and mutant BCR-ABL, JAK-2, and FLT-3. *Methods.* The inhibitory activity of MK-0457 was evaluated against a panel of ~200 kinases *in vitro* (at Km for ATP). The effect of MK-0457 on cells expressing wild type and mutant forms of either BCR-ABL, JAK-2, or FLT-3 was characterized in (i) viability assays, (ii) FACS analyses, and/or (iii) western blot for autophosphorylation and substrate phosphorylation. *Results.* MK-0457 (200 nM) exposure resulted in at least 50% inhibition of ABL, T315I ABL, ABL-2, FLT-3, FLT-3 D835Y, BMX, JAK-2, YES, LYN, and LCK kinases in addition to the AUR kinases. Pharmacodynamic assays in cells indicated nanomolar inhibition of AUR kinases, BCR-ABL and FLT-3 and low micromolar inhibition of JAK-2. Thus, at clinical doses of 24-32 mg/m^2/hr (achieving patient plasma exposure of >1 μM), MK-0457 is expected to inhibit these tyrosine kinases in addition to the AUR kinases. In spite of its cross-reactivity profile, MK-0457 induced similar cytotoxicity (IC_{50} ~ 300 nM) and exhibited an AUR B-like inhibitor phenotype of failed cytokinesis, leading to endoreduplication and apoptosis in BaF3 cells transfected with ABL or FLT-3 (mutant and wild type) kinases. *Conclusions.* MK-0457 demonstrates potent AUR kinase inhibitory activity and additional activity against a small subset of tyrosine kinases. Although the cellular phenotype of MK-0457 is most consistent with inhibition of AUR B, inhibition of these tyrosine kinases may contribute to its observed clinical activity in T315I mutant chronic myeloid leukemia and acute lymphocytic leukemia as well as in JAK-2 positive myeloproliferative diseases.

0739

ANTI-TUMOR ACTIVITY OF ORALLY BIOAVAILABLE INHIBITORS OF THE 20S PROTEASOME

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Background. Pharmacological agents that inhibit the proteasome have shown clinical efficacy in the treatment of several hematological malignancies. Bortezomib, a dipeptide boronic acid, is an approved treatment for relapse/refractory multiple myeloma and mantle cell lymphoma and PR-171, a tetrapeptide epoxyketone, has shown promising activity in early clinical trials in multiple myeloma. Clinically, both bortezomib and PR-171 are administered intravenously and although this route of delivery results in potent systemic proteasome inhibition, it may limit the clinical application of these inhibitors in part due to the necessity of frequent administrations. *Aims.* Our goal was to develop an orally-bioavailable proteasome inhibitor that may offer greater dosing flexibility and patient convenience compared to existing therapeutics. *Methods.* We synthesized a series of tripeptide epoxyketone analogs of PR-171 and tested those with potent (IC_{50} <100 nM) *in vitro* inhibitory activity against the chymotryptic like (CT-L) activity of the 20S proteasome for bioavailability following oral administration in mice. Bioavailability was measured using pharmacokinetic analysis of plasma levels of compounds following intravenous and oral administration and by a pharmacodynamic assay that measures the CT-L activity in tissue extracts and blood one hour after dosing. An assessment of oral bioavailability of select compounds was also determined in rats and dogs. Compounds were tested *in vitro* for solubility, intestinal cell permeability, metabolism (intestinal fluid, liver microsomes, and hepatocytes) and sensitivity to the multidrug resistance protein 1 (MDR1) efflux pump in order to determine which properties were associated with oral bioavailability. Toxicity studies were done in mice using a two day daily dosing regimen. Anti-tumor

activity was assessed in immunocompromised mice bearing established human tumor xenografts. *Results.* Over 80 compounds were tested for exposure following oral administration using the pharmcodynamic assay and 17 were identified that achieve >80% inhibition of the proteasome in blood and tissues. Pharmacokinetic and pharmacodynamic analysis showed the bioavailability of the most active compounds to range from 5-30%. In comparing the oral bioavailability determined in mice with a variety of properties measured *in vitro*, we found that oral bioavailability is associated with increased intrinsic solubility and metabolic stability and reduced MDR1 sensitivity. The oral bioavailability of a subset of these compounds was also determined in rats and dogs and found to be comparable to mice both by pharmacodynamic and pharmacokinetic analysis. Toxicity studies in mice showed that repeated oral administration was well tolerated at doses that resulted in >80% proteasome inhibition in most tissues. Anti-tumor activity of these orally bioavailable inhibitors was assessed in two human tumor xenograft models using cell lines derived from hematological malignancy: RL (non-Hodgkin's lymphoma B cell) and HS-Sultans (Burkitt's lymphoma). Oral administration of several of these proteasome inhibitors resulted in anti-tumor responses equivalent to intravenously administered PR-171 when given on the same schedule. *Summary.* Based on these studies, a number of potent, orally bioavailable proteasome inhibitors with potential for the treatment of malignant diseases have been identified for further pre-clinical and clinical development.

0740

MARKERS OF VIRAL INFECTIONS DURING RITUXIMAB TREATMENT OF PATIENTS WITH RESISTANT AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)

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Background. Standard AIHA treatment is based on application of corticosteroids. Recently rituximab treatment was found to be quite efficient, especially when AIHA is resistant to standard therapy. Earlier we have found high incidence of EBV and HBV viral markers in AIHA patients. Some publications report the cases where rituximab treatment bear high risk of complications due to viral and bacterial infections. *Aims.* to monitor markers of viral infections during treatment of AIHA patients, in particular using Rituximab. *Materials and Methods.* EIA (IgM-VCA-EBV, IgG-EA-EBV, IgG-NA-EBV, IgM-HCMV, IgG-HCMV, IgM-HSV1-2,IgG-HSV1-2, HBsAg, a-HCV). PCR (DNA-HBV, DNA-EBV, DNA-CMV, DNA-HSV1-2, DNA-HHV-6, RNA-HCV). Viral DNA(RNA) in plasma and peripheral blood mononuclear cells (PBMC), saliva and broncheo-alveolar lavage (BAL) fluid, spleen and liver bioptates was detected by PCR. 41 AIHA patients were followed. Eight of forty one patients, diagnosed with resistant AIHA were treated by rituximab and followed more closely. *Results.* In 26 patients (including all 8 patients with resistant AIHA) out of 41 DNA EBV was detected in PBMC. With standard therapy DNA EBV was detected during all follow-up period (up to 6 years). In 8 patients treated with rituximab CD19, CD20 and CD22 cells were not detectable about one week after injection. Gradual recovery of this cell population took about 2-8 months. DNA EBV ceased to be detectable within 4-8 month after rituximab injection. Patients are in the state of clinical remission for a period from 6 months up to 3 years. HCV viral load in plasma of one patient (genotype 1b) was not essentially influenced by rituximab therapy. High titer of IgG-HSV1-2 (1:6400, 1:12800) was detected in all 8 patients. It was not changed during the course of treatment. IgG-HCMV titer (initially in the range 1:400 to 1:12800 depending on the patient) was not changed as well. IgG-EA-EBV was found in serum of 2 patients before, in the course and after rituximab treatment. *Conclusions.* Rituximab treatment leads to clinical remission in patients with resistant AIHA. In this group of patients we did not detected any signs of activation of viral infections, moreover DNA EBV was below detectable levels during remission period. Any considerable reduction of viral antibodies levels was not detected.

0741

PHARMACEUTICAL DEVELOPMENT OF HIGH DOSE RADIOLABELLED RITUXIMAB AS CONSOLIDATION TREATMENT FOR PATIENTS WITH RELAPSED OR REFRACTORY CD20 POSITIVE B-CELL NON-HODGKINS LYMPHOMA

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Background. Radioimmunotherapy is a promising target-oriented strategy for treatment of B-cell non-Hodgkin´s lymphoma. In this study, the CD20 targeted monoclonal antibody rituximab was coupled to the radionuclide 131-Iodine. Rituximab induces antitumour activity through antibody-mediated mechanisms, while 131-Iodine promotes cytotoxic activity by the crossfire-effect to neighbouring tumour cells with none or insufficient antigen expression. Disposition of this agent in the patient can be visualised by gamma imaging. This study was performed to optimise the pharmaceutical preparation of 131-Iodine labelled rituximab and the subsequent quality control. Due to the required high dose of radioactivity, employed for myeloablative treatment, the safety of the involved personnel is of crucial importance. In addition, the integrity of rituximab must be maintained. *Aims.* The aim of this study was to develop clinical grade 131-Iodine labelled rituximab, maintaining its properties, with minimum exposure to involved personnel. *Methods*. A previously developed labelling method was used as a starting point. First, the 131-Iodine was pretreated to adjust the pH and to protect the radionuclide from oxidation. The labelling was provoked by addition of 35 μg of Iodogen in acetonitril (1 mg/mL) to a reaction vial with rituximab and 131-Iodine. After 3 minutes, the reaction was terminated by addition of ascorbic acid. Subsequently, the 131-Iodine rituximab conjugate was purified. All labelling steps were carried out under aseptic conditions and a sterile filtration was performed as final step. The labelling process consisted mainly of a remote system, operated with underpressure to promote the radiation safety. The labelling procedure was performed under good manufacturing practice (GMP) conditions. Radiation exposure was monitored by wearing a thermoluminescence detector (TLD) and an electronic personal dosimeter. Exposure to the fingers was assessed by attaching TLDs to the fingertips. The annual maximum permissible dose to radiation workers is 20 mSv. The final product was characterized by size exclusion chromatography (SEC-HPLC) and by instant thin layer chromatography (iTLC). The immunoreactive fraction was determined using a cell-binding assay at antigen excess using Epstein Barr Virus-transformed human B-lymphocytes (EBV-LCL). *Results.* This method resulted in a labelling yield of 80% and a radiochemical purity of >99%. SEC-HPLC analysis showed that during the labelling the integrity of the antibody was maintained, although a small increase in formation of aggregates was observed (<5%). The immunoreactivity, determined by cell-binding assays, was 70%. 131-Iodine labelled rituximab, conjugated according to a more aggressive reaction procedure with an excess of Iodogen, showed an immunoreactivy of less than 40%. The exposure due to the radioactivity during the radiolabelling was 10 μSv per labelling procedure, which is acceptable for radiation safety. *Summary and conclusions*. Rituximab can be radiolabelled with 131-Iodine with high efficiency while the integrity and reactivity of rituximab are preserved. The labelling process was accompanied with a minimal exposure to the labelling personnel. With the developed method 131-Iodine labelled rituximab can be safely and reproducibly prepared for clinical applications.

0742

SNS-032 EXHIBITS DOSE-DEPENDENT MECHANISM-BASED INHIBITION OF CDK7 AND CDK9 IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH ADVANCED CANCERS TREATED IN AN ONGOING PHASE 1 TRIAL

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Background. SNS-032 (formerly BMS-387032), a potent, selective inhibitor of cyclin-dependent kinases (CDK) 2, 7 and 9, inhibits both transcription and the cell cycle. SNS-032 is in phase 1 clinical trials for both hematologic and solid malignancies. SNS-032 is hypothesized to have anti-cancer effects by decreasing expression of cytokines and survival factors that promote and maintain malignancies (short T? tran-

scripts and proteins most affected by transcriptional blockade) and by inhibiting deregulated proliferation. We recently reported SNS-032 mediated target modulation and cytotoxicity in a multiple myeloma cell line, RPMI-8226 (Conroy AACR poster 2007), and have now benchmarked SNS-032 against flavopiridol. Flavopiridol, a pan-CDK inhibitor, demonstrated activity in CLL. However, target modulation consistent with CDK inhibition has not been observed in flavopiridol-treated patient PBMCs or in CLL cells exposed in whole blood (Bible 2005, Lucas ASH poster 2006). Flavopiridol may exert clinical activity via DNA intercalation rather than by CDK inhibition (Bible 2005). We obtained PBMCs from SNS-032 treated patients as part of the correlative science component of an open-label, multi-center, dose-escalation trial of SNS-032 for the treatment of advanced solid malignancies. Presented here are preliminary results of the correlative science studies. *Aims*. 1) Profile SNS-032 vs. flavopiridol in a cancer cell line to understand differences in target modulation (TM) related to plasma protein binding in human vs. bovine serum; 2) assess TM consistent with CDK inhibition in patient PBMC; 3) explore pharmacodynamic (PD) relationships with dose, pharmacokinetics (PK). *Methods*. TM and MTT cytotoxicity assays were performed on RPMI-8226 cells treated with SNS-032 or flavopiridol in the presence of 10% human or bovine serum. SNS-032 was administered to patients with advanced solid cancers as 1-hr IV infusions qd x5, q3wks. TM and plasma PK were quantified on Days 1-4. Isolated PBMC were frozen on site for analysis by Western blot. PK levels were assessed by LC/MS/MS. *Results.* SNS-032 and flavopiridol had similar potency in bovine serum. However, flavopiridol was less active in human serum whereas SNS-032 was equipotent. Consequently, in human serum, SNS-032 is a 3-fold more potent inhibitor of CDK9 and 5-fold more potent in cytotoxicity compared to flavopiridol. In the clinical study, on Day 1 RNA Pol II CTD pS2 and pS5 decreased, indicating inhibition of CDKs 9 and 7, with uniform TM at 3hr post dose; the rate and extent of TM increased with dose, though on Day 4 there was some evidence of rebound. A dose-dependent decrease in actin was observed on Day 1. MCL-1 survival-protein reduction was first noted at 48 mg/m^2. TM was sustained with SNS-032 levels below IC10. *Conclusions*. SNS-032 is a more potent CDK9 inhibitor and pro-apoptotic agent than flavopiridol when assayed in human versus bovine serum. We successfully demonstrate mechanism-based, dose-dependent inhibition of CDKs 7 and 9 via decreased phosphorylation of pS5 and pS2 of RNA Pol II CTD. Decreased MCL-1 suggests inhibition of survival proteins. These data differentiate SNS-032 from flavopiridol and indicate that transcriptional inhibition may be a fundamental mechanism by which SNS-032 causes apoptosis in solid and hematologic cancers.

0743

ACHIEVEMENT OF THE GOALS OF THERAPY FOR PATIENTS WITH GAUCHER DISEASE ON ENZYME REPLACEMENT THERAPY IS HIGHER AMONG EARLIER-TREATED PATIENTS AND IS NOT INFLUENCED BY DISEASE SEVERITY AT PRESENTATION

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Background. Enzyme Replacement Therapy (ERT) has dramatically changed the clinical course and prognosis of patients with Gaucher disease. *Aims.* To retrospectively evaluate the achievement of the goals of treatment in a cohort of homogeneously treated patients with Gaucher's disease. *Patients and Methods.* Expanding the goals of treatment, initially established by Pastores *et al.,* we retrospectively evaluated the achievement of 35 goals, related to 7 fields of disease's features (anemia, thrombocytopenia, hepatomegaly, splenomegaly, bone manifestations, pulmonary manifestations, quality of life) in a cohort of 57 patients with type I Gaucher disease, receiving ERT at an initial dose of 30 IU/kg/month. Patients were 27 females and 30 males with a median age at diagnosis of 30 years (range 2-77 years) and at treatment start of 39 years (range 5-79 years). The median duration of treatment was 94 months (range 24-155 months). Patients on ERT for < 24 months were not included in the analysis. *Results.* The median applicability was 22 goals or 63% per patient (range 17-28 goals or 49-82%). The median overall success rate was 20 goals or 86.5% per patient (range 12-27 goals or 61-100%). The success rate for 8 goals was 100% (restoration of normal hemoglobin 1 year post-splenectomy, no major bleeding 1 year after ERT start, duplication of platelet count in severely thrombocytopenic patients 2 years after ERT start, maintenance of platelet count in non-thrombocytopenic patients, prevention of bone crises, reversal of hepatopulmonary syndrome and dependence on oxygen, amelioration of pulmonary hypertension and prevention of rapid deterioration of pulmonary disease). Conversely, success rate for 4 goals was <75% (elimination of the shortness of breath, reduction/withdrawal of analgesics, improvement of MRI findings, and improvement of physical function for normal activity). Higher success rates were achieved for the goals related to thrombocytopenia (97.9%) and pulmonary manifestations (97%) although in the latter case the applicability was low. Lower success rate was achieved for the goals related to bone manifestations (78.7%). There was no significant difference in the success rate between males and females, splenectomised and non-splenectomised patients, and between patients diagnosed earlier or later than the age of 5 years. However patients started on ERT before the age of 40 years exhibited significantly higher success rate as compared to patients started later (p=0.047) and there was a significant inverse correlation between overall success rate and patient's age at treatment start (r=-0.437). Finally, the success rate was higher among double heterozygotes, carrying one N370S allele, as compared to patients homozygous for the N370S mutation (90.2±9.3% vs. 81.7±12.9%, p=0.021). Conversely, patients carrying the L444P mutation had comparable success rate with those not carrying this mutation. *Conclusions.* The achievement of the goals of therapy for patients with Gaucher disease, receiving ERT is higher among earlier treated patients and among patients not carrying homozygosity for the N370S mutation, and is not influenced by disease severity at presentation.

0744

SPANISH REGISTRY OF RADIOIMMUNOTHERAPY: ANALYSIS OF OUTCOMES OF PATIENTS WITH RECURRENT OR REFRACTORY FOLLICULAR LYMPHOMA (FL) TREATED WITH 90Y IBRITUMOMAB TIUXETAN (90Y-RIT)

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Background. 90Y-RIT is approved for the treatment of refractory or recurrent FL. The aim of this study was to collect data of FL patients (p) treated with RIT within the clinical practice setting, to retrospectively analyze treatment effectiveness and tolerability, in order to provide a pragmatic approach to experimental data. *Methods.* Multicenter, retrospective study. FL p treated, since commercial availability in Spain, with 90Y-RIT, were registered. Effectiveness endpoints retrospectively studied were: objective response rate (ORR), time to progression (TTP) overall survival (OS) and safety. Clinical prognostic factors were collected to asses their possible influence upon treatment effectiveness, by multivariate analyses. *Results.* 55 p from 26 centres were registered: M/F, 58.5/41.5%; mean age, 57 years (22-83); ECOG 0-1, 73.3%. Median time since FL diagnosis was 4.8 (1-24) years. Before 90Y-RIT, most of the cases were refractory to previous treatments (76.3%) with a median number of previous treatments of 3 (range 1-8). FLIPI distribution was: low-risk 51.9%, intermediate-risk 18.5% and high-risk 29.6%. Median administered dose of 90Y-IT was 0.4 mCi/Kg. ORR was 74.6% (95% CI: 63.1, 86.1), with 84.4% in p younger than 60 years old and 59.1% in elderly p. According to FLIPI ORR was: LR: 92.9%; IR: 50%; HR: 74.1%. With a median follow-up time of 6 months, median TTP was 17.7 months (95% CI: 14.3, 21.1) and median OS has not been achieved, with an estimated OS at 1 and 2 years of 79.8% and 60.8%, respectively. Bone marrow involvement and high FLIPI score were significantly associated with higher risk of progression disease: hazard ratio 3.71 (95% CI: 1.00, 13.68) and 21.12 (95% CI: 2.37, 187.91), respectively. Median time to G3-4 haematological toxicity ranged between 27-38.5 days, being the most frequent thrombocitopenia (46.2%) and neutropenia (33.8%). 17 p required red blood cell transfusions (median, 4 U) and 19 required platelet transfusions (median, 7 U). The most frequent G3-4 non haematological toxicity was asthenia (10.8%). *Conclusions.* Despite the limitations of the retrospective design of the Registry, these results obtained with 90Y-RIT for FL patients treated within the clinical practice setting are similar to that obtained in clinical trials. Updated data will be presented at the meeting.

0745

IN VITRO ACTIVITY OF TYROSINE KINASE, FARNESYL TRANSFERASE AND AKT KINASE INHIBITORS ON C-KIT POSITIVE/NEGATIVE AND MDR-PGP POSITIVE/NEGATIVE LEUKAEMIA TUMOR CELL LINES

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Imatinib is a target inhibitor of the tyrosin kinase (TK) activity associated to P210/190-BCR-ABL chimeric protein and to platelet-derived growth factor receptor (PDGF-R) α and β or c-kit receptor. Actually, few haematologic responses were observed in the c-kit positive acute myeloid leukaemias (AMLs) patents treated with Imatinib. The sensitivity of Imatinib to several proteins involved in the mechanisms of multidrug resistance (MDR), such as the over-expression of P-glycoprotein (Pgp), has been suggested as one the causes of failure of Imatinib in c-kit positive AML. In this study, we evaluated the effects of MDR-Pgp expression on the *in vitro* activity of Imatinib and other TK inhibitors such as Nilotinib (AMN107, Novartis Pharma) and Dasatinib (BMS-354825, Bristol-Myers Squibb) on c-kit positive (HL60/HLA60-DNR) and c-kit negative (CCRF-CEM/CEM-VLB) human leukaemic cell lines. Secondly, we tested the *in vitro* activity of farnesyl-transferase inhibitors (FTIs) Tipifarnib (R115777, Zarnestra, Johnson & Johnson) and Lonafarnib (SCH66336, Sarasar, Schering-Plough) and of AKT inhibitor (A-443654, Abbott) either alone or in combination with Imatinib on c-kit positive/MDR-Pgp positive cell line (HLA60-DNR) to investigate alternative strategies against c-kit positive AML blasts. By using the MTT-microcultured tetrazolium colorimetric assay, we measured a consistent growth inhibitory effect of TK inhibitors in MK1/BCR-ABL positive cell line and only in the HL60 DNR/c-kit positive cell line, even though this latter was over-expressing MDR-Pgp. Both in MK1/BCR-ABL positive and HL60 DNR/c-kit positive cell lines, Nilotinib and Dasatinib resulted 10 and 100 folds more potent than Imatinib, respectively. In all of the leukaemic cell lines, Tipifarnib (ID50 range = 0.0065 to 0.21 μM) was more toxic than Lonafarnib (ID50 range = 0.3 to 6.5 μM), while AKT inhibitor (A-443654) showed a very low growth inhibitory effect (ID50 range = 0.1 and 0.35 microM). Neither FTIs nor AKT cytotoxic activity was influenced by the MDR-Pgp over-expression. When we tested the combinations of Imatinib with Tipifarnib or other inhibitors on the HL60 DNR/c-kit positive/MDR-Pgp positive or on the other leukaemic cell lines we did not measure any synergistic effects, but only additive effects. 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 Imatinib or to other TK, FTIs or AKT inhibitors. To improve the inhibitory effects on c-kit positive cells may be more useful to employ Nilotib or Dasatinib. As we did not observe any synergistic effects, the use *in vivo* of combinations with 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.

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RADIOIMMUNOTHERAPY AS A PROMISING THERAPEUTIC OPTION IN PATIENTS WITH RELAPSED OR REFRACTORY CD20+ NON-HODGKINS LYMPHOMA

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90Y ibritumomab tiuxetan is a murine anti-CD20 monoclonal antibody developed for radioimmunotherapeutic targeting of CD20+ lymphoma cells. As it emits therapeutic β radiation a wide ranging crossfire effect can be achieved. Our aim was to evaluate the effectiveness of 90Y ibritumomab tiuxetan (Zevalin®) as a treatment option for patients with CD20+ NHL, especially for those with follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLCL). Forty patients with CD20+ NHL were treated with Zevalin® between March 2004 and June 2006 in this cohort. Twenty-two patients had relapsed or refractory FL, 11 patients had relapsed or refractory DLCL, 2 patients suffered from Immunocytoma, one patient had mantle cell lymphoma and one suffered from Burkitts lymphoma. One of the patients had a post-transplantation B-lymphoproliferative disorder after 9 years of immunosuppressive therapy after heart transplantation. All of them were extensively pretreated with a median of 3 prior regimen of polychemotherapy (range 1 to 6). Two patients with FL and one patient with DLCL received Zevalin® as first-line therapy. The median age was 61 in FL (range 41-79) and 69 in DLCL (range 36-86). The Zevalin® treatment regimen consisted of pre-treatment with Rituximab (250 mg/m^2 intravenously on days 1 and 8) to deplete peripheral blood B-cells followed by Zevalin® infusion on day 8. A mean of 1084 MBq (range 680-2040) 90Y ibritumomab tiuxetan (Yatrics-Zevalin, Schering AG) was administered. Remission state was evaluated in DLCL three months after therapy. Additionally we calculated overall survival and disease-free survival for patients with FL. For FL overall survival was 87,5% (21/24) after a median follow-up of 16 months (range 2-30) and disease-free survival was 79% (19/24) after a median follow-up of 15 months (range 3-30). Out of eleven patients with DLCL, six developed progressive disease, three achieved complete remission, one partial remission and one patient remained in stable disease. One patient in CR died due to myocardial infarction. Thus 73% (8/11) DLCL patients did not respond to this therapeutic strategy. All of them were extensively pretreated. The main side effects were thrombocytopenia followed by mild leukocytopenia and anemia. Treatment associated myelodysplastic syndrome was diagnosed in one patient twenty-nine months after therapy. Our results confirm that Zevalin® is a good treatment option in patients with relapsed or refractory FL whereas extensively pretreated DLCL patients show hardly any response to this therapeutic strategy. Possibly these patients would benefit in an earlier use of Zevalin® in the course of the disease.

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TARGETING THE RAF/MEK/ERK SIGNALING PATHWAY BY THE NOVEL MEK INHIBITOR PD0325901: MOLECULAR AND FUNCTIONAL EFFECTS IN PRE-CLINICAL LEUKEMIA MODELS

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Background. The Raf/MEK/ERK pathway plays a pivotal role in the regulation of cell proliferation, survival and differentiation. As reported by our and other groups, this pathway is frequently deregulated in hematological malignancies, particularly in AML, representing an attractive target for therapeutic interventions. *Aims.* Here we investigated the effects of PD0325901, a novel MEK inhibitor, on phospho-protein expression, gene expression profile, cell proliferation, and apoptosis in AML, ALL and multiple myeloma (MM) cell lines, in oncogene-transformed hematopoietic murine myeloid cell lines, as well as in *ex vivo*-cultured primary AML blasts. *Results.* AML cell lines (OCI-AML2, OCI-AML3, HL-60) showed a high sensitivity to PD0325901 (IC_{50}: 5-19 nM), NB4 (APL) and U266 (MM) showed an intermediate sensitivity (IC_{50}: 822 and 724 nM), while all the lymphoid cell lines tested and the myeloid cell lines U937 and KG1 were resistant (IC_{50} >1000 nM). Cell cycle analysis and Annexin V assay demonstrated that cell growth inhibition was caused by cell cycle arrest and apoptosis induction. The effects of PD0325901 were also examined on primary cells from 18 AML patients. A statistically significant reduction in the percentage of S-phase cells (p=0.01) and increase in the percentage of apoptotic cells (p=0.019) was also observed in primary AML samples, *in vitro* exposed to 100 nM of the MEK inhibitor. Next, PD0325901 effects were examined in a panel of IL-3-dependent murine myeloid FDC-P1 cell lines transformed to grow in response to 11 different oncogenes in the absence of IL-3. Fms, Ras-, Raf-1-, B-Raf-, MEK1-, IGF-1R- and STAT5a-transformed FDC-P1 cells were very sensitive to PD0325901 (IC_{50}: ~ nM), while A-Raf-, BCR-ABL-, EGFR- or Src-transformed cells were 10- to 100-fold less sensitive (IC_{50}: 10 to 100 nM); the parental, IL-3 dependent FDC-P1 cell line had an IC50 >1000 nM. Semi-quantitative analysis of the phosphorylation status of 18 different target proteins on OCI-AML3 cells after treatment with 10 nM PD0325901 showed a 5- to 8-fold reduction in ERK-1/2 and a 2-fold reduction in JNK and STAT3 phosphorylation. Conversely, increased phosphorylation in response to PD0325901 was observed for Raf-1 (2.5-fold), MEK1/2 (2.4-fold), AKT (2-fold) and p70S6K (2-fold). The gene expression profile of the sensitive cell line OCI-AML3 was also profoundly altered from PD0325901 (10 nM) treatment: 96 genes were

modulated after 24 h (37 up- and 59 down-regulated), most of which involved in cell cycle regulation. Changes in cyclin D1 and D3, cyclin E and cdc 25A were also validated at the protein level. *Conclusions.* The strong growth-inhibitory and pro-apoptotic activity induced *in vitro* by PD0325901 suggests that MEK may be an appropriate therapeutic target in hematological malignancies, particularly in myeloid leukemias.

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LONG TERM EFFICACY AND SAFETY OF MIGLUSTAT THERAPY IN TYPE 1 GAUCHER DISEASE

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Background and Objective. Gaucher disease (GD) is the most common lysosomal storage disease. Miglustat ZAVESCA, is a synthetic iminosugar which acts as an inhibitor of the enzyme glucosylceramide synthase (SRT). This therapy offers an alternative approach for GD based on the indirect effect reducing the burden of glycolipids delivered to the macrophage system after phagocytosis of formed blood cells. We present the results after more than 24 months on an everyday clinical use of oral therapy in type 1 GD patients. *Design and Methods.* The Spanish group on GD design a structured project named with the acronymus ZAGAL. It includes a set of recommendations in a structured protocol for collecting safety, efficacy and QoL data at 6, 12, 24 months and beyond. The project's aim is to guarantee the safe and proper use of Zavesca in an everyday clinical use. Baseline assessment: complete clinical, analytical and imaging evaluation, detailed neurological exam and superficial electroneurogram in sural and peroneal nerve. Cognitive test and memory impairment screen for dementia assessment were used. A free lactose and low carbohydrate diet (FLLCD) were recommended and applied in first weeks on therapy. *Results.* 41 GD patients (females 56.1%). mean age 46.4.y (range: 21-74), SSI 5.8 (range: 2-9), spleen removal 9.5%, chitotriosidase activity 3,286 nM/mL.h (range 468-10,553), CCL18/PARC 533 (range 102-1,219). Ten patients was naïve to SRT, mean age: 46.7y. Thirty one patients were included on SRT once stabilising their disease with Imiglucerase during a mean of 3.8 y (range: 2-11), dose 30-60 U/kg, mean age 39.2 y; mainly heterozygous for N370S. In February 2007 15 patients had completed 24 months on SRT, 21 patients 12 months and 10 naïve patients/13 switch 6 months. Response: all patients with anaemia improved haemoglobin concentration (mean 0.8 g/dL). Platelet count improved in patients with lowest values and it was maintained in patients with counts in normal limits. Chitotriosidase activity was maintained in switched patients and slightly decreased in naïve patients. The response was observed at 6 months on therapy and it is maintained after 24 months on therapy. In 7 naïve patients bone marrow MRI improvement was documented. No new symptoms were developed, three patient discontinued SRT due to poor compliance. Gastrointestinal disturbance appeared sporadically in five patients and became normal when they followed the FLLCD. One patient had a significant weight loss (<10%); mild tremor was observed in five patients. no cognitive impairment or another neurological problems appeared. *Conclusions.* In our experience type 1 GD patients with mild or moderate disease had a satisfactory clinical, analytical and bone-marrow response to SRT with scarcely adverse events. At six months in naïve patients the response is similar to that observed in clinical trials and in patients treated with Imiglucerase and remained stable at 12 and 24 months.

Platelets and thrombocytopenia

0749

PREVALENCE AND CLINICAL SIGNIFICANCE OF ELEVATED ANTINUCLEAR ANTIBODY TEST IN CHILDREN AND ADULT PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background and Aims. Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by persistent thrombocytopenia due to autoantibody binding to platelet antigens causing their premature destruction by the reticuloendothelial system. The antinuclear antibody (ANA) titer is considered to be the best laboratory screening test for SLE, juvenile rheumatoid arthritis, and other systemic autoimmune disorders. A positive ANA result is not completely sensitive or spesific, because it also can be detected in normal children or adults. Nevertheless, ANA production can be the harbinger of autoimmune disease. We performed a study to determine the likelihood of children and adults with ITP to later developed SLE and other connective tissue disease (CTD). Patients and Methods. The prevalence and clinical significance of ANA were investigated in 365 childhood and 108 adult patients with ITP. At the time of diagnosis of ITP, patients with CTD were excluded. Results. Out of 365 childhood ITP; 301 (82.4%) patients were acute, 64 (17.6%) patients were chronic ITP. ANA titer ≥1:80 were positive in 33 (9.04%) of 365 patients with childhood ITP; 21 patients (6.9%) were in acute, and 12 patients (18.7%) were in chronic groups. Out of 108 adult patients with ITP; 31 (28.7%) patients were acute and 77 (71.3%) patients were chronic ITP cases. ANA titer ≥1:80 were positive in 36 (33.3%) of 108 patients with adult ITP; 12 patients (38.8%) were in acute, and 24 patients (31.2%) were in chronic groups. Mean follow-up 3.6 years (range: 2.1 to 7 years) for all patients. At the end of follow-up period Sjogren's syndrome (SS) was diagnosed in only one adult chronic ITP cases. None of the other high titer ANA positive patients developed SLE or other CTD. Conclusions. Our results showed that there is a statistically significant difference in terms of ANA positivity between childhood acute and chronic ITP patients (p=0.003). Our results showed that there is a statistically difference in terms of ANA positivity between childhood acute and chronic ITP patients (p=0.003). We think that ANA positivity may be an indicator in terms of chronicity for childhood ITP. The detection of high-titer ANA is not enough to identify those patients with ITP who are at risk of developing SLE. However, large scale studies should be considered to determine the significance of ANA positivity and their utility in differentiating acute from chronic ITP.

0750

ITP IN CHILDREN: UNICENTRIC EXPERIENCE ON 265 CASES

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Background. Acute Immune Thrombocytopenic Purpura (ITP) affects about 2-8/100.000 children/year. ITP is an immune mediated disorder related to autoantibodies against major platelet membrane antigens, resulting in shortened platelet survival. A spontaneous recovery is described in about 50% of children. The first line treatment, when necessary, includes oral or intravenous corticosteroids and/or high doses of intravenous immunoglobulins (IVIG). *Aims.* The aim of this study is to describe a large unicentric experience on 265 children in different therapeutic approaches, and to contribute to the problems of whether, when, and how to treat ITP in children. *Methods.* Between January 1995 and December 2005, according to the different protocols in use during this period, 265 children (0-15 years) have been consecutively observed. Diagnosis was made on well established criteria, excluding other haematological disorders by bone marrow aspirate showing normal to increased megakaryocytes. In all the children we studied autoimmunity, to exclude systemic autoimmune disorders, and serology for common viral infections (CMV, EBV, HIV). Twenty eight children have been treated with High Doses of Methylprednisolone (HDMP: 15 mg/kg × 4 days), 63 with HDMP at 7.5 mg/kg × 4 days, 37 with pulses of HD Dexamethasone (DXM), 29 with low doses of methylprednisolone (MP), 51 with different doses of IVIG (0,4 g/kg or 0,8 g/kg). According to our usual strategy, 57 children have not been treated (wait and see) because of a platelet count ≥ 10×10^9/L without significant bleeding. *Results.* Two hundred forty four (92.1%) children reached a persistent Complete Response (CR), 237 (89.4%) after a first line treatment or the wait and see strate-

gy. There are not significant differences between different therapeutical approaches in terms of the percentage of CR. IVIG and HDMP at 7.5 mg/Kg for 4 days seem to be the best treatments to reach as soon as possible a safe platelet level ≥30×10^9/L (3-6 days), and a CR (7-11 days). Among NR (chronic) patients, seven have been splenectomized and only 3 reached a stable CR. We never observed a significant toxicity, nor adverse events related to the treatment. *Conclusions.* Our experience shows that there are no statistically significant differences in terms of CR between the different treatments, even considering that more than 70% of relapses and 39% of NR children can be cured with further treatments. However, the main target of the treatment is to reach safe platelet levels ≥30×10^9/L as soon as possible to avoid life-threatening risks (spontaneous bleeding and trauma for young children) or parent anxiety. Therefore, IVIG (0,4 mg/kg) and HDMP (7,5 mg/kg/day) seem to be the best options; the low costs of HDMP and its safety need to be considered. The high percentage of CR after any kind of first line treatment, let us to suppose that it is possible to obtain a spontaneous recovery in the great majority of patients also without treatment. Therefore, the extension of the 'wait and see' strategy for as many patients as possible could be important. In children, in comparison to ITP adults, the splenectomy cannot be considered an usual therapy and not always a successful procedure.

0751

ITP INCIDENCE AND MORTALITY IN UK GENERAL PRACTICE RESEARCH DATABASE

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Background. ITP is an autoimmune disease characterized by low platelet counts and an increased risk of bleeding. The epidemiology of ITP in the UK has not been well characterized. The General Practice Research Database (GPRD), which comprises computerized general practice medical records for a population-based cohort of approximately five million residents of the UK, provides an opportunity to quantify the occurrence and outcomes of ITP in the general population. *Aims.* 1. Estimate the incidence of ITP in the UK; and 2. Estimate the survival of patients with incident ITP compared to the population without ITP. *Methods.* Incident cases of ITP during 1990-2005 were identified in the GPRD and matched to comparison subjects by sex, year of birth, and index date. Total person-time contributed by all subjects in the GPRD population was used as the denominator for incidence calculations (with censoring when patients developed ITP, transferred out of their practice, or died). We analyzed changes in incidence over time using Poisson regression. We estimated survival by the Kaplan-Meier method and compared survival among incident ITP cases and their matched comparison subjects using the log-rank test and proportional hazards regression. *Results.* We identified 1,146 incident ITP cases and 5,715 matched comparison subjects without ITP. The crude (average) incidence of ITP was 3.9/10^5 person-years (py). The incidence was 4.4/10^5 py in females and 3.4/10^5 in males. The incidence by age was bimodal with highest values under 18 years and in the elderly. The age- and sex-adjusted incidence of ITP increased an average 5% per year (cumulative increase approximately two-fold during the 15-year study period). During a median 3.4 years of follow-up in the GPRD (interquartile range 1.5-6.0 years, maximum 15 years), 140 ITP cases (12.2%) and 482 comparison subjects (8.4%) died (p=0.0001). The estimated survival for ITP cases at 5 years was 87% (95% confidence interval [CI] 84-89%) and at 10 years was 78% (95% CI 74-82%). Adjusted for age and sex, the hazard ratio for death associated with ITP was 1.6 (95% CI 1.3-1.9). Ninety-six percent of deaths among ITP cases occurred in those older than 45 years. There was no significant survival difference between males and females. *Conclusions.* This study provides estimates of ITP incidence and mortality in the UK. ITP incidence recorded by general practitioners has increased substantially during the past 15 years. Further work is needed to determine whether this reflects a true increase in the incidence of ITP in the UK population, better detection, changing criteria for diagnosis, increased recording by general practitioners, or a combination of factors. Patients with ITP have approximately 60% higher mortality than sex- and age-matched comparison subjects without ITP. This increased risk of death with ITP is largely concentrated in middle-aged and elderly patients. We are extending our study to explore causes of death among patients with ITP in the UK.

0752

A CASE OF AMEGAKARYOCYTIC THROMBOCYTOPENIA WITH RADIO-ULNAR SYNOSTOSIS SYNDROME, SUCCESSFULLY TREATED WITH ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background and Aims. Bone marrow failure syndromes can be associated with abnormalities of skeletal defects. Since Thompson et al. (2000) have described two families of amegakaryocytic thrombocytopenia with radio-ulnar synostosis at first, which has been distinguishable from other congenital hyporegenerative thrombocytopenic disease or syndromes such as congenital amegakaryocytic thrombocytopenia, Fanconi's anaemia, thrombocytopenia and absent radii (TAR), and is said to be associated with a point mutation in exon2 of HOXA11 gene. Currently, stem cell transplantation is considered to be the only curative approach. We describe the case of an 1-year old girl with amegakaryocytic thrombocytopenia with radio-ulnar synostosis syndrome. *Methods and Results.* The patient was born to parents of Japanese and presented systemic petichiae at birth. Laboratory findings showed with leukocyte count of 17.1×10^9/L with no abnormal cells. The hemoglobin was 12.9 g/dL and the platelet count was 8.0×10^9/L. She had clinodactylies of the fifth digit on both hands and limited pronation and supination of both upper extremities. A bone marrow at 3 days of age showed normocellular marrow with the normal maturation of erythroid and myeloid elements, but the absence of megakaryocytes and radiographic examination revealed bilateral proximal radial-ulnar synostosis (Figure 1). To make sure the diagnosis, DNA sequence analysis was also performed in search of HOXA11 mutation. With those findings, she was diagnosed as having amegakaryocytic thrombocytopenia with radio-ulnar synostosis syndrome. The platelet count remained bellow 1.0 ×10^9/L and pancytopenia progressed gradually at the age of 5-months. At the age of 1-year and 6-months, she underwent allogeneic bone marrow transplantation (allo-BMT) from an HLA-identical unrelated donor. The Conditioning regimen consisted of total lymphoid irradiation, fludarabine, cyclophosphamide and anti-thymocyte globulin. FK506 and short-term methotrexate were used for graft-versus-host disease (GvHD) prophylaxis. The number of infused nucleated cells was 6.9×10^8/kg. Time to achieve a granulocyte count > 0.5×10^9/L and a platelet count > 50×10^9/L was 16 days and 27 days, respectively. One month after BMT, a bone marrow examination showed complete remission with 100% donor type chimerism. During the course, she developed acute GvHD(skin; grade II) and was treated with prednisolone, successfully. A bone marrow examination continues to show complete remission with follow-up of 9 months after allo-BMT. *Conclusions.* Allo-BMT from an HLA-identical unrelated donor is a curative and suitable approach for patients with amegakaryocytic thrombocytopenia with radio-ulnar synostosis, who do not have HLA-identical sibling donors. In our case, the point mutation in HOXA11 gene was not found, which may suggest that there is a co-factor modulating HOXA11 expression and this case is possibly a subtype of this syndrome. Careful clinical course watching is required if another complications or the disease relapse will occur again.

Figure 1. Forearm radiograph shows raio-ulnar synostosis.

0753

A SUSTAINED REMISSION OF IDIOPATHIC THROMBOCYTOPENIC PURPURA AFTER HELICOBACTER PYLORI ERADICATION: A LONG TERM FOLLOW-UP

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Background. Several studies have described increase of the platelet count among patients with chronic idiopathic thrombocytopenic purpura (ITP) following *H. pylori* eradication. However, in most of these studies the median follow-up after eradication was less then a year. *Aims.* A long term monitoring of the platelet count after *H. pylori* eradication in adult *H. pylori*- positive ITP patients is performed in order to evaluate whether the eradication of H. pylori is associated with sustained ITP remission. *Patients and Methods.* 39 *H. pylori*-infected adult ITP pts. (9 male, 30 female; median disease duration 7 years; median age 54 years; mean platelet count 68×10^9/L; 7refractory; median follow-up 36 months) entered the prospective study between February 2002-December 2006. The diagnosis of ITP was made according to the ASH Guidelines. *H. pylori* infection was assessed by C14-urea breath test (UBT) in all patients and in 24/39 was also confirmed by histology of gastric biopsy. All immunosuppressive drugs were withdrawn at least 1 month before examination. 32 H. pylori-positive patients were treated with: clarithromycin 500 mg BD, amoxycillin 1 g BD and pantoprazole 40 mg BD for 7 days. Amoxycillin was replaced with metronidazole 500 mg TDS in penicillin-allergic patients. Eradication of infection was assessed by UBT 2 months after treatment completion and at the end of follow-up. Platelet count was monitored monthly and assessed at 3 and 6 months after the end of treatment, then every 3 month. A complete response (CR) was defined as a platelet count of $>150\times10^9$/L, and a partial response (PR) as a platelet count of $>50\times10^9$/L with an increase of $>30\times10^9$/L with respect to the pretreatment value. The remaining patients were considered with no response (NR). Data were analyzed by t test; percentages were compared by χ^2 test (Fischer exact test for value ″5); a p-value <0.05 was considered statistically significant. *Results.* Successful eradication was confirmed in 26/32 (81.25%) patients. A significant platelet recovery is registered after *H. pylori* eradication: $60.4\pm27.2\times10^9$/L vs. $95.2\pm60.68\times10^9$/L (p=0.006*). Stabile platelet recovery was registered in 7/26 (26.9%) successfully eradicated ITP patients within 6 months following treatment (4CR/3PR). No platelet recovery was registered in either *H. pylori*-positive unsuccessfully eradicated patients (0/6) or H. Pylori-positive untreated patients (0/7). There were no difference in response rates between previously untreated patients, those previously treated with prednisolone, and in patients refractory to corticosteroids (p=0.76). After a median follow-up of 36 months the mean platelet count further increased, although not significantly, up to $107.27\pm83.98\times10^9$/L (p=0.20). At the end of the follow-up 9/26 (34.6%) *H. pylori* eradicated patients showed response (5 CR/4PR). Two patients, initially nonresponders achieved remission: 1 CR and 1 PR. None of the initial responders relapsed. The response duration was 11-50 (median 24) months. *Conclusions.* The long term follow-up confirms the efficacy of *H. pylori* eradication in H. pylori-infected ITP patients. Even nonresponders may achieve sustained remission during the follow-up thus suggesting that autoimmunity against platelets may be reverted after *H. pylori* eradication.

0754

HETEROGENEITY OF TERMINOLOGY AND CLINICAL DEFINITIONS IN ADULT IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) : A CRITICAL APPRAISAL FROM A SYSTEMATIC REVIEW OF THE LITERATURE

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Background. Meta-analyses and systematic reviews, pooling and comparing data from different studies, have the potential to improve the quality of knowledge in many fields of medicine. This opportunity is unfortunately severely hampered in the case of (ITP), where there is an intrinsic weakness in the clinical studies due to the diffuse variability of clinical definitions used by the different authors. *Aims.* Objective assessment of magnitude of this heterogeneity, providing a basis for consensus or harmonization on ITP terminology. *Methods.* A systematic review of the recent (January 2000 to June 2006) literature on ITP in adults was carried out, focusing on the heterogeneity and variability in the terminology and critical definitions relevant for management. The platelet cut-off levels to define ITP, to start treatment and to define type of response; the timing for assessing the response to treatment; the grading of bleeding symptoms; the criteria to define initial, chronic and refractory forms were extracted from the articles for comparison. Major disagreement was considered in case of less than 75% agreement in the different studies. *Results.* A total of 79 papers were considered eligible. Major disagreement was found concerning the platelet count to define ITP; the grading of severity of ITP; the definition of refractory ITP; the platelet threshold to start treatment and to assess the response and the timing for the assessment of response to therapy. There was a general consensus only on the minimum interval time required for a diagnosis of chronic ITP (6 months); the indication to splenectomy (restriction to patients failing first-line therapy); the definition of refractory ITP (restriction to cases needing treatment after failing splenectomy to maintain a safe platelet count). *Conclusions.* A confounding terminology and an unacceptable heterogeneity on clinical definitions relevant to management decisions and outcomes reporting were evident in recent ITP literature. This disagreement makes very difficult to compare different studies and to share data and clinical experiences. Major efforts towards consensus and harmonization in terminology and definitions used in ITP are urgently needed, even more considering the renewed interest in its management, after the introduction of new drugs, like thrombopoietin agonists and anti CD-20 antibodies

0755

THE UTILITY OF AUTOMATED RETICULATED PLATELET COUNT ESTIMATION IN CLINICAL PAEDIATRIC HAEMATOLOGY

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Background. Reticulated Platelets were described in 1969 as the platelet reticulocyte. Residual RNA in reticulated platelets can be detected using various methods and the number of these reticulated platelets has been shown to reflect thrombopoesis. Clinical utility of reticulated platelets has not been demonstrated, partly because of the difficulty of flow cytometric estimation of reticulated platelets. *Aims.* The first aim of this study was to compare the flow cytometer technique and the automated Sysmex XE2100 (upgraded software) for the measurement of these reticulated platelets. Our other aim was to analyse the utility of automated reticulated platelet count estimation in different clinical settings. *Methods.* Two methods of analysis were used. The first was the Sysmex XE2100 (Upgraded software) haematology analyser which quantifies reticulated platelets by a flow cytometric technique in which two fluorescent dyes penetrate the cell membrane and stain the RNA of these immature platelets. The second method of analysis was the Beckman Coulter Flow Cytometer. For this a thiozole orange dye which crosses the platelet membrane and a CD61 PerCP antibody which binds to glycoprotein IIIa on the platelets was used to estimate the reticulated platelets. *Results.* Poor correlation was found between the two methodologies (r squared=0.045). We found that the Sysmex method was very rapid, precise and reproducible whereas the flow cytometry method was an imprecise, slow and a subjective method to estimate reticulated platelets. Notwithstanding we found that the mean reticulated platelet count for Idiopathic Thrombocytopenic Purpura (ITP) patients was raised (n=10, reticulated platelet percentage (RPP)=44.1%) compared with normal's (platelet count between $150\text{-}453\times10^9$/ where n=87, RPP= 4.4%). p<0.0001. We also found the RPP to be significantly raised in inherited thrombocytopenias including Bernard Soulier Disorder (RPp=35.6%) and May-Hegglin (RPp=55%). The RPP was also elevated at diagnosis in Acute Lymphoblastic Leukaemia (RPp=11.8%, p<0.05 that different from normal) but not Acute Myeloid Leukaemia. We analysed the peripheral blood reticulated platelet count in 13 successive children undergoing haemopoietic stem cell transplantation. A clear surge in reticulated platelets was seen in most patients around the time of white cell engraftment. Only in cord blood transplant recipients was this surge poorly defined. To express this surge quantitatively we compared the mean RPP from all patients in the 8 days prior to white cells > 0.5 (n=13, RPp=4.8) with the mean in the 8 days following WBC engraftment (n=13, RPp=8.3, p<0.001). *Conclusions and Summary.* We therefore describe that automated reticulated platelet counting is possible in a clinical haematology laboratory and yields results that are reproducible and meaningful. A high reticulated platelet percentage is not solely diagnostic of ITP and may be found in other conditions including ALL. A rise in reticulated platelets accompanies engraftment in transplant but this finding is probably unlikely to influence clinical practice. It was also noted that patients with low platelet counts who subsequently received a platelet transfusion dropped their RPP which could suggest that platelet transfusions actually suppress platelet production.

0756

HEPARIN-INDUCED THROMBOCYTOPENIA: INCIDENCE, THROMBOTIC COMPLICATIONS AND TREATMENT

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Background. Heparin-induced thrombocytopenia (HIT) occurs in 0,5-5% of heparin-treated patients, is due to anti heparin/PF4 antibodies (HIT Ab) and significantly increases the risk of thrombotic events. HIT related thromboses are reported in 17.5-45% of patients treated with unfractionated heparin (UFH) and 0.6-5.9% of those treated with low molecolar weight heparins (LMWH). Hallmark of HIT is the rapid decline in platelet number (5-7 days) down to less than 50% of basal value. It has recently been published that the presence of HIT even in the absence of thrombocytopenia, raises the risk of thrombotic events; therefore, it has been suggested in these patients to withdraw heparin and start an alternative anticoagulant (AC) strategy even with no evidence of thrombosis. *Aims.* Evaluation of: the incidence of thrombosis in HIT patients, the response to alternative therapy and the role of a additional pro thrombotic risk factors. *Methods.* 53 patients (mean age 67.2±13.9 yrs) with HIT Ab were evaluated, of whom 8 (15%) had received UFH and 45 (85%) LMWH. In the UFH group, 5 (62,5%) received prophylactic doses; in the LMWH group, 28 (62,2%) were on prophylaxis. Heparin was stopped in all patients when HIT Ab were detected. Additional risk factors were: polytrauma (3 patients), diabetes (6), coronary artery disease (15), atrial fibrillation (4), cerebral vasculopathy (4), cardiac valvular prosthesis (5), congenital thrombophilia (5), neoplasia (8). Antiphospholipid antibodies (APA) were assayed in 66% of patients. After heparin withdrawal, 11 patients (23%) were started on: dermatan-sulphate (DS) (Mistral, Mediolanum, Italy), 7 (15%) DS + warfarin, 6 (13%) warfarin alone, 5 (11%) antiplatelet therapy, 1 (2%) defibrotide + warfarin; 15 subjects (32%) received no further anticoagulant treatement. 2 patients died (4%); death was related to thrombosis in 1 case. *Results.* 6 patients (11%) in the LMWH group/prophylaxis developed HIT-associated thrombosis (4 venous and 2 arterial) and 1 of them consequently died, 2 of them had polytrauma, 1 diabetes. No patient with cancer developed thrombosis. 4/35 subjects were positive for APA; none developed thrombosis. In patients with thrombosis, mean platelet fall was 60.2% of basal value; subjects asymptomatic for thrombosis had a mean platelet fall of 53%. *Conclusions.* Thrombotic incidence was 11% in our group. No correlation was found between thrombosis and APA, neoplasia or other risk factors. No patient was treated with direct thrombin inhibitors. Alternative AC therapy, expecially DS, showed efficacy and safety. 32% of patiens received no further AC, without complications. In the light of these results, the need for AC therapy with lepirudin, a direct thrombin inhibitor that is approved worldwide for the treatment of patients with HIT, seems questionable, expecially in those without thrombosis. Risk factors leading to thrombosis in patients with HIT remain to be elucidated.

0757

DETECTION OF SPECIFIC IGG ANTIBODIES IN HEPARIN-INDUCED THROMBOCYTOPENIA TYPE II

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Background. Heparin-induced thrombocytopenia type II (HIT II) is caused by antibodies (ab) against Heparin/platelet factor 4 (HPF4) complex. Commercially available ELISAs detect ab of IgG-, IgA- and IgM-class without differentiation whereas it is assumed that only IgG ab are responsible for typical clinical sequels. *Aims.* This study was performed to compare detection of IgG ab with two different serological assays in patients with surgical and medical diagnoses suspected for HIT II. *Materials and Methods.* Serum samples of 165 patients (64 surgical, 30 male; mean age 65.5 years, range 1.4-90.3; 101 medical. 41 male; mean age 66.3 years, range 2.6-96.6) with clinically suspected HIT II were tested by a gel particle test (ID-PaGIA H/PF4, DiaMed, Cressier, s/Morat, Switzerland) and by ELISA (GTI PF4 HAT45, GTI, Waukesha, WI, USA) using combined anti-IgG/A/M and an anti-IgG conjugate (GTI) only. *Results.* The gel particle test revealed positive reactions in 39/165 patients, 35/39 had positive IgG/A/M ELISA reactions, and IgG ab were detected in 25/35 cases. In the group of patients with negative gel test results (n=126) we found 42 patients positive in the IgG/A/M ELISA and 12/42 had IgG ab. A positive correlation between the gel particle test and detection of IgG ab was found (phi =0.556; $p<0.001$). Additionally, comparing surgical and medical patients, we found a higher correlation of a positive gel particle test with the detection of specific IgG ab in the surgical (phi=0.852; $p<0.001$) than in the medical patients (phi =0.397; $p<0.001$). In surgical patients the gel particle test had a higher sensitivity (62%) to detect patients with positive IgG/A/M ELISA than in the medical patients (39%). Specificity of the gel test was lower in medical (91%) than in surgical patients (100%), as four patients with positive gel test had negative results in the IgG/A/M ELISA. Furthermore, OD values of positive IgG ELISA were higher in samples with positive gel test (n=25; median 1,916; Q1 924 - Q3 2,950) than in those with negative results (n=12; median 621; Q1 527-Q3 1,027; $p<0.002$). *Conclusions.* The use of the gel particle test for screening for clinically relevant HPF4-ab has a low sensitivity in medical patients. Our data show that detection of IgG ab correlates with positive gel test *Results.* Serological testing for HPF4 ab to confirm clinical diagnosis of HIT II depends on using a variety of test systems. There are significant differences in surgical and medical patients concerning the occurrence and detection of relevant IgG ab which demands further prospective studies.

0758

SEVERE BLEEDING IN PATIENTS WITH SEVERE CHRONIC ITP: RESULTS FROM TWO DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED CLINICAL TRIALS

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Background. Idiopathic thrombocytopenic purpura (ITP) is caused by increased platelet clearance and inadequate platelet production. There is a paucity of data on the incidence of severe bleeding in this patient population. *Aims.* To analyze the severe bleeding events in 231 patients with chronic ITP. *Methods.* Two multi-center, double-blind, placebo-controlled, randomized clinical trials including 117 patients with chronic ITP in phase II, and 114 in phase III were analyzed. In both studies, subjects with platelet counts <30,000/μL received different doses of eltrombopag, an oral thrombopoietin-receptor agonist, or placebo for up to 6 weeks and were observed for 6 weeks after discontinuation of study treatment. Bleeding was assessed weekly during treatment and biweekly after discontinuation of study drug using the World Health Organization (WHO) bleeding scale as follows: Grade 0 (no bleeding), Grade 1 (mild), Grade 2 (moderate), Grade 3 (gross), and Grade 4 (debilitating blood loss). In this analysis, *severe bleeding events* were defined as any cerebral bleeding, or any Grade 3 or Grade 4 bleeding event. *Results.* In the 231 patients followed for 12 weeks, 9 severe bleeding events were observed in 7 females and 2 males with a median patient age of 50 years (range: 21-79): 2 cerebral (Grade 2, and Grade *not determined*), 1 subarachnoid (Grade 4), 1 gastrointestinal (Grade 4), 1 gingival hemorrhage (Grade 3), 1 rectal hemorrhage (Grade 3), 1 epistaxis (Grade 3), and 2 menorrhagia (Grade 3). All these events occurred in patients who either did not have a platelet response ≥50,000/μL on eltrombopag, discontinued treatment or were on placebo. The median platelet count at the time of bleeding in these patients was 5,000/μL (range: 2,000-16,000). *Conclusions.* Despite the brief observation period of only 12 weeks, 9/231 patients experienced a total of 9 severe or life-threatening bleeding episodes including 3 intracranial hemorrhages. All episodes occurred in patients when their platelet counts were <16,000/μL. No severe bleeding event occurred in patients who responded to eltrombopag while on treatment. These prospective data confirm that ITP is a serious, potentially life-threatening disease for patients with persistently low platelet counts.

0759

CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA IS NOT BENIGN TO PATIENTS TREATED WITH STANDARD OF CARE: REPORT ON DECREMENT TO HEALTH-RELATED QUALITY OF LIFE

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Background. The Health-Related Quality of Life (HRQoL) burden of Idiopathic Thrombocytopenic Purpura (ITP), with associated symptoms

and treatment, has rarely been systematically assessed or compared with the HRQoL of those living with other chronic disease. To appreciate the impact that new treatment options for ITP may have on HRQoL, it is imperative to understand the relative influence of ITP on patients, even while managed according to current standards of care. *Aims.* To characterize HRQoL in patients with ITP, treated with at least one prior therapy and enrolled in a double-blind, randomized, placebo-controlled, dose-ranging, phase III study of eltrombopag, an oral platelet growth factor, and to compare the impact of ITP on health status and HRQoL with that of common chronic diseases. *Methods.* In this global study of adults with chronic ITP and entry platelet counts <30,000/μL, the secondary endpoints of HRQoL and bleeding were measured at baseline and up to 42 days of dosing. Patient self-report of health status and HRQoL was captured using the Short-Form 36v2. Mean domain scores and component scores were calculated and scaled from 0 to 100, with higher scores indicating a better health status. *Results.* A total of 114 patients were randomized to placebo (n=38) or eltrombopag, 50 mg once daily (n=76): mean age 49.9 years; 61% female; 74% white; and 52% had received ≥3 prior ITP therapies. At baseline, 43% of patients were receiving a stable dose of concomitant ITP therapy, primarily corticosteroids and immunoglobulins. Clinically significant bleeding (WHO Grades 2-4, mild or debilitating blood loss) was observed in 21.5% of patients at screening in placebo and active treatment arms (approximately equally distributed between groups), with median platelet counts of 17,000-19,000/μL. HRQoL data further substantiated this demographic, bleeding and platelet patient profile (Table 1). ITP domain scores were within the range of scores observed with common chronic diseases such as chronic hypertension and chronic obstructive pulmonary disease (COPD); patients with ITP reported less overall health-state disability than those with COPD, but greater negative impact compared with those with chronic hypertension. When compared with population norms for men and women aged 45-54 years, over half of the domains were affected by ITP. The greatest decrements, ≥10 points, were for physical functioning, physical role, general health emotional role and bodily pain. The impact of ITP was less evident for social functioning and mental health. No differences in baseline domain scores were observed between those randomized into active treatment and placebo arms. *Conclusions.* These data suggest substantial, disease-specific HRQOL effects on patients with ITP, which may improve when platelet counts are elevated and stabilized, and bleeding risk is reduced.

Table 1. Comparison of mean (SD) Short-form 36v2 scores between patients with ITP and US general population norms.

	ITP Patients mean score (SD)		US general population mean score (SD)			
Health status domain	Placebo (n=38)	Eltrombopag (n=76)	Hypertension	Type II diabetes	COPD	Age 45-54 years
Physical Functioning	70.4 (30.52)	69.8 (27.48)	73.43 (26.41)	67.69 (28.66)	56.91 (29.14)	84.61 (21.13)
Role-Physical	68.2 (28.99)	68.5 (32.51)	62.01 (39.40)	56.75 (41.72)	34.38 (38.73)	82.65 (33.08)
Bodily Pain	64.3 (29.05)	73.2 (27.79)	72.31 (24.44)	68.52 (26.48)	54.82 (26.14)	73.12 (24.04)
General Health	58.0 (22.24)	57.9 (19.35)	63.3 (19.69)	56.11 (21.12)	45.29 (18.94)	71.76 (19.39)
Vitality	54.4 (23.88)	56.2 (22.85)	58.34 (21.38)	55.73 (21.58)	44.95 (19.55)	61.79 (20.91)
Social Functioning	77.0 (24.74)	76.7 (26.06)	86.70 (20.67)	82.04 (24.96)	71.82 (31.40)	84.07 (21.84)
Role-Emotional	72.7 (23.78)	76.5 (29.42)	79.69 (35.74)	75.60 (36.63)	59.73 (44.61)	83.60 (31.44)
Mental Health	67.8 (19.59)	71.5 (18.68)	77.86 (17.39)	76.74 (18.32)	68.06 (19.68)	75.33 (17.86)

0760

UNCOMPLICATED OUTCOME OF A MASSIVE SINGLE OVERDOSE OF ELTROMBOPAG (SB-497115-GR), A NOVEL ORAL THROMBOPOIETIN RECEPTOR AGONIST, IN A PATIENT WITH CANCER ON CHEMOTHERAPY

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Background. Eltrombopag is an orally bioavailable, selective thrombopoietin receptor agonist that stimulates platelet production by inducing proliferation and differentiation of megakaryocytes. It is well absorbed in the gastrointestinal tract, >99% protein bound in the circulation, has a half-life of about 12 hours, and metabolized in the liver. With daily administration, increased platelet count is apparent after 7'10 days following initiation of treatment with return to baseline 2 weeks after the discontinuation of treatment. *Aims.* To report a single case of an overdose of eltrombopag in a patient receiving carboplatin and paclitaxel (CTx) for ovarian cancer. *Methods.* (Case Report) A 49-year-old woman with ovarian cancer scheduled to start CTx treatment once every 21 days was included in a phase II randomized, double-blind, placebo-controlled trial of eltrombopag treatment for chemotherapy-induced thrombocytopenia. In a suspected attempt to commit suicide, she ingested 5000 mg of eltrombopag, 50 times the assigned daily dose of 100 mg once daily, the day after the first dose of CTx. Serial pharmacokinetics, blood chemistry and platelet counts were measured. *Results.* Initial treatment for the eltrombopag overdose included gastric lavage, intravenous fluids, omeprazole, furosemide, oral calcium, laxatives, one plasmapheresis (12 hours post-overdose) and cessation of eltrombopag treatment. Platelet counts and corresponding plasma concentrations of eltrombopag following overdose are given in the table. The peak plasma level of eltrombopag was 286,480 ng/μL on Day 1; the estimated area under the curve was 1776 μg.hr/mL, ~10-fold that of steady-state values typically observed with a 100 mg once-daily dose. Platelet count peaked at 929,000/μL on Day 14. Liver enzymes measured between Days 2 and 31 peaked at a 2.5-fold increase in alanine aminotransferase activity and a 6-fold increase in aspartate aminotransferase activity, with slight anaemia and granulocytopenia. With the exception of a short episode of bradycardia (30 bpm), which was treated with atropine during the initial treatment for overdose, and mild asthenia, the patient remained asymptomatic and continued on CTx treatment. *Conclusions.* The high exposure to eltrombopag did not produce life-threatening toxicity. Following the ingestion of one single massive dose, an increase in platelet count was seen that followed the same temporal pattern when compared with the standard 50 mg once daily dose.

Table 1.

0761

PLATELET RESPONSE TO THE ORAL PLATELET GROWTH FACTOR ELTROMBOPAG IN A PATIENT WITH MYH9 SYNDROME (MAY-HEGGLIN ANOMALY): A CASE REPORT

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Background. May-Hegglin anomaly and Sebastian, Fechtner and Epstein syndromes are rare autosomal dominant disorders that are characterized by thrombocytopenia and giant platelets resulting from mutations of the MYH9 gene. Patients often have an increased bleeding tendency and require platelet transfusions when faced with a haemostatic challenge. Alport-like features, including nephropathy, cataracts and/or hearing loss, can also be present. *Aims.* To present a case report of platelet response in a woman with MYH9 syndrome treated with eltrombopag. *Methods.* The patient was enrolled in a Phase III eltrombopag study of patients with chronic idiopathic thrombocytopenic purpura (ITP) and platelet counts <30,000/μL. *Results.* Prior ITP treatments for the subject, which included steroids, intravenous immunoglobulin, splenectomy and rituximab, resulted in no satisfactory response. Medical history was notable for hysterectomy secondary to menorrhagia. The subject presented with mild bruising at baseline and was randomly assigned to eltrombopag 50 mg. Platelet counts increased to >200,000/μL within 3 weeks after initiation of eltrombopag. Clinical evidence of bruising disappeared during the period when platelet count was >20,000/μL (day 8). After discontinuing eltrombopag platelet count slowly decreased. During follow-up, it was found that bruising returned when the platelet count was 27,000/μL. Peripheral smears before and following treatment showed the presence of numerous giant platelets. Family medical history revealed that eight paternal family members had a history of macrothrombocytopenia. Therefore, molecular characterization was performed to define the genetic basis of the familial disorder by directly sequencing the MYH9 gene in the patient. Two mutations were identified that predict amino acid changes at R702W (exon 30) and R1933X (exon 40). Although amino acid substitutions at position R702 and truncation at R1933 have been documented, this is the first description of a patient having both mutations and substitution of a tryptophan to replace the arginine at position 702. *Conclusions.* This case report provides scientific rationale for future studies of the oral platelet growth factor eltrombopag in individuals with this inherited form of thrombocytopenia.

Figure 1.

Quality of life, palliative care

0762

PATIENT INFORMATION SHEETS FOR CLINICAL HEAMATOLOGY TRIALS ARE TOO COMPLEX FOR MOST PATIENTS TO UNDERSTAND

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Background. Informed consent is a critical part of clinical research as protection for both the patient and the researcher. For consent to be valid the patient must be aware of the risks, benefits and alternatives to participating in clinical research. This information is provided in the form of a written patient information sheet (PIS) given along with a consent form. Understanding of the written word is based on *reading age*. This is a reflection on the number of school years a patient must have to understand the text they are reading. *Readability scores* are a reflection of reading ease and use sentence length and number of syllables to produce a score. PISs should have a target reading age of 13, and a readability score above 60. *Aim.* The study formally investigated the readability of PISs as an indication of the ability of the PIS to facilitate informed consent. *Methods.* The PISs for all clinical trials that were open for recruitment during November 2006 to haematology patients from the Western Infirmary, Glasgow, were considered for the study. For ease of analysis only those trials with electronic copies of the PIS were included. Readability scores and reading ages were generated for each information sheet based on the established Flesch-Kincaid Calculation using a Microsoft Word function. *Results.* Twelve studies were eligible for consideration. Of the 12 studies, 9 were MRC, 1 BNLI, 1 SNLG, and 1 UKMF. The average reading age was 15.8 (range 14.7-16.7) and the average reading ease score was 51.1 (range 44.6-55.1). *Conclusions and Summary*. All of the PISs were above the recommended reading age (13) and failed to reach the target readability score (>60). These results demonstrate that written information given prior to obtaining consent for clinical trials is too complicated for patients to easily read and understand. This raises important ethical questions in terms of obtaining truly informed consent. We suggest that a) the reading age of the PIS is calculated before submission to the ethics committee, b) that the reading age of the PIS is adjusted to fit the recommended criteria as mentioned above, and c) that the reading age and readability score of the PIS is available to the ethics committee at the time of submission.

0763

EPIDEMIOLOGY, FEATURES AND OUTCOME OF BLEEDING IN PATIENTS WITH ADVANCED HAEMATOLOGICAL MALIGNANCIES FOLLOWED AT HOME: AN ITALIAN SURVEY

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Background. Patients with haematological malignancies in advanced phase of disease, experience a wide range of symptoms including major bleeding, which can heavily afflict the quality of life (QoL) and represent a difficult problem solving in the home care (HC) management. *Aims.* In order to address this issue, a prospective study in a haematological HC setting has been conducted and the results are hereby reported. *Methods.* Regularly symptom assessment and clinical complications, including haemorrhagic episodes, was recorded. Laboratory evaluation, including complete bloods counts, were frequently executed (3687 blood collections performed during 33.698 home care days, 0.11 blood collection/ day / patient). Patients were followed at home until death for a mean period of 72+144 (1-1132) days. Out of 33.698 days of HC, in 7018 (21%), we recorded a platelet count $<20\times10^9$/L (severe thrombocytopenia) occurring in 217 (46%) of 469 patients. Severe thrombocytemyc patients received prophylaxis with prednisone and tranexamic acid; 108 (50%) of them, presented major haemorrhages. No prophylactic platelet transfusion was administered in thrombocytopenic asymptomatic patients or with minor bleeding such as pethechae and ecchimosis. A doctor of our service and a Transfusion Service for the red blood cells (RBC) and platelets supply on continuous duty are available 24 hours daily all year around. In the event of any haemorrhagic complications, patients received treatment with local haemostasis, tranexamic acid and, if necessary and available, platelet concentrates. In case of

uncontrolled massive bleeding affected patients perceived to be close to death and for whom resuscitation is not appropriate, palliative sedation was adopted, if consent was obtained. *Results.* Out of 469 patients, 123 (26%) developed 232 major bleeding episodes. Severe thrombocytopenia, younger age, diagnoses of acute leukaemias, active infections and mucositis, were significantly correlated with the occurrence of bleeding. Out of 232 major bleedings, 210 (91%) occurred in patients with severe thrombocytopenia. This finding occurred most frequently in patients with acute myeloblastyc leukaemia (AML), acute lymphoblastyc leukaemia (ALL) and blastic crises than in those affected by others haemophaties ($p<0.00005$). Between acute leukaemias, the incidence of bleeding was higher in AML than in ALL ($p=0.049$) and in younger patients than in others (median age, years, 50 vs. 72, $p<0.00005$). Bleeding patients received specific treatment (local hemostatic measures, platelet units, hemostatic drugs, RBC packed) with control of bleeding at home in 206 (88%) of cases. Bleeding was fatal in 26 (6%) of 447 died patients (11 brain haemorrhage, 2 hematemesis, 3 hemoptisis, 6 melena), 22 at home and 4 after hospital admission. *Conclusions.* In our experience, a close correlation between the incidence of haemorrhages and the progressively lower platelet count was found, although only one half of severe thrombocytemic patients experienced almost one bleeding episode, which can be effectively manageable at home by a skilled and haematological-based HC team.

0764

EPIDEMIOLOGY, FEATURES AND OUTCOME OF PAIN IN PATIENTS WITH ADVANCED HAEMATOLOGICAL MALIGNANCIES FOLLOWED IN A HOME CARE PROGRAM: AN ITALIAN SURVEY

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Background. Although recent studies have shown a high occurrence of pain in the haematological population and specific pain features and syndromes have been described, no study on this topic concerning patients with blood-related neoplasm in 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 and the results are hereby reported. *Methods.* Each pain syndrome was properly assessed and classified, by the treating physicians. 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); in less reliable patients, only a verbal description scale including four items: *no pain*, *mild pain*, *moderate* and *severe pain*11 was applied. A treatment protocol based on the World Health Organisation (WHO) analgesic ladder was applied in association with causal measures if required. *Results.* There were 258 (55%) males, median age was 67 (4-95) years and the median Karnofsky Performance Status (KPS) was 50 (10-70). They were followed at home for a mean of 72+144 (range: 1-1132) days. At the end study, 433 patients died; of which, 368 (85%) died at home and 65 (15%) after a hospital admission. Out of 469 patients, 244 (52%) experienced a total of 284 pain syndromes, intensity of which was rated from mild to moderate in 31% and from moderate to severe in 69% of them. The causative pain mechanism was diagnosed as follows: 56% deep somatic, 15% superficial somatic, 14% visceral, 8% mixed and 7% neuropathic of the 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 mucositis. Pain management was provided at home. 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, which were associated with a poorer control of pain compared to continuous nociceptive pain states (46% vs. 98%, $p=0.0001$). *Conclusions.* Pain is a relevant problem in patients affected by haematological malignancies in the advanced phase and its management is 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. In our experience, an approach based on the association of causal therapies and analgesics allowed an optimal control of most pain syndromes.

0765

OUTPATIENT MANAGEMENT OF BONE MARROW APLASIA FOLLOWING HIGH DOSE MELPHALAN AND PERIPHERAL BLOOD STEM CELL INFUSION IN PATIENTS WITH MULTIPLE MYELOMA

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High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) represents standard practice for newly diagnosed patients with multiple myeloma (MM). Accordingly, there is a growing demand for ASCT resulting in increased pressure on available hospital beds. The main advantages of at-home ASCT, whenever possible, are the improvement in the quality of life and the reduction in the number of days during which patients need hospitalization, with a consequent reduction of costs, risk of nosocomial infections and more rational utilization of beds. The aim of this study was to confirm the feasibility and safety of performing ASCT on an outpatient basis, according to an early discharge method. A total of 107 MM patients out of 126 consecutively autografts executed in MM patients in PR or CR were selected to receive ASCT on an outpatient basis after treatment with 4 cycles of VAD or thalidomide/dexamethasone depending on the period of observation. The remaining 19 cases were excluded from the program because of poor performance status (n=5), conditioning with BEAM regimen (n=8), geographical distance and/or lack of caregiver (n=6). All selected patients accepted the outpatient-based procedure. The median age was 58 years (range 35-73). Fourty patients (37%) were in CR, while 67 (63%) in PR according to EBMT criteria. We adopted a mixed in-out patient procedure; in particular, after conditioning with high-dose melphalan (200 mg/sqm or 140 mg/sqm for patients aged up to or over 65 years, respectively) and stem cell infusion, patients were programmed to go home and to be rehospitalized in the case of febrile neutropenia or other severe toxicities. All 107 patients were discharged as programmed on day +1, and 83/107 (78%) did spend the aplastic phase entirely at home following high-dose chemotherapy and stem cell infusion. A second hospital admission was required in 24 cases (22%). Febrile neutropenia and severe mucositis needing total parenteral nutrition were the most frequent causes of hospitalization (13 and 9 cases, respectively). However, there were no documented infections and either fever or mucositis was easily resolved at the time of hematopoietic recovery in all patients. Overall, no case of transplant related mortality was recorded. Of note, percent of second admission was of 43% in the initial 30 patients and 14% in the following 77 patients (p:0.002). Finally, comparing this group of patients with a historical group of MM patients autografted in a completely inpatient model, we demonstrated a significant reduction of costs, accounting for more than 40% as well as a remarkable patient satisfaction. We conclude that ASCT on an outpatient basis is feasible and safe in patients with MM and more than 75% of patients are manageable at home, provided that a caregiver is available. A progressive increase in expertise results in a significant reduction of hospitalization.

0766

LATE EFFECTS AMONG CHILDHOOD CANCER SURVIVORS-A SINGLE CENTER EXPERIENCE IN KOREA

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Background. Owing to the recent advances in treatment, nearly 80% of childhood cancer patients become long-term survivors. There are a few report on the health status of childhood cancer survivors in the West. However, there are a limited number of reports on late effects of childhood cancer survivors in the Asian-Pacific region. *Aims.* We tried to determine the frequency and severity of late effects in childhood cancer survivors in Korea, including the survivors who received hematopoietic stem cell transplantation (HSCT). *Methods.* Data were obtained from the patients who were registered at the Long-Term Follow-Up Clinic (LTFUC) of Severance Children's Hospital in Seoul, Korea from July 2005 to January 2007. We included the survivors of cancer who were diagnosed at less than 21 years of age. The survivor-specific follow-up schedule was made according to the individual health risk. Follow-up guideline of the institution was developed based on the Long-term Follow-up Guidelines of Children's Oncology Group (COG) and Practice Statement of United Kingdom Children's Cancer Study Group (UKCCSG). Severity scorings were based on Common Terminology Criteria for Adverse Effects (CTCAE) version 3. *Results.* One hundred eighty-two

survivors were collected. The mean age of the survivors was 12.1±5.6 years. The mean age at diagnosis was 5.4±4.4 years. The mean period between the completion of treatment and enrollment to the study was 4.9±3.9 years. Diagnosis included leukemia (n=78), lymphoma (n=25), brain tumor (n=18) and Wilms tumor (n=18), and others. Among the 182 survivors, 54.4% had at least one late effect and 23.1% had 2 or more side effects; 8.2% had severe late effects (CTCAE grade ≥3). Complication rates of brain tumor and Wilms tumor survivors were 89.9% (16/18) and 27.8% (5/18), respectively (p<0.05). The most common late effect was endocrinologic, i.e., thyroid hormone abnormality, reproductive or sexual function abnormality, or delayed growth. By multivariate analysis, HSCT (OR 3.3; 95% CI 1.3~8.5) and radiation (OR 4.6; 95% CI 2.0~10.6) were independent risk factors associated with late effect. Transplantation, radiation, brain tumor, age at cancer diagnosis, and age at the visit to LTFUC were factors associated with the severity of late effects. Radiation had significant relationship with reproductive or sexual function (p<0.05). Transplantation or brain tumor was related with thyroid hormone abnormality (p<0.05). The number of survivors who had undergone HSCT was 38 (20.8%). The survivors with HSCT had more frequent and severe late effects compared to the survivors without HSCT; 76.3% had at least one late effects, 34.3% have 2 or more late effects; 13.2% have severe late effects. Endocrinologic abnormalities were also the most common late effects in HSCT survivors. *Summary and conclusions.* This is the first report on the late effects among childhood cancer survivors in Korea. We found that HSCT and radiation were independent risk factors of late effects. It is necessary to provide risk-based health care for the high-risk survivors. Since endocrinologic abnormalities are the most commonly encountered late effect, prompt detection and management of such late effects will improve the quality of life of childhood cancer survivors.

0767

QUALITY OF LIFE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS): A REVIEW OF QUESTIONNAIRES ASSESSING DISEASE BURDEN AND TREATMENT IMPACT

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Background. Several new therapies for MDS have been developed in recent years, and the assessment of quality of life (QoL) is becoming increasingly important. Anemia and thrombocytopenia are the most common cytopenias experienced by MDS patients, and common symptoms associated with these conditions include fatigue, dyspnea, bleeding, and bruising, are likely to impact the QoL of MDS patients. *Aims.* To identify patient-reported outcome (PRO) instruments used to assess disease burden and treatment impact. *Methods.* A review of the published literature using MEDLINE, Cochrane, EMBASE, and EconLit databases from 1980-2006 identified articles focusing on QoL and MDS. Hematology and QoL websites were also searched for abstracts presented in 2004 - 2006. *Results.* Of 600 references, relevant studies were selected for detailed review (i.e., assessed QoL in MDS patients, contained original data). A representative summary of the key studies are included below: *FACT-AN (4 studies).* Low risk MDS patients (n=11 and n=30 in 2 studies) who responded to rHuEPO therapy reported greater improvements on the FACT-An compared to non-responders. There was a positive correlation (r=0.247, p=0.025) between hemoglobin levels and FACT-An scores among 53 low/intermediate risk MDS patients receiving Darbepoetin alfa. FACT-An scores improved at 4 and 8 weeks among 133 low-risk MDS patients using epoetin alfa. *EORTC QLQ-C30 (3 studies).* Compared to an age and sex'adjusted control group, 53 MDS patients reported worse fatigue, dyspnea, and physical function (p<0.01). Patients receiving azacitidine (n=99) reported greater improvements in fatigue, dyspnea, and physical function than patients receiving supportive care (n=92) (p<0.05). Patients receiving decitabline plus supportive care (n=89) had better EORTC scores compared to 81 patients receiving supportive care alone (p<0.05). *Functional Living Index- Cancer (2 studies)*. Bone marrow patients (n=89) reported worse functioning vs. stem cell patients (n=282) on the FLIC. *VAS and EQ-5D (2 studies).* VAS scores were lower in 50 MDS patients vs. healthy age and sex-matched controls (61.2 vs 80.0; p=0.05), and EQ-5D scores were lower among transfusion-dependent (TD) MDS patients vs. transfusion free (TF) MDS patients (0.62 vs. 0.85, p<0.001). *SF-36 (1 study)*. 50 TD patients had worse scores than the general US population (n=2,474) and patients with ITP. *Multidimensional Fatigue Inventory (1 study)*. 50 MDS patients had scores ranging from 8.4 (mental fatigue) to 13.6 (physical fatigue) on scales which ranged from 4 to 20 (4=optimal). *Mental Health Inventory (1 study)*. Patients receiving azacitidine (n=99) reported less psychological distress (p=0.015) and higher positive affect (p=0.077) vs supportive care patients (n=92). *QOL-E (1study)*. TD patients (n=13) had worse scores compared to TF patients (n=27) on the physical (p=0.02), social (p=0.03), and MDS-specific domains (p=0.02). *Summary and Conclusions*. MDS adversely affects QoL in patients compared to the general population and other chronic diseases (including patients with diabetes and ITP). Current MDS treatments generally resulted in less fatigue, improved physical functioning, and less psychological distress. Transfusion dependency also adversely impacts QoL; however, the specific impact of bleeding on QoL has not been assessed.

0768

ANALYSIS OF QUALITY OF LIFE RESPONSES BY EFFICACY RESPONSE STATUS IN CANCER PATIENTS WITH CHEMOTHERAPY-INDUCED ANAEMIA WHO RECEIVED DARBEPOETIN ALFA 500 MCG EVERY 3 WEEKS AND IV IRON

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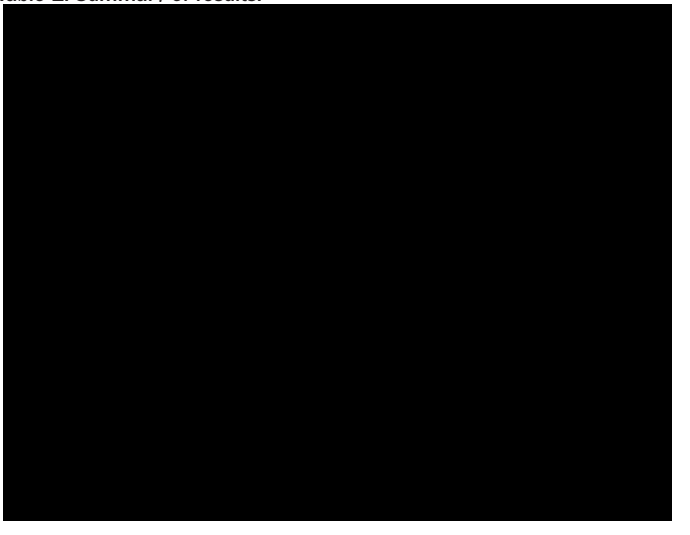
Background. Intravenous (IV) iron supplementation in cancer patients with chemotherapy-induced anaemia (CIA) receiving erythropoiesis-stimulating agents (ESAs) may help optimize response to ESAs. *Aims.* Examine quality of life (QOL) outcomes based on efficacy response status in an exploratory analysis from a phase IIIb, randomized, open-label study in patients with CIA who received darbepoetin alfa (DA) 500 mcg every 3 weeks (Q3W) and who received either IV iron or oral iron/no iron. *Methods.* Eligible patients had a non-myeloid malignancy and CIA (baseline haemoglobin <11 g/dL) and had provided informed consent. Patients were randomly allocated (1:1) to receive either DA plus 200mg IV iron (200 mg Q3W with DA Q3W or two 100 mcg doses within 3 weeks) or DA plus oral iron/no iron, with stratification by tumour type (lung/gynaecological or other) and baseline haemoglobin category (<10 or ≥10 g/dL). Change in FACT-Fatigue from baseline to the end of the treatment period (EOTP) was assessed based on whether a patient did or did not respond to treatment with DA. The analysis was performed separately for 3 different definitions of an efficacy responder: haematopoietic responder (haemoglobin ≥12 g/dL or a ≥ 2-g/dL increase in haemoglobin from baseline), haemoglobin responder (achievement of haemoglobin of ≥11 g/dL), and transfusion responder (no RBC transfusions required between week 5 and EOTP). An ANCOVA model was fitted including baseline FACT-Fatigue as a continuous covariate and factors of haemoglobin at randomisation (<10, ≥10 g/dL), tumour type (lung/gynaecological, other), treatment group (IV iron vs oral/no iron), response (responder, non-responder), and treatment group by responder interaction. From this model, least squares (LS) means (and associated standard errors [SE]) were calculated for each combination of treatment and responder/non-responder.

Table 1. Summary of results.



Results. A total of 396 patients were randomized and received ≥1 dose of DA (IV iron arm =200; oral iron/no iron arm = 196). Patients had a mean (SD) age of 61.0 (11.5) years; most (61%) were women. As presented elsewhere, at the end of the study, significantly more patients receiving IV iron and DA exhibited a haematopoietic response than did those receiving oral/no iron and DA (Kaplan-Meier estimates [95%CI], 86% [79-92] vs 73% [66-80], respectively; difference, 13% [3-23], p=0.011). The LS mean (SE) changes from baseline to EOTP in FACT-F score were 2.4 (0.79) and 2.17 (0.77), respectively, for the IV iron and oral/no-iron arms. Changes in FACT-F score from baseline to EOTP by efficacy responder status are presented in the Table 1. *Summary and conclusions* In this study, within each treatment arm, QOL improvements were better for patients who demonstrate an efficacy response than those who do not. Most groups of efficacy responders exhibited an adjusted mean change in FACT-F score that was close to or exceeded the 3-point threshold identified as clinically significant (Cella *et al.,* J Pain Symptom Manage 2002;24(6): 547-61). These data provide further evidence, suggesting a link between quality of life improvements and clinical responses.

0769

QUALITY OF LIFE ASSESSMENT IN HAEMOPHILIC PATIENTS WITH INHIBITORS: RESULTS OF THE COHIBA STUDY

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Background. Haemophilia with inhibitors is a rare condition, with a prevalence of 1 of 100,000 inhabitants. The development of inhibitors requires a substantial amount of economical and human resources and represents one of the most challenging complications of haemophilia treatment in terms of life-threatening bleeding, severe arthropathy and physical disability. Quality of life and self-management abilities of patients are affected by this complication. Health-Related Quality-of-Life (HRQoL) and treatment satisfaction are so-called patient-rated outcomes (PROs), which provide an opportunity for patients to report their own experience of functioning, well-being and treatment. In the frame of the Italian COHIBA Study haemophilia patients with inhibitors were asked about their treatment preferences, HRQoL and treatment satisfaction. *Aims.* Description of HRQoL and treatment satisfaction in adult and paediatric haemophilic patients with inhibitors across age groups and clinical conditions. *Methods.* HRQoL and treatment satisfaction was assessed in 30 patients from 9 Italian Haemophilia Centers. HRQoL was evaluated with the Core Instrument of the haemophilia-specific Haem-A-QoL consisting of 25 items (allowing the comparison between children and adults), treatment satisfaction was assessed with the Hemo-Sat questionnaire (for adults and parents of haemophilic children) consisting of 34 items pertaining to 6 dimensions (ease & convenience, efficacy, burden, specialist/nurses, centre/hospital, general satisfaction). *Results.* In total 23 adult and 7 paediatric inhibitor patients, severely affected by haemophilia A, were enrolled in the COHIBA Study. 56% received on-demand treatment, 22% prophylaxis and 22% ITI. The median age in adults was 40 years (17-60), in children 10 years (4-16). Patients as well as parents of haemophilic children were mainly unsatisfied with haemophilia treatment in terms of *efficacy* (MA=56.25, SDA=18.7; Mp=72.22, SD p=11.7) and *ease* (MA=53.38, SDA=10.42; Mp=61.21, SDp=8.6), even though parents seemed to be more unsatisfied than adult patients. Adult patients showed main impairments in their HRQoL in the dimensions *sport, physical health* and *future*; most of them (87%) had to refrain *often* or *always* from sports like soccer, pain in the joints was reported *often* or *always* by 65% and concerning their *future* 43% thought *seldom* or *never* that things get better in the future. Whereas children were less impaired in their HRQoL than adult patients; areas of main impairments were *sport* (100% could *seldom* or *never* do as much sports as others), *dealing* (33% were *seldom* or *never* able to tell whether they were bleeding) and *view* (33% found *seldom* or *never* that their life was more difficult because of haemophilia). *Conclusions.* Adult haemophilic patients were more impaired in their HRQoL than paediatric patients, even though both were mainly limited in sports activities. Assessment of PROs is important to understand specific problems of haemophilic patients and to improve their individual treatment.

0770

EFFECTIVENESS OF EPOETIN B 30,000 UI ONCE WEEKLY FOR TREATMENT OF ANEMIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES TREATED WITH CHEMOTHERAPY

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Background. Anemia is the most frequent hematological complication of chemotherapy in cancer patients with a profound impact on their quality of life. Furthermore, hematological malignancies (HM) by themselves are frequently a cause of anemia. Epoetin β (E) is an effective treatment of chemotherapy-induced anemia in patients (pts) with non myeloid HM. *Aims.* To evaluate the efficacy and safety of E 30 000 IU once weekly (QW) in pts with non-myeloid HM and more specifically in subgroups of lymphomas (L) and multiple myelomas (MM). *Methods.* This was a multicenter, single-arm trial. Eligibility criteria were: informed consent, age ≥18 yrs, WHO performance status 0-2, malignant non-myeloid malignancies, on-going chemotherapy and anemia: hemoglobin (Hb) <12 g/dL. E 30 000 IU QW was administered SC for up to 16 weeks. Follow-up visits were scheduled after each chemotherapy cycle. Primary endpoint was Hb response defined as an Hb increase of ≥2 g/dL (whatever the baseline Hb level) and/or an achievement of Hb level of ≥12 g/dL or 13 g/dL (pts with Hb levels <11 g/dL or ≥11 g/dL at baseline respectively). Delay of Hb response, transfusion requirement, mood and cognitive functions were also assessed. Here we focus specifically on the subgroup of pts with HM. *Results.* In total, 326 pts with HM were enrolled including 191 L, 92 MM and 43 other HM. 53% of pts were male and 48% were above 70 years old. Median Hb level at baseline was 10.0 g/dL [95IC 9.8 10.0]. At the 12th week (W12), median Hb level increased to 12.8 g/dL [95IC 11.9-12.7] in all HM pts and was quite similar with regard to the tumor type (12.8 g/dL in L pts, 12.7 g/dL in MM pts) or the Hb level at inclusion (12.5 g/dL, 12,7 g/dL, 13 g/dL in pts with baseline Hb level < 9.5 g/dL, between 9.5 g/dL and 11 g/dL or ″11 g/dL respectively) in MM. Hb response rate was: 60% (IC95: 55-66%) in all HM, 63% (IC95: 56-70%) in male, 57% (IC95: 49-65%) in female, 56% (IC95: 49-64%) in pts < 70 years, 65% (IC95: 57-72%) in pts > 70 years, 59% (IC95: 52-66%) in L pts and 67% (IC95: 58-77%) in MM pts. E treatment was well tolerated. Thromboembolic events occurred in 6,7% of pts, a rate consistent with information provided in the current label for E. *Conclusions.* Epoetin β 30 000 IU once weekly is effective and well tolerated in anemic pts with HM chemotherapy. Hb level achieved is quite similar with regard to the tumor type (MM or L) or the baseline Hb level.

0771

ASSESSMENT OF HEALTH-RELATED QUALITY OF LIFE AND PHYSICAL PERFORMANCE IN ADULT PATIENTS WITH HAEMOPHILIA ATTENDING A SPORTS THERAPY PROGRAMME (HEP)

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Background. Sport activities are considered beneficial for patients with haemophilia in terms of physical aspects, such as possible protection of joints and prevention of bleeds and deformity, and non-physical aspects, such as quality of life, socialisation and self-esteem. Patients' report of their own experience of well-being and functioning becomes more and more important; therefore subjective evaluation should be combined with objective measurements. In the frame of the Haemophilia & Exercise Project (HEP) health-related quality of life (HRQoL) and subjective physical performance were evaluated together with objective measures such as orthopaedic joint score and EMG in haemophilia patients Germany. *Aims.* Assessment of HRQoL and subjective physical performance in adult patients with haemophilia attending the HEP sport camp. *Methods.* HEP is a sport camp, where adult haemophilia patients are trained twice a year. Participants were tested both objectively and subjectively concerning their physical performance, evaluated with the newly developed performance-specific HEP-Test-Q consisting of 25 items pertaining to 4 dimensions (mobility, strength & coordination, endurance, body perception), as well as regarding their HRQoL assessed with the generic SF-36 and the haemophilia-specific Haem-A-QoL questionnaire consisting of 46 items pertaining to 10 dimensions. *Results.* In total 33 haemophilia patients were enrolled in the HEP with a median age of 45 years (19-65). Almost all patients had haemophilia A (90.9%) and were severely affected by haemophilia (87.9%), 9.1% had inhibitors, 65.6% had chronic hepatitis C and 12.1% had HIV infection. Patients reported in average 6 bleeds in the previous 12 months (0-24) and 42.4% had target joints; 45.5% of all patients were on prophylaxis. HRQoL of these patients was significantly impaired in physical dimensions of the SF-36, as well as for *emotional role functioning* compared to the general population. In the haemophilia-specific Haem-A-QoL impairments were mainly found in the dimensions *sport & leisure*, *future* and *physical health*, 57.6% of patients reported that they *couldn't do as much sports as others*, 51.5% suffered *often/always* from *pain in joints*. In the performance-specific HEP-Test-Q patients showed good values for *mobility*, but high impairments in *coordination* and *endurance*; 63.7% reported *often/always* problems in *walking down stairs*, 63.6% could *never/seldom do exhausting activities*. With the HEP-Test-Q 52% of the variance of the physical component of SF-36 could be explained, 34% of the mental component and 64% of the total score of Haem-A-QoL. *Conclusions.* Since most impairments in HRQoL were found in physical domains, especially in the dimension *sport & leisure* the additional assessment of subjective physical performance can be helpful in understanding specific problems of haemophilia patients, which can be measured by the newly developed HEP-Test-Q. It makes sense to combine objective assessments of physical performance with such subjective instruments in order to reveal aspects, which can not be measured yet objectively such as body perception. Since up to 64% of the variance of HRQoL could be explained by subjective physical performance it is important that haemophilia patients attend a supervised sport training which might improve physical status and body perception and consequently has a positive impact on quality of life.

0772

THE ROLE OF SERUM CYTOKINES ON THE DEVELOPMENT OF FATIGUE DURING ALLOGENEIC STEM CELL TRANSPLANTATION

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Purpose of the study. To prospectively study the role of serum pro-inflammatory cytokines in the development of multiple symptoms in patients during the acute phase of allogeneic hematopoietic stem cell transplantation (allo-HSCT). *Methods.* Thirty patients with acute myelogenous leukemia or myelodysplastic syndrome reported symptoms using the M. D. Anderson Symptom Inventory approximately weekly during the first 100 days of allo- HSCT. Serum was collected at multiple time points during the study and assayed for a panel of inflammatory cytokines (interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-12p40p70), IL-1 receptor antagonist (IL-1RA), and soluble receptor 1 of tumor necrosis factor (sTNFRI). Random effects modeling was used to analyze the longitudinal measures. *Results.* Over the first 100 days following allo-HSCT, the most severe patient-reported symptom was fatigue, followed by poor appetite, pain, drowsiness, dry mouth, and disturbed sleep. Fatigue increased rapi y and reached their highest severity levels at Day +11 of HSCT (3 days after nadir) and remained as the most severe symptom in 100 days.

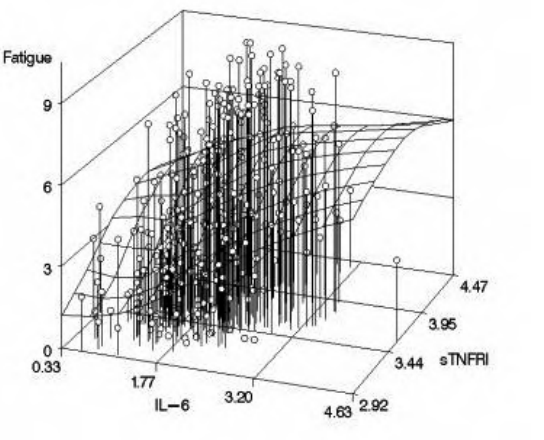

Figure 1.

Serum IL-6, IL-8, and sTNF-RI increased significantly from baseline to nadir. Controlled for age, gender, race, disease status, infusion cell service, conditioning regiment, and infusion dose of cd34 in the mixed effects model, the changes serum IL-6 and sTNFRI levels from baseline to 100 days were positively associated with fatigue severity during the first 100 days of allo-HSCT ($p<0.01$, see Figure 1), with equation of Fatigue=-10.666+1.65*race+1.02 * log10 (IL-6)+2.6 * log10 (sTNFRI). *Conclusions.* The changes in the serum levels of primary inflammatory cytokines was associated with the development of cancer-related fatigue, the most severe nonspecific symptoms in the acute phase of allo-HSCT.

0773

ONLY MINOR IMPAIRMENT OF HEALTH RELATED QUALITY OF LIFE IN ADULT LONG-TERM SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Compared to survivors of childhood leukemia, only little is known about late effects and HRQL of adult patients with Acute Lymphoblastic Leukemia (ALL). With the improvement of survival-rates up to 40% in the last 10 years, the status of long-term survivors of adult ALL is of increasing interest. The German Multicenter Study Group for Adult ALL (GMALL) has conducted 7 consecutive prospective studies for de-novo ALL since 1981. All patients received intensive chemotherapy (CT) with or without stem cell transplantation (SCT). This is the first interim-analysis of Health Related Quality of Life (HRQL) in long-term survivors of GMALL studies 02/84-06/99 *Methods.* A questionnaire with 191 questions was sent out to patients of 5 consecutive studies, alive at least 5 years after diagnosis. HRQL was evaluated with the EORTC-QLQ-

C30 questionnaire, and the correspondent Q-Leu Module. In addition, questions concerning health-status, fertility-issues, social-family-life and working-conditions were enclosed. Results were described and if possible compared to the German normal population. *Results.* For this interim analysis, questionnaires from 152 patients were evaluable. Median age was 40 years (21-70). 64% of the replying patients were male. Median-time after diagnosis was 10 years. EORTC functional scales - esp. cognitive and social function - were slightly reduced, compared to the German normal population, but overall QOL was even better (Figure 1).

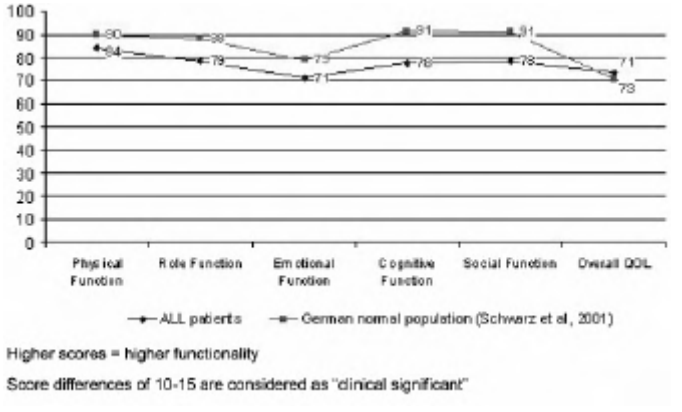

Figure 1. EORTC QLQ C30 Functional Scales.

EORTC-symptom scales showed that fatigue and low physical-functioning increased particularly in elderly patients. Gender-differences concerned pain and insomnia, whereas women were less impaired than men. No significant differences were found between patients undergoing SCTvsCT. Women underwent significantly more often joint-surgery due to osteonecrosis (25v s 11%). Men significantly more often reported hypertension (28 vs 9%), bowel- (15%vs6%), liver- (15 vs 6%) and kidney-diseases (9 vs 2%). The most frequently reported health-problems were back-pain (37%), allergies (24%) and hypertension (20%). Amazingly, the reported QOL did not correlate negatively with obvious health-problems. 85% of the patients estimated their own activity just as well as before their disease (ECOG 0 or 1) and 83% estimated their chance to stay healthy as *very-good* or *good*. Most patients reported closer relationship to their friends and family after the disease. Reduced mental-capacity, loss of concentration and limited physical-function were major self-reported complaints. Fertility after therapy is a major concern of patients, but preservation opportunities were offered only in 23% of men and in none of the women. However, more than half of all patients with desire to have children could realise this wish after therapy. 66% of the patients who were employed prior to their disease also worked afterwards part- or fulltime. *Conclusions.* Overall HRQL of patients 10-years after ALL is only slightly impaired compared to the normal population. Major differences concerned cognitive-, and social-functioning. HRQL of SCT-patients did not differ compared to CT-patients. Although fertility appeared to be preserved in over half of the patients, sperm-cryopreservation should be offered to all men before treatment. High QOL-scores, also in patients with health problems, suggest that coping-strategies play an important role. Therefore evaluation of QOL at several time-points (before, during and after therapy) is preferable to optimize supportive-care and to improve psycho-social support.

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Red cells, iron and hemolysis

0774

TEN-YEAR EXPERIENCE WITH PARTIAL SPLENECTOMY (PSX) FOR HEREDITARY SPHEROCYTOSIS (HS) IN CHILDREN

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Background. Though effective in the management of HS, total splenectomy (TSx) carries a life-long risk of overwhelming post-splenectomy infection (OPSI) despite preventive vaccination & penicillin prophylaxis. PSx may be quite effective while avoiding OPSI. *Aims.* To review own experience with PSx for HS in children. *Methods.* HS was classified as mild, moderate, or severe (T. Cynober). Upon informed consent, pts with moderate/severe disease were immunized against encapsulated bacteria. PSx (G. Tchernia), with cholecystectomy (Cx) if needed, was performed 1-6 wk later. Antibiotic therapy was given prn and revaccination recommended after 3-5 yr. The increase in mean Hb level & decrease in mean absolute reticulocyte count (ARC) were assessed in intent-to-treat & as-treated analysis. Moreover, the mean changes in Hb level & ARC were compared between successful PSx (9) and factual TSx (5). Comparisons were made by t-test, with $p<0.05$ indicating significance. One pt was excluded from analysis because of short follow-up (FU). Morbidity, quality of life, activity, & academic performance were systematically documented. *Results.* Between 02/96 and 02/07, 14 children (M:F=8:6) with moderate or severe HS underwent laparotomy with the intent of PSx with or w/o Cx. Their median age at operation was 7.6 yr (range, 2.4-18.4; 11 pts >5 yr). PSx was indicated for anemia (7), cholecystolithiasis (3), or both (4). Another pt underwent elective TSx aged 6.7 yr. PSx was not feasible in 1 pt due to a huge spleen (1176 mL) with unclear and complex vasculature, necessitating conversion to TSx. One girl developed hemoperitoneum (300 mL) caused by injury of the retained vascular pedicle and massive pleural effusion, requiring removal of the remnant and management at PICU. 80 & 100 mL of blood were drained in 2 cases. In 3 pts, 3 viral and 2 bacterial infections occurred. The median hospital stay was 8 d (range, 5-13). Two pts lost splenic remnant in 2 & 18 months. Regrowth and/or activity of the remnant were demonstrated post-PSx in the remaining pts, however w/o deleterious effects on blood counts. In intent-to-treat analysis, the mean Hb increased from 99.4 to 137.8 g/L, while the mean ARC decreased from 346,700 to 136,500/μL (p=0.000001 for both). In as-treated analysis, the mean Hb increased by 32 g/L (p=0.00002), and mean ARC decreased by 182,300/ĪL (p=0.0001) post-PSx. However, PSx elicited less spectacular changes than TSx: -Hb 32 vs 50.2 g/L (p=0.01) & -ARC 182,300 vs 309,000/μL (p=0.02). Nine pts were FU a median of 5.11 yr post-PSx (range, 4.10-10.5) for 54.9 pt.yr overall. 43 febrile episodes, mostly viral upper RTI, were encountered, of which 2 required hospitalization. Nevertheless, antibiotics, most commonly broad-spectrum oral penicillin, were given in 23 cases. Except for 1 boy with IBD developing >3 yr post-PSx & <2 yr post-Cx, all children managed perfectly, enjoyed normal physical activity & performed well. *Conclusions.* PSx combined with other measures appears to offer a reasonable alternative to TSx in children with HS. However, more pts & longer FU are needed to fully appreciate its true role in the management of this disorder.

0775

INTERIM REPORT FROM THE UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME FOR GENERAL HAEMATOLOGY (UK NEQAS (H)) ON A WEB PILOT SCHEME FOR DIGITAL MORPHOLOGY

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Background. UK NEQAS (H) collaborated with Manchester Royal Infirmary and Greater Manchester Universities to develop a Web based pilot scheme for Digital Morphology(DM). The DM scheme was registered with the Institute of Biomedical Science to enable laboratory Biomedical Scientists to collect evidence and points towards Continuing Professional Development (CPD). *Aims.* To promote quality standards and improve consensus of morphology by education rather than individual assessment. Process: In April 2005 UK NEQAS (H) invited participating

centres of the conventional glass slide Morphology scheme to submit a named individual as a CPD registrant for the DM scheme. In April 2006 the number of registrants was increased from 221 to 412 individuals from 14 countries (85% are UK based). For each exercise two Web based morphology cases were released (four releases per year, fourteen cases to date). Each case consisted of multiple digital images from smears previously released as glass slide morphology assessment surveys. Revised coded comment report sheets and reflective feedback forms were placed on the Web (www.ukneqas-haem.org.uk) One CPD point was awarded per completed case. *Results.* On average 51% of registrants completed the exercises (range 43%-69%). The majority of participants (72%) spent <30 minutes reviewing each case but additional time on background reading. The cases included Haemoglobinopathies, Disseminated Intravascular Coagulation and both chronic and acute leukaemias. Of those who gave additional feedback >70% stated the exercises had improved their awareness of the haematological conditions whereas <20% said their knowledge had not changed (variation in improvement depended upon clinical diagnosis). General comments from 28% were used to develop the scheme format; optical magnifications now appear with the images and presentation of clinical data has been streamlined. With reference to education aspects participant feedback was positive, many commented that their overall knowledge of a condition improved, as cases were presented with relevant additional data (cell markers, cytogenetics, immunochemistry) and expert opinion highlighting the significance of specific morphological features e.g. appearance of granulation or nuclear structure. Participants stressed the usefulness of images for teaching and education purposes, particularly for rare haematological cases seen less frequently in some smaller laboratories and for bone marrows. *Development.* Additionally the collaboration has blended (or stitched) sequential high power (x60 objective) quality images to create larger composite images (virtual slides) which were viewed using appropriate software. This allows the user to move across images creating the feel of a microscope whilst maintaining high resolution. Stitched images ensure all users see exactly the same cells and enable the scheme to present rare cases that have insufficient material for the conventional glass slide survey. *Summary.* Future development by UK NEQAS (H) and the collaboration, include the introduction of electronic reporting for participants and improved access to viewing software allowing larger composite images. The Digital Morphology scheme is currently aimed at educating individuals. With the key theme of personal professional development to promote improvement to the quality of haematological morphology the scheme has the potential for further expansion across the UK and internationally. Further information can also be found at www.manlab.co.uk

0776

NEUTROPHIL ACTIVATION MARKERS IN CHRONIC RENAL FAILURE PATIENTS UNDER HAEMODIALYSIS AND RECOMBINANT HUMAN ERYTHROPOIETIN

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The interaction of blood with nonbiological materials of the extracorporeal circuit during hemodialysis, leads to the activation of several noncellular and cellular systems, including polymorphonuclear leukocytes (PMN). PMN activation leads to desgranulation and release of proteases and of oxygen free radicals, and may be associated with resistance to recombinant human erythropoietin (rhEPO) therapy. The aim of this work was to evaluate the neutrophil activation state in chronic renal failure (CRF) patients under haemodialysis, and its linkage with resistance to rhEPO therapy, by measuring circulating levels of elastase and lactoferrin. These two substances are contained in primary and secondary neutrophil granules, respectively, and are frequently used as indirect markers of neutrophil activation *in vivo*. We studied 50 CRF patients (32 males, 18 females; mean age 64.5±15.4), 25 responders and 25 non-responders to rhEPO therapy. CRF patients were dialysed three times per week for 3 to 5 h, for a median period of time of 36 months. All patients used the high-flux polysulfone FX-class dialysers of Fresenius, 25 with FX60, 23 with FX80 and 2 with Fx100 dialyser type. Twenty-five individuals were included in a control group, age and gender-matched with CRF patients. Total leukocyte count was measured using an automatic counter (Sysmex K1000, Hamburg, Germany) and leukocyte differential counts were evaluated in Wright-stained blood films. Plasma levels of elastase and lactoferrin were evaluated by enzyme immunoassays (human PMN Elastase ELISA, Bender MedSystems; Lactoferrin ELISA Kit, Calbiochem, respectively). Compared with controls, CRF patients presented with significantly higher neutrophil counts and elastase levels ($p<0.05$). No significant differences were observed for lactoferrin and for total leukocyte count. Elastase per neutrophil and lactoferrin per neutrophil presented similar values for controls and CRF patients. No statistically significant differences were found between responders and non responders to rhEPO therapy concerning total and differential leukocyte counts, as well as elastase and lactoferrin plasma levels. The higher neutrophil counts and elastase plasma levels in CRF patients seem to reflect the undergoing inflammatory process. The rise in those values may result from the mobilization of the marginal pool of neutrophils or of an increased neutrophil production to face the chronic inflammatory process associated with the disease and/or with the regular haemodialysis process. Actually, no differences were observed between responders and non-responders to rhEPO therapy.

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0777

NON-INVASIVE COLOR VISUALIZATION OF BLOOD CELLS

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Background. Our understanding of biological organization of a live matter and its cellular process we can mainly get from microscopy. But visualization of a biological structures is based on interaction of electrons and photons with sample what leads to destruction of a sample. In some cases to get color contrast image of separate elements of biological sample need to use a variety of dyes and fluorescent substances but it leads to artificial staining of sample, destructive modification and loss very important structural information its native structure. For instance to get color image of morphological structure of blood smears are usually using staining of living blood smears by dyes. But this method leads to disruption of sample caused by the specimen preparation and viewing conditions.[1] Other ways of generating color image contrast is based on visualization of phase gradients within unstaining specimens, as realized by phase contrast[2] and differential interference contrast.[3] Usually in medical practice conventional bright-field microscopy let us see black and white image of separate morphological elements of blood smears only (Figure 1).

Figure 1. Red blood cells in native color under optical microscopy.

Aims. In present paper we would like to offer the new nondestructive method of optical microscopy capable of examining the structures of living cells in their natural colors without staining them, using a specially designed substrate for deposition of biological sample and observing native structure in reflected light. *Methods.* Offered approach based on physical phenomena of white light interference reflected from sample surface and special supporter on which this sample is deposited. As distinct from phase contrast[2] or differential interference contrast[3] microscopy there we have interference picture not for passed through sample and transparence object-plate two light rays but for two reflected light rays on the sample surface and substrate respectively. It allows to occur at the image plane converting previously invisible gradients of refractive index within the specimen in to intensity gradients in the image. Color interference contrast image is achieved due to special condition of experiment is connected with chose of angle of incidental light,

wave length of light of reflected ray, chemical composition of sample, thickness of sample, refractive index of sample, refractive index of substrate, chemical composition of substrate. The setup for color reflected interference microscopy was centered around ordinary optical microscope (Carl Zeiss, Germany) equipped for digital photo camera (Sony) and substrate which serve as object-plate for sample and as source of coherent light for scattering on morphological structures of sample. Light from a 100 watt xenon source was directed on to the specimen. Microscopic images were obtained with Zeiss lens and digital camera and recorded on a personal computer using commercially available software. *Results.* To demonstrate the potential usefulness of this method, we provide qualitative data describing color image of healthy and pathological damaged cells for alive and dry blood smears. Comparison Figure 1A and Figure 1B for same samples but obtained by conventional bright-field microscopy and by using new method correspondently showed distinguishing in color not only separate red blood cells but distinguishing difference parts in area separate erythrocytes too. Usually for healthily individuals a albuminous aureole around the erythrocytes are mainly white-yellow but for cancer cells (core rectal cancer) the aureole color is quite different and reflects significant changes in chemical composition of both internal, and external contents of erythrocytes (Figure 1D, 1E). Easy detection of organic shells around blood cells in our case is evident. Operations by fixing, smear coloring, prolonged processing, the availability for phase-contrast or interference microscope, special illuminators, radiating the exciting short-wave light beams are not required. Interferometric coloring of blood elements occurs on a surface of specially selected substrate. Corresponding colored images of blood elements are formed due to interference phenomena occurring under interaction of light beams reflected from front and back surfaces of blood elements, smeared on a substrate. As it is seen from the given micro photos, the character of the colored image is the same, as though they were investigated with phase-contrast or interference universal microscopes.

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0778

ANALYSIS OF SLC40A1 (FERROPORTIN 1) GENE AT MRNA LEVEL REVEALS RAPIDLY THE CAUSATIVE MUTATIONS IN PATIENTS WITH HEREDITARY HEMOCHROMATOSIS TYPE IV

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Background. Mutations in the SLC40A1 (ferroportin 1) gene result in a dominant genetic disorder [ferroportin disease; hereditary hemochromatosis type (HH) IV], characterized by iron overload. In all previous studies, the mutational analysis of SLC40A1 gene has been performed at genomic DNA level by PCR amplification and direct sequencing of all coding regions and flanking intron-exon boundaries (usually in 9 PCR reactions). The aim of this study was to analyze the SLC40A1 gene at the mRNA level, for the rapid detection of ferroportin 1 gene alterations. *Methods.* Two female patients displayed hyperferritinemia, normal transferrin saturation and iron accumulation predominantly in macrophages and Kupffer cells (typical ferroportin disease phenotype). mRNA was extracted from PBMCs by standard protocol. Afterwards, the entire coding sequence of the SLC40A1 gene was amplified in only two RT-PCR reactions, following by direct sequencing or/and NIRCA (non-isotopic RNase cleavage assay). *Results.* RT-PCR-sequencing analysis showed that one patient displayed the previously described alteration V162∅ and the other one the novel mutation R178G. NIRCA analysis demonstrated the results of sequencing analysis in both cases, confirming that it could be used as a first screening (but not necessary) step for the detection of SLC40A1 gene alterations. *Conclusions.* This protocol turned out to be rapid, sensitive and reliable, facilitating the detection of SLC40A1 gene mutations in patients with hereditary hemochromatosis type IV. The broad application of this procedure may facilitate the rapid molecular analysis of SLC40A1 gene contributing to the understanding of the Fpn disease pathogenesis.

0779

MOLECULAR CHARACTERIZATION OF A NEW LONG DELETION IN THE FERROCHELATASE GENE

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Background. Erythropoietic protoporphyria (EPP, MIM 177000) is an autosomal dominant disease with incomplete penetrance, due to reduced activity of ferrochelatase (FECH; EC 4.99.1.1). The enzyme is located in the inner mitochondrial membrane and catalyzes the chelation of ferrous iron into protoporphyrin IX, the final step in the heme biosynthetic pathway. Clinical manifestations have a childhood onset and include skin photosensitivity and mild anaemia. The human ferrochelatase gene (FECH) maps to chromosome 18q21.3; it spans 45kb with a total of 11 exons encoding for a precusor of 423 amino acid residues, the first 62 of which are the putative mitochondrial leader sequence. So far molecular analysis of FECH gene has allowed the identification of more than 100 different mutations responsible for EPP. Phenotypic expression of EPP requires coinheritance of a null FECH allele and a wild-type low expressed allele; there are evidences suggesting that an entire haplotype (-251G, IVS1-23T, IVS3-48C; GTC haplotype) is involved in reducing the allele expression. *Aims.* We analysed a Canadian family of Italian origin in which the proband showed clinical signs of EPP but no mutations were found in the promoter and in the entire coding region. Moreover, the proband carried the GTC haplotype and the -251G polymorphism in apparently homozigosity. Family studies established absence of mendelian segregation for the -251G polymorphism, suggesting an emizigosity in this region and a possible deletion. The aim of this study was to identify the size of the putative deletion. *Methods.* The promoter, the entire coding region and the intron-exon boundaries of FECH gene have been amplified by PCR and submitted to direct sequencing. In order to identify an heterozygous region, we extended the SNPs analysis upstream the FECH gene and we performed XL-PCR analysis to establish the size of the deletion. *Results.* Two heterozygous polymorphisms were found on asparaginyl-tRNA synthetase (NARS) gene. Thus we conducted an XL-PCR using primers situated in the NARS gene and in the intron 2 of FECH gene respectively. The XL-PCR showed a wild type fragment of 14569bp and a shorter fragment of about 4000 bp. Sequence analysis on the isolated abnormal fragment showed a 10377bp deletion. The deletion includes an intergenic region of about 6500bp, the promoter, the exon 1 and part of intron 1 of the FECH gene. The family study identified also two asymptomatic carriers of the same mutation. *Conclusions.* This deletion causes the loss of the entire promoter which probably prevents the expression of the mutated allele. Nevertheless this allele could code for an mRNA lacking the first 22 aa of the leader sequence, causing the retention of the protein in the cytoplasm and thus the blockage of its translocation to the inner mitochondrial membrane. Quantitative RNA analysis is under investigation to confirm these hypothesis. The deletion is probably caused by unequal intragenic recombination between two Alu sequences which have been found close to the breakpoints. The presence of several Alu in the FECH gene suggests the high probability of deletions as a cause of EPP.

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MOLECULAR HETEROGENEITY OF PORPHYRIA CUTANEA TARDA IN ITALY: IDENTIFICATION OF THREE NOVEL MUTATIONS IN THE UROPORPHYRINOGEN DECARBOXYLASE GENE

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Background. Porphyria cutanea tarda (PCT, MIM 176100) is a human metabolic disorder due to the acquired or genetic impairment of uroporphyrinogen decarboxylase (URO-D, E.C. 4.1.1.37) activity, the fifth enzyme of the heme biosynthetic pathway. A classification of inherited and non-inherited forms is based on the enzyme activity levels in red blood cells (RBC). Among the diagnostic criteria, the most powerful is the URO-D deficiency and the presence of a plasma fluorescent peak at 620 nm. The main clinical findings observed in PCT are skin lesions on light-exposed areas of the body, skin fragility, vesicles and bullae formation after sun exposure. Clinical manifestations of PCT are often precipitated by triggering factors such as alcohol, drug abuse, estrogens, virus infections, hepatotoxic chemicals and hepatic siderosis. Nowadays more than 70 molecular abnormalities have been so far identified in the URO-

D gene as responsible for PCT, showing a high molecular heterogeneity. Few data are presently available on the Italian population. In a previous work, we have identified 18 different molecular defects among 22 unrelated Italian PCT patients. *Aims.* The aim of this study was to identify the molecular defect in the URO-D gene in fifteen new families with typical clinical and biochemical signs of PCT. *Methods.* The promoters, the entire coding region and the intron-exon boundaries of URO-D gene has been amplified by polymerase chain reaction and submitted to direct automated sequencing. *Results.* The plasma peak at 620 nm was positive in all subjects. PCR analysis and automated direct sequencing identified seven molecular defects in URO-D gene, three of which are new finding. In this study we analyzed a total of 22 subjects, 20 of which carry a mutation. Table 1 summarized the mutations identified. *Summary.* These results allowed the identification of three novel URO-D mutations. In a previous work, we have identified other thirteen new molecular defects for a total of sixteen different mutations restricted to the Italian population; the 243-245 del CAT and the 1107G>A are the most common. So far our group identified a total of twenty one different mutations among Italian patients. This study confirmed the high heterogeneity of molecular abnormalities responsible for PCT phenotype and the presence of clusters of mutations in particular geographic areas.

Table 1. Mutations identified in the italian population. The mutations identified by our group are indicated with*.

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FERROPORTIN DISEASE OR TYPE IV HEMOCHROMATOSIS: SHOWING A HETEROGENEOUS PHENOTYPICAL EXPRESSION

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Introduction. Hereditary hemochromatosis (HH) is the most prevalent form of iron overload in caucasians. This term comprises a group of disorders that lead to abnormalities in iron homeostasis, leading to increased intestinal absorption of iron and tissue deposition. If not treated, the accumulated iron can result in tissue damage and lesions such as liver cirrhosis, diabetes mellitus, arthropathy, myocardiopathy, endocrine disorders and hepatocellular hepatocarcinoma. A wide majority of clinical cases of HH are associated to homozygosity from the C282Y mutation of the HFE gene (type I hemochromatosis), although some other mutations of this gene can also lead to iron overload. A recently discovered molecule (in the year 2000), Ferroportin 1, FPN1, is the only known exporter of cellular iron. In 2001, some families with atypical autosomal dominant HH were reported, in whom missense mutations in the FPN1 gene were found (SLC40A1). This disorder has been named type IV hemochromatosis, or Ferroportin disease, and its clinical presentation seems to be heterogeneous. *Objectives.* To describe the phenotypic expression in members of a family suffering from type IV HH. Among them, in 2005, a novel mutation resulting in a single nucleotide substitution (c.263G>C) in exon 3 of the FPN1 gene was found. *Results.* From 1998 up to the present, 6 members of this family have been included in a weekly or fortnightly phlebotomy program. There were four males and two females . No symptoms were present, except arthralgia in the oldest man. They began phlebotomies at the ages of 56,50,46,22 for the males and 24 and 19 for the females. A slightly low platelet count was presented in four cases (115×10^3/L; 141×10^3/L; 113×10^3/L and 140×10^3/L). The glucose level was normal in all of them and also the liver enzymes, except the ALT and GGT levels, which were slightly higher in the oldest man. All of their ferritin levels were elevated: 9,075 ng/mL; 2,830 ng/mL; 5,291 ng/mL; 1,870 ng/mL in the males, and 1,524 ng/mL and 724 ng/mL in the females. Their transferrin saturation was 91%; 43.4%; 71.2%; 63.7%; 41% and 60% respectively. Liver biopsies were done on the four males with Grade IV iron overload in the oldest man and Grade III in the rest. Fibrosis was present in three cases but none of them presented a cirrhosis state. Two of the male cases, in the biweekly phlebotomy program, showed a tendency to anemia. Several other members of this family, who have rejected periodical phlebotomy therapy, show extremely high serum ferritin levels but no related symptoms. *Conclusions.* 1. Ferroportin disease, or type IV hereditary hemochromatosis, must be suspected in cases of familial asymptomatic iron overload that present at early ages, even in women, with disproportionately low transferrin saturations considering the greatly elevated ferritin levels. 2. A standard biweekly phlebotomy program may cause a tendency to anemia and therefore may not be well tolerated. 3. The response to phlebotomies is heterogeneous, even between members of the same family with the same molecular lesion. 4. Prospective population studies are required to define the most appropriate therapy in this type of hemochromatosis, in view of the apparently benign nature of this particular iron overload.

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IRON REGULATING GENES AND CHELATION

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Background. Iron-chelating therapy has been used in clinical practice for over 20 years and has contributed in a significant decrease in mortality and morbidity of patients with transfusion-dependent anemias and chronic iron overload. Many aspects of the mechanisms of action of chelating agents have yet to be elucidated. So far, no studies have identified the intracellular iron pathways affected by both chelators and little is known on their effects on gene expression and translational modulation. A*ims.* The aims of the present study were to evaluate the effects of iron chelation on the expression of iron-regulating genes. *Methods.* To study the effects of iron chelation on the expression of iron-related genes, normal and iron loaded human hepatic cells HepG2, were treated with the iron chelating agents, desferrioxamine (DFO) and deferasirox (Exjade, ICL670), which were provided in pure form by Novartis Pharma AG, Switzerland. The cells were initially cultured with either ferric ammonium citrate (FAC) 50 μm or normal medium. After 24 hours DFO or deferasirox was added in the cultures for further 12-hour period. The transcriptional expression of the iron-related genes, namely DMT1 (divalent metal transporter 1), TfR1 (transferin receptor 1), HAMP (hepcidin) and Ireg1 (ferroportin), was studied using quantitative Real-Time PCR (qRT-PCR). Statistic analysis was performed using Student's paired t-test. *Results.* Expression of both iron importers DMT1 and TfR1 is induced by both DFO and deferasirox, indicating effective depletion of intracellular iron with chelation. Regulation is most probably mediated via the IRE/IRP system and stabilization of mRNA transcripts. Ireg1 mRNA expression was increased by DFO and greater by deferasirox mainly in non-iron loaded cells. Only deferasirox resulted in mild up-regulation of ferroportin's transcription in iron-loaded cells. Hepcidin transcription was up-regulated in low iron conditions following chelation. The induction of HAMP mRNA expression was more pronounced in iron-loaded hepatic cells, especially with deferasirox. *Conclusions.* We conclude that DFO and deferasirox modulate the transcription of iron-regulating genes in the human hepatic cells HepG2. The effects on gene expression depend on the initial iron status of the cell. A more pronounced response was observed with deferasirox compared to DFO, suggesting that deferasirox could prove to be more effective in mobilizing intracellular iron and in controlling iron overload. Further studies are required to fully understand the underlying mechanisms of DFO and deferasirox mode of action and to explore the possibility that chelation therapy affects iron homeostasis via alternative pathways, like the modulation of gene translation and/or transcription.

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PREVALENCE OF MEMBRANE PROTEIN DEFICIENCIES IN PORTUGUESE PATIENTS WITH HEREDITARY ELIPTOCYTOSIS AND HEREDITARY SPHEROCYTOSIS

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The erythrocyte owes its unique shape and mechanical proprieties to its membrane, which is a complex structure involving interconnections between transmembrane proteins, the lipid bilayer and cytoskeleton proteins. Defects in membrane proteins lead to pathologies known as red cell membrane disorders, being Hereditary Spherocytosis (HS) the most common. HS and Hereditary Eliptocytosis (HE) are hemolytic anemias caused by deficiencies in several erythrocyte membrane proteins (spectrin, ankyrin, band 3 and protein 4.2 for HS; spectrin and protein 4.1 for HE). The presence of spherocytes in peripheral blood smears, the increase in osmotic fragility and in reticulocyte count are screening hallmarks of HS. For HE the presence of eliptocytes in peripheral blood smears is the major screening hallmark. The aim of our work was to identify and quantify the membrane protein deficiency underlying HS and HE, in order to estimate the prevalence of each deficiency in Portuguese patients. We studied 87 Portuguese patients of the northern region of Portugal, diagnosed with HS or HE after standard screening tests. The protein deficiencies that underlie both HS and HE cases were identified and quantified by standardized electrophoretic erythrocyte membrane protein analysis. Only 7 patients, out of the 87 patients studied, presented HE. In HE, 6 patients (85.7%) presented protein 4.1 deficiency and one patient (14.3%) spectrin deficiency. Considering the HS cases, we observed band 3 deficiency in 51 (63.7%) patients, ankyrin deficiency in 20 patients (25.0%), spectrin deficiency in 7 patients (8.8%) and protein 4.2 deficiency in 2 patients (2.5%). Considering the total of patients with HE and HS, the prevalence of erythrocyte membrane protein deficiencies was found to be 58.6% for band 3, 23.0% for ankyrin, 9.2% for spectrin, 6.9% for protein 4.1 and 2.3% for protein 4.2. Our sample Portuguese population showed band 3 deficiency as the most prevalent protein deficiency in HS, followed by ankyrin deficiency. The literature also refers these deficiencies as the most prevalent, although ankyrin deficiency is the most prevalent in other populations. The prevalence found for HE in our studied population differs from what is described by the literature, which states spectrin deficiency as the major cause. This probably reflects region specificities, and the distribution of the ethnic groups living in the North of Portugal. *A*

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PYRUVATE KINASE DEFICIENCY: BIOCHEMICAL CHARACTERIZATION OF TWO MUTATIONS CAUSING INABILITY OF THE ENZYME TO PROPERLY BIND SUBSTRATES

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Background. Pyruvate kinase (PK) deficiency is the most common enzyme defect of erythrocyte glycolytic pathway causing hereditary nonspherocytic chronic haemolytic anaemia. PK deficiency is transmitted as an autosomal recessive trait and 180 mutations associated with the disorder have been so far reported in the gene encoding the red cell pyruvate kinase (RPK). The severity of the disorder is highly variable, ranging from mild to severe anaemia, which can be life-threatening and require continuous transfusion therapy. RPK catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate with the synthesis of ATP. It is activated homotropically by PEP and heterotropically by fructose 1,6-bisphosphates (FBP), and is inhibited by ATP and alanine. The three-dimensional structure of RPK has been elucidated and ten mutations targeting distinc regions of RPK structure have been investigated at protein level. Characterization of mutant proteins may serve as a valuable tool to assist with diagnosis and genetic counseling. *Aims.* To improve knowledge on molecular basis of the haemolytic anaemia caused by PK deficiency two additional mutant forms of RPK have been subjected to biochemical characterization. The variants investigated Gly159Val (c.475G>T) and Arg163Cys (c.487C>T) have been described in two young PK-deficient patients affected by severe haemolytic anaemia.[1,2] *Methods.* The DNA bearing the desired mutations were obtained from the cloned wild-type cDNA using standard methods of site-directed mutagenesis. The mutant enzymes were expressed in E.coli DH5α and purified to homogeneity following the procedure developed for recombinant wild-type protein.[3] *Results.* Arg163Cys RPK showed a drastic reduction of the catalytic efficiency expecially versus ADP (3 orders of magnitude) due to the increased Km value (42-fold higher). Gly159Val had kinetic properties altered either vs ADP or PEP (Km values, 8- and 4-fold higher; catalytic efficiency, about 15-fold reduced). Moreover, both mutant enzymes did not gain the fully active conformation in the presence of 1mM FBP (nH 1.3 and 1.5 for Arg163Cys and Gly159Val, respectively, vs 1.05 of the wild-type enzyme) and resulted quite insensitive to ATP inhibition (IC_{50} at least 20-fold higher). *Conclusions.* The mutation c.487C>T affects an arginine essential for ADP binding, and thus prevents the enzyme from accomplishing his function. The mutation c.475G>T affects a functionally crucial residue involved in the allosteric transition, thus impairing the substrates binding site to adopt the appropriate geometry required for the catalytic cycle. The altered properties displayed by the mutant forms account for the reduced activity observed in RBCs of patients affected by this pathology.

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IDENTIFICATION AND CHARACTERIZATION OF THE FIRST MUTATION IN THE RED BLOOD CELL SPECIFIC HEXOKINASE PROMOTER IN A PATIENT WITH MILD HEXOKINASE DEFICIENCY

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Background. Hexokinase (HK) is one of the key enzymes of glycolysis and catalyzes the phosphorylation of glucose to glucose-6-phosphate. HK-I, that is encoded by the HK-I gene (HK1), is the predominant isozyme in a variety of tissues including brain, kidney, erythrocytes, and platelets. Red blood cell hexokinase (HK-R) is a HK isozyme that is transcribed from HK1 by use of an erythroid-specific promoter. HK-R is mainly expressed in reticulocytes and young erythrocytes and its half life is much shorter than that of HK-I. Thus HK-I replaces HK-R as the erythrocyte matures. Hexokinase deficiency is a very rare cause of hereditary non-spherocytic haemolytic anaemia. To date less than 20 cases have been described. *Aims.* The aim of this study was to investigate the molecular basis for HK deficiency in a patient with chronic haemolysis. *Methods.* HK activity, and activity of the red blood cell age-related enzymes, was determined according to standardized procedures. Functional studies were performed using transient transfection of HK promoter constructs in human K562 erythroleukemia cells. DNA-protein interaction at the promoter of HK was studied using Electrophoretic Mobility Shift Assay's (EMSA) with nuclear extracts from K562 cells. DNA analysis and RT-PCR were performed according to standardized procedures. *Results.* In the patient, HK activity was 61% of the mean reference value, whereas PK and G6PD activities were normal. Consequently, we interpreted the HK activity as too low. By DNA sequence analysis we identified on the paternal allele in the erythroid-specific promoter of HKI two novel mutations in cis: -373A>C and -193A>G (relatively to the start codon). These mutations were absent in a Caucasian reference population. Transfection of promoter constructs into K562 cells showed that the most upstream 373A>C mutation was nonfunctional. In contrast, the 193A>G mutation reduced promoter activity by 92%. EMSA using K562 nuclear extracts indicated binding of a trans-acting factor. On the maternal allele we identified a novel mutation in exon 3 (c.278G>A). Three PCR products were amplified from the patient's mRNA by HK-R specific RT-PCR: one normal PCR product, one lacking exon 3 and one alternatively processed transcript. These results suggested that the exon 3 mutation compromises normal pre-mRNA processing. *Summary and conclusions.* We investigated the molecular basis for HK deficiency in a patient with hexokinase deficiency. The -193A>G mutation was shown to cause a dramatic decrease in promoter activity *in vitro*, and is therefore the first mutation known to cause HK-R specific HK deficiency. Furthermore, the c.278G>A exon 3 mutation on the second allele was

shown to cause aberrant splicing of both HK-R and HK-I. We postulate that the reduced transcription of HK-R and the aberrant splicing of HK-I and HK-R collectively resulted in hexokinase deficiency and mild chronic haemolysis, as observed in the patient here described.

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PROTEIN DEFICIENCIES AND CLINICAL OUTCOME OF HEREDITARY SPHEROCYTOSIS. IS THERE A RELATION AFTER ALL?

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Hereditary Spherocytosis (HS) is the most common non-immune hemolytic anemia in individuals of northern European ancestry, ranging from an asymptomatic condition to a severe life-threatening anemia. HS is usually classified as mild, typical or severe according to the severity of the symptoms and analytical presentation. The common features of HS include mild anemia, jaundice and splenomegaly. Splenectomy, when performed, corrects the anemic state. Mutations in the genes encoding several distinct erythrocyte membrane proteins - spectrin, ankyrin, band 3 and protein 4.2 - underlie HS. The aim of our work was to identify and quantify the membrane protein deficiency underlying HS, in order to look for a correlation between the type and amount of protein deficiency with the severity of the disease. We studied 80 Portuguese patients diagnosed with HS by standard screening tests. The protein deficiencies were identified and quantified by standardized electrophoretic erythrocyte membrane protein analysis, for each case of HS. The patients were classified as having *mild* (n=35), *moderate* (n=31) and *severe* (n=14) HS, according to Guidelines for the diagnosis and management of Hereditary Spherocytosis (Bolton-Maggs *et al.*; Br. J. Haematol.; 2004). The clinical classification of the splenectomized patients reports to HS presentation prior splenectomy. We found isolated protein deficiencies for protein 4.2 (2 patients) and for spectrin (7 patients). Combined protein deficiencies were also observed, namely, a primary band 3 deficiency combined with a protein 4.2 secondary deficiency (51 patients), and a primary ankyrin deficiency associated with secondary deficiencies in spectrin and protein 4.2 (20 patients). Patients with isolated spectrin deficiency presented a significantly positive correlation ($r=0.850$, $p<0.05$) between the amount of protein deficit and HS severity. In patients presenting combined protein deficiencies, the amounts of protein deficits were compared; we observed that the greater the unbalance between combined protein deficiencies, the greater was the HS severity ($r=0.489$, $p<0.001$, for band 3 primary deficiency and $r=0.653$, $p<0.01$, for ankyrin primary deficiency). Our data suggest that in case of isolated spectrin deficiency, the amount of the deficit is responsible for the clinical severity of HS. In cases of combined protein deficiencies, it is the unbalance between the amounts of deficiencies that seems to be responsible for the clinical outcome of HS, probably reflecting an enhanced membrane protein destabilization.

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SCREENING FOR PK-LR GENE LARGE DELETIONS IN PYRUVATE KINASE DEFICIENT PATIENTS WITH UNDETECTED MUTATIONS

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Background. Red cell pyruvate kinase (PK) deficiency is the most frequent glycolytic abnormality causing chronic haemolytic anaemia. The gene encoding erythrocyte PK (PK-LR) is located on chromosome 1 and 180 different mutations, mostly missense, have been reported. The failure to detect one or both mutations in some PK deficient patients has been attributed to technical reasons, giving rise to the assumption that some mutations, such as large deletions, could not be detected by the normal panel of primers used for PCR of genomic DNA analysis. To date only a few PK-LR gene large deletions have been described: the *Gypsy* deletion of 1149bp which results in the loss of exon 11, PK 'Viet' (del 4-10), and a deletion of 5006bp. *Aims.*The aim of the study was to investigate the presence of PK-LR gene large deletions in a series of PK patients with undetected mutations, using a quantitative multiplex PCR of short fluorescent fragments (QMPSF) assay. *Methods.* 11 patients were studied. PK deficiency was diagnosed by the demonstration of a reduced PK activity and/or thermostability, the other red cell enzymes showing normal or increased activity in relation to reticulocytosis. PK-LR gene sequencing showed the presence of only one mutated allele in 3 patients, whereas in 7 cases no mutations were detected. In one patient the failure of PCR amplification of exon 11 was observed; parents were studied by sequencing and no mutation was found. We applied the QMPSF assay described by Costa et al (2005), slightly modified. A 9-fragment multiplex PCR, including one control fragment not located on chromosome 1 (P5'N-1, 7p15-p14), was performed and run on an ABIPRISM 310 capillary sequencer. After adjusting PCR efficiency with the normalization control fragment, the chromatograms of each patient were compared with a median of 6 normal subjects; deleted regions can be identified by a peak height roughly half that of the control sample. *Results.* In one patient PCR analysis failed to amplify exon 11. QMPSF assay performed in the parents showed an abnormal pattern corresponding to an heterozygous absence of exon 11; molecular characterisation of the deleted region showed a new large deletion of 1431bp extending from intron 10 to intron 11, identified at the homozygous level in the patient and heterozygous in the parents. The new mutation also present an insertion of 2 bp (ct). In the remaining patients no abnormalities were found. *Conclusions.* Molecular diagnosis of PK deficiency, usually done by sequencing the exons, flanking regions and erythroid promoter of PK-LR gene, can fail to detect the mutations in some patients. We applied the QMPSF assay in a series of patients with undetected mutations and find the presence of a new homozygous large deletion of 1431 bp of in one case. Even if rare, PK-LR gene large deletions may account for some of the undetected mutations in PK deficient patients, and QMPSF assay can be useful as a screening technique to identify them

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G6PD TONDELA: A NEW CLASS I VARIANT DUE TO 18 NUCLEOTIDES DELETION IN EXON 10

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Background. Glucose 6-phosphate dehydrogenase (G6PD) is the product of a housekeeping X-linked gene that appears to be ubiquitous in its expression in mammalian tissues. The enzyme catalyzes the first step of the pentose phosphate pathway and G6PD deficiency is the most common human enzyme defect. More than 140 mutations have been identified associated with the deficiency, the great majority consisting in single missense point mutations causing amino acids replacements. The exceptions are 6 small deletions of 1 to 8 amino acids. This is probably due to the fact that total absence of G6PD activity is not compatible with life. *Aims.* To characterize, at the molecular level, a chronic haemolytic anaemia of a G6PD deficient patient and her relatives. *Patients*. A 74 years old Portuguese woman was referred to our Haematology Unit to elucidate the aetiology of a chronic haemolytic anaemia aggravated in the past decade. Her spleen was palpable 5 cm below the left costal margin and she had a macrocytic anaemia with high reticulocytosis. Hb=105 g/L, MCV=117 fL, reticulocytes=29%, serum bilirubin: total 26.8 micromol/L (N:<22), unconjugated 20.8 micromol/L (N:<17); G6PD activity=16.6% of normal. She was the single daughter of unrelated parents and has three healthy daughters, with no signals of chronic haemolysis. Daughter 1 has a mild reticulocytosis (3.7%) and Hb=123 g/L; the G6PD activity level was 83.4% of normal. Daughter 2 has Hb=120 g/L, mild reticulocytosis (2.8%) and the G6PD activity level above the normal range (120% of normal). Daughter 3 has normal haematological parameters and normal G6PD activity. Daughters 1 and 2 have no children and there is no reference to affected males or miscarriages in the family. *Methods.* After informed consent, genomic DNA was extracted from EDTA peripheral blood samples and G6PD gene was studied by PCR, SSCP and sequencing analysis. *Results.* Patient and daughter 1 and 2 G6PD gene showed a PCR-SSCP mobility shift in exon 10. Subsequent automatic sequencing revealed an 18-bp deletion, involving nucleotides 1076-1094, at the heterozygous state. The deletion does not alter the reading frame, and a mutant protein 6 amino acids shorter (360-365 residues KALNER) is predictable produced. *Summary and conclusions.* The molecular study of a Portuguese patient with chronic haemolytic anaemia enabled us to identify a G6PD class I variant due to a new G6PD nucleotide deletion. The deletion involves 6 residues near the dimmer interface of the G6PD enzyme, which certainly affects its conformation and stability. The consequent decrease in G6PD activity is certainly responsible for the patient's chronic haemolysis. Het-

erozygous mother has a more severe clinical phenotype than daughters 1 and 2, most probably due to the X inactivation process in females: the chromosomes X selective inactivation favoured the wild type allele in the mother's erythropoietic cell line while in daughter 2 the process favoured the mutant allele. This unbalanced X lionization is known to aggravate with aging.

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BAND 3 AS A MARKER OF ERYTHROCYTE CHANGES IN CHRONIC RENAL FAILURE

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Band 3 protein is the major integral protein of the red blood cell (RBC) membrane. It is known as the senescent neoantigen, as modifications in band 3 protein, by proteolytic cleavage, clustering or exposure of unusual epitopes, trigger the binding of specific anti-band 3 autoantibodies and complement activation, marking RBC for death. An abnormal band 3 profile [% of band 3 monomer; high molecular weight aggregates (HMWAg); proteolytic fragments (Pfrag)] has been associated with RBC damage/aging in inflammatory conditions associated with oxidative stress, namely in cardiovascular disease, pregnancy and acute physical exercise. Chronic renal failure (CRF) has also been associated with both inflammation and oxidative stress. A deficient renal erythropoietin secretion underlies the development of an anaemia, which is usually corrected by therapy with recombinant human erythropoietin (rhEPO). However, about 25% of the patients do not respond to this therapy. Our aim was to study the band 3 profile, as a cumulative marker of RBC damage, in CRF patients under haemodialysis and rhEPO therapy. We studied 44 CRF patients, 22 responders and 22 non-responders to rhEPO therapy, and 25 healthy individuals matched for age and gender. We evaluated the band 3 profile, membrane bound haemoglobin (MBH), RBC count, haematocrit (Ht), haemoglobin concentration (Hb), haematimetric indices, red cell distribution width (RDW), reticulocyte count and reticulocyte production indice (RPI). CRF patients (patients vs control) showed a statistically significant decrease in RBC count, Hb and Ht; a significant increase in reticulocyte count, RDW and RPI was also observed; band 3 profile presented a significant reduction in HMWAg and Pfrag and a significant increase in band 3 monomer. No difference was found in MBH, though a trend to lower values was observed. A positive correlation was found between Pfrag and Hb (r=0.352, p=0.019) and Ht (r=0.384, p=0.010). When comparing responders to non responders, we found a statistically significant reduction in Hb, Ht, mean cell haemoglobin and mean cell haemoglobin concentration. A statistically significant increase in RDW was found in non-responders patients. Concerning band 3 profile, we found differences statistically significant in Pfrag (lower in non-responders). CRF patients presented an anaemia, which was enhanced in non-responders; this anaemia is regenerative, as patients presented twice the reticulocyte control value, being higher in non-responders. The higher RDW observed in patients, may reflect this rise in reticulocyte count; however, the higher RDW value for non-responders may also reflect a higher RBC damage. Actually, the changes observed in band 3 profile presented by CRF patients, a decrease in HMWAg and a rise in Pfrag, seems to reflect a younger erythrocyte population, However, the higher HMWAg presented by non-responders, when compared to responders, suggest a higher RBC damage in that patients. Our data suggest that in CRF patients there is an underlying oxidatixe stress, which is linked to RBC damage, triggering the enhancement of the reticulocyte production. Band 3 changes suggest an increase in damaged RBC, but also an increase in younger RBCs. Band 3 profile could be used as a marker of RBC changes in these patients and in understanding the mechanism of resistance to rhEPO therapy.

0790

IRON DEFICIENCY IN CHILDREN WITH MALABSORPTION

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Background. The malabsorption represent a frequent cause of iron deficiency (ID) in children and adolescents unresponsive to iron therapy. *Aims*. In these patients an early diagnosis is necessary for an appropriate therapy. *Methods.* A retrospective study on 240 subjects with ID, age range 7.5 months- 16 years was performed. In patients unresponsive to iron therapy the causes of malabsorption were studied. ID was established by Hb, MCV, serum iron, serum transferring saturation and serum ferritin (EIA) determination. Parasitologic stool analysis, fecal blood test, measurement of antigliadin IgA and IgG, antiendomysial IgA and antitransglutaminase IgA and IgG were performed in unresponsive patients and in patients with gastrointestinal (GI) symptoms. The biopsy of the small intestine via upper GI endoscopy confirmed diagnosis of celiac disease (CD). *Results.* Our retrospective analysis shows that 41.5% of patients of both sexes aged between 3 and 16 years had ID due to malabsorption. The cause of malabsorption were: CD: 37 cases - 91% children (3-10 years), 8% adolescents (11-15 years). Helycobacter Pylori infections (HPI): 39 cases- 51% children(3-10 years), 49% adolescents (11-16 years). Enteromonas infection: 15 children (3-10 years). HPI+CD: 8 children (3-10 years). *Conclusions.* An abnormal iron absorption leading to ID, often in the absence of G.I. symptoms and without other manifestations of malabsorption syndrome is increasingly being recognized. We found that in childhood and adolescence the most important cause of ID unresponsive to specific therapy is a reduced absorption due to C.D. and/or H.P.I. H.P.I. can produce gastro-duodenal lesions with acute or chronic blood loss, but it mainly influences iron absorption, lowering the concentration of gastric juice ascorbic acid. Another cause of unresponsive iron deficiency in our patients was enteromonas infection probably through low intestinal absorption. The gluten free diet, improving bowel function, corrected the secondary iron deficiency and mild anemia, while, in the cases of more severe anemia (Hb< 7g/dL), specific parenteral iron therapy was necessary. The eradication of HPI or enteromonas parasitic infection improved the ID with mild anemia while in the cases with more severe anemia specific iron therapy was necessary. In patients unresponsive to oral iron therapy the diagnosis of malabsorption should always be suspected.

0791

EXPRESSION LEVELS OF CD47, CD35, CD55, CD59 ON RED CELLS, AND SIRP-ALPHA, BETA ON PERIPHERAL MONOCYTES FROM PATIENTS WITH WARM AUTOIMMUNE HEMOLYTIC ANEMIA (WAIHA)

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AIHA is defined as an increased destruction of red cells (RBCs) in the presence of anti-RBC autoantibodies. CD47 is an integrin-associated protein expressed on all cells including RBCs. Signal-regulatory proteins (SIRPs) comprise a novel transmembrane glycoprotein that is especially abundant in macrophages. Animal models show that the binding of RBC CD47 to SIRP-α on macrophages contributes to the inhibition of phagocytosis, and CD47-deficient mice develop severe AIHA, while CD35 (CR1- complement receptor 1), CD55 (decay accelerating factor) and CD59 (membrane inhibitor of reactive lysis) are complement inhibitory proteins. Using flow cytometric analyses, in this study we evaluated the expression of CD47, CD35, CD55, CD59 on RBCs and SIRP-α,β on peripheral monocytes of patients with wAIHA. The study population consisted of 38 patients with AIHA, 23 with active AIHA [M:F = 7:16; median age: 43 yrs (3-73)], 15 AIHA in remission without required medical treatment [M:F=0:15; median age 48.5 yrs (18-82)] and 20 healthy controls [M:F = 8:12, median age: 36.5 yrs (25-71)]. Thirty patients presented idiopathic AIHA while 8 patients had secondary AIHA (2 systemic lupus erythematosus, 3 non-Hodgkin lymphomas and 3 HCV infection). The median Hb levels of patients with active AIHA was 9.5 mg/dL (range: 2.9 to 13.5 mg/dL) and median absolute reticulocyte counts was 170×10^3/L (range: 54 to 756×10^3/L). At the time of the analyses, 22 patients had a positive direct antiglobulin test, 22 (95.6%) had IgG on their RBCs, 9 (39.1%) had IgG plus C3. The RBC eluates (dichloromethane technique) from cell samples were positive in 22 patients, but the retrieved autoantibodies were pan-reactive showing no specific reactivity. The mean fluorescence intensity (MFI) of the

expression of CD47, CD35 and CD55 on RBCs and SIRP-α,β on monocytes of active AIHA patients, AIHA in remission and healthy controls were not statistically different. Active AIHA patients showed significant lower CD59 expression on RBCs than healthy controls, while CD59 expression in patients whit AIHA in remission was not significantly different from that of healthy controls (Table 1). Four patients presenting life threatening AIHA were treated with high dose of steroids and RBC transfusions, but three patients evolved to death. The expression of CD59 on RBCs of 3 AIHA patients who died were significantly lower than that seen on RBCs healthy controls (MFI=433.6±69.6 and 553.74±36.6, *p=0*.0001). Although experimental studies have suggested that CD47 has a profound influence on the severity of AIHA in mice, by binding of RBC CD47 to SIRP-α on macrophages, the present human data do not demonstrate significant difference on RBCs of patients warm AIHA or healthy individuals. On the other hand, complement regulatory proteins (CD35, CD55 and CD59) may play an important role in protecting RBC destruction through the activation of complement. Our results suggest that patients with active wAIHA may present significant CD59 deficiency on their RBCs that may increase the susceptibility of cells to complement-mediated lysis resulting in severe clinical hemolysis. (Funding by FAPESP).

Table 1.

	Group 1 active AIHA (n=23)	Group 2 AIHA in remission (n=15)	Group 3 healthy controls (n=20)	P
CD47	461.1 ± 40.9	461.0 ± 23.7	464.4 ± 26.7	NS
CD35	184.6 ± 28.8	171.7 ± 29.8	194.3 ± 22.6	NS
CD55	371.5 ± 76.3	333.9 ± 72.4	381.1 ± 48.4	NS
CD59	512.5 ± 59.6	536.0 ± 46.1	553.7 ± 36.6	1 vs 3 P= .009
SIRP-α,β	480.9 ± 36.1	479.6 ± 43.1	488.6 ± 27.7	NS

0792

IDENTIFICATION OF A NOVEL TRUNCATING MUTATION OF HEMOJUVELIN (HFE2) GENE LEADING TO SEVERE JUVENILE HEMOCHROMATOSIS

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Background. Juvenile hemochromatosis (JH) is a rare and devastating disorder of iron metabolism leading to severe and early end organ damage threw iron overload. Homozygous mutations of HFE2 and less frequently HAMP gene lead to early onset severe hemochromatosis. The function of hemojuvelin is not perfectly elucidated. It is accepted that HJ positively modulates the iron regulator hepcidin. We report the case of a 26 year old north african male consulting for erectile dysfonction. With hypogonadotropic hypogonadism. Serum ferritin level was 5069 ng/mL and transferrin saturation > 90% leading to high suspiscion of hemochromatosis. Hepatic MRI assessed hepato- pancreatic iron overload and liver biopsy showed evidence of cirrhosis.No diabetes was diagnosed. Cardiac MRI showed ventricular hypertrophy and muscular iron deposits without contractile dysfunction. *Methods and Results.* Genetic testing was performed but failed to identify mutation neither of the HFE nor the HAMP gene. Direct sequencing of the 3 coding exons of the HFE2 (hemojuvelin) gene displayed homozygous p.Arg257 X (g.3147 C>T) mutation. The genomic reference sequence is NC_000001.8. *Discussions.* This mutation has not been reported yet , nevertheless, out of it's truncating nature it can be considered responsible for the clinical presentation. Truncating mutations account for 50% of the HFE2 mutations. Our patient underwent hormonal substitution, weekly 500 ml phlebotomy and daily iron chelation with deferrioxamine. After 18 weeks of treatment , SFL was still 2505 ng/mL and TfSat >90% with elevated hepatic enzymes accounting for the dramatic iron overload and the severity of the underlying iron metabolism disorder. *Conclusions.* we describe a novel homozygous truncating mutation p.arg257X (g.3147 C>T) mutation of the HFE 2 (hemojuvelin) gene leading to severe juvenile hemochromatosis with multiple end organ damage consecutive to iron overload.

0793

TRIOSE-PHOSPHATE ISOMERASE DEFICIENCY: MOLECULAR STUDIES IN TWO PATIENTS

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Background. Triosephosphate isomerase (TPI, EC 5.3.1.1) catalyzes the reversible isomerisation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P). TPI deficiency is the most severe autosomal recessive disorder of the glycolytic pathway associated with neonatal jaundice, chronic haemolytic anaemia, progressive neuromuscular dysfunction and increase propensity to infection. Almost all reported cases are of European origin (15/18) and lethality was common in early childhood. To date 14 different mutations in the human TPI gene have been identified. The most frequent, 11 out of 14 (79%) mutant alleles, is the 315G>C mutation at exon 3 leading to the amino acid change 104Glu>Asp. This mutation has been found in apparently unrelated families all over the world, in contrast to the other known TPI mutations reported in individual families. *Aims.* To establish the molecular basis of TPI deficiency in 2 patients. *Patients*. Case 1: Full-term female newborn with severe haemolytic anaemia requiring exchange transfusion and temporary assisted ventilation. Erythrocyte TPI activity was 31% of normal. The parents were unrelated and healthy, of Spanish origin, both with erythrocyte TPI activity around 60%. At the age of one month presented with seizures and severe respiratory failure, dependent of assisted ventilation, and died at age of 50 days. Case 2: A 33 weeks premature newborn male with severe haemolytic anaemia and respiratory distress. Erythrocyte TPI activity was markedly reduced (45% of normal, but in the presence of transfused donor erythrocytes). The parents, of Portuguese origin, were unrelated; they did not present clinical symptoms but TPI activity was 44% and 35% of normal. The patient became transfusion dependent and developed progressive neurological (especially motor) impairment which progressed to respiratory failure, needing assisted ventilation by the third year of life. He died at the age of three years. *Methods.* After informed consent, genomic DNA was extracted from peripheral blood samples. The entire coding sequence and adjacent regions of TPI gene were amplified by PCR and sequenced. *Results.* Propositus 1: TPI gene sequencing showed compound heterozygosity for the common 315G>C (E104D) mutation and a previously unknown mutation in exon 2, the transversion 188C>A predicting the amino acid change 62Ala>Asp. Sequencing of the entire TPI coding regions and splicing site boundaries did not showed any other nucleotide mutations. Screening for mutation 188A in 50 healthy controls was negative. Both parents sequencing confirmed the presence of the 315C allele in the mother and the 188A allele in the father. Propositus 2: TPI gene exon 3 sequencing demonstrated a homozygous 315G>C (E104D) mutation. *Conclusions.* In 2 unrelated TPI deficient patients the most frequent TPI mutation E104D was found in 3 alleles. The importance of Glu104 to enzyme structure and function was indicated by its conservation in the TPI protein of all species characterized to date. The other mutation, 188C>A, previously not described, predicts a drastic non-conservative replacement of the nonpolar Ala by the acidic Asp at residue 62. The evolutionary conservation of Ala62 from C. elegans to humans indicates that this mutation certainly influences the TPI conformation, stability or kinetic properties, reducing the enzymatic activity.

0794

CARDIAC MANIFESTATIONS IN A BRAZILIAN FAMILY WITH HEREDITARY XEROCYTOSIS

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Background. Hereditary xerocytosis (HX), a dehydrated form of hereditary stomatocytosis, is a rare hematological disorder, characterized by abnormal mechanisms of cellular volume regulation. Clinical presentation includes variable degrees of hemolytic anemia, non-transfusional iron overload and high risk for thrombotic events, especially after splenectomy.It has been described few families around the world. *Aims.*To describe echocardiographic and electrocardiographic alterations in a Brazilian family with HX in which some members had heart failure. *Methods.* The diagnosis of HX in 8 patients from the same family was performed by complete blood count (Technicon-Bayer H3), ectacytometry (LORCA Mechatronics) and iron profile. Hereditary hemochromatosis HFE-related was excluded in all patients. None of the 7 patients had received blood transfusions. Twelve-lead electrocardiogram was performed in order to analyze QTc interval dispersion. Also, standard echocardiographic studies including septal, lateral, inferior, anterior left ventricular (LV) and right ventricular (RV) lateral systolic and diastolic annular velocities by tissue Doppler imaging (TDI), and biventricular pulsed-wave myocardial performance index (MPI) were performed in 7 patients. *Results.* One patient was excluded because he died of heart failure before the TDI echocardiogram. Four patients were female and the mean age was 31±7 years. Six patients had heart failure (HF) functional class I by New York Heart Association (NYHA) classification and 1 patient had HF class II. Patients had a mean hemoglobin of 11.4±1,33 g/dL, mean MCV of 108.3±6 fl, mean ferritin of 1670±582 mg/dL and mean transferrin saturation of 94,5±4,5%, reflecting mild macrocitic anemia and severe iron overload. The electrocardiogram showed increased QTc interval dispersion, when compared to a historic control group. As for the echocardiographic results, the patient who had HF functional class II presented eccentric hypertrophy. One patient had mild LV dilation and systolic dysfunction, two patients had increased of left atrium (LA) volumes, 3 patients had decreased total LA emptying volume and 4 patients had decreased LA ejection fraction. One patient presented biventricular impaired relaxation. All patients presented decreased systolic and/or diastolic TDI regional velocities. One patient present increased RV MPI and 4 LV MPI. *Conclusions.* Nevertheless patients with HX did not receive blood transfusions; they had iron overload and cardiac disease which could lead to heart failure and sudden death. The increased QTc interval dispersion may be related to electrical instability and ventricular arrhythmias. All patients with HX should be followed carefully with periodic cardiac evaluations, electrocardiogram and echocardiogram even if asymptomatic, in order to detect these manifestations in an early stage, treat heart disease and prevent heart failure and death. Further studies are needed to establish the pathophysiology of heart disease in these patients and its relation with iron overload.

Thalassemia and sickle cell disease

0795

SICKLE CELL DISEASE: THE LEBANESE EXPERIENCE

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Background. Sickle cell disease (SCD), the commonest single gene disorder worldwide, is an inherited disease that has different clinical and hematological manifestations in different populations. *Aims.* Identify the demographic, clinical and hematological manifestations of SCD in a registry of patients regularly followed in 3 Lebanese centers experienced in inherited hemoglobin disorders. *Methods.* Retrospective patient data were retrieved from medical records at enrollment and at semi-annual visits and from patient and parent interviews. The definitions of clinical events were the same as those adhered to by the cooperative study of sickle cell disease. All events were analyzed prior to any therapeutic intervention. *Results.* Information on 387 patients with sickle cell anemia (SS) and sickle β-thalassemia (ST) was collected. The mean (±SD) age for this cohort was 17.9 (±12.5) years, and the mean (±SD) follow up was 9.3±6.9 years. 55% of patients were males and SS/ST distribution was 3:1. The disease was clustered in 2 geographic areas in North and South Lebanon. Nearly all patients were Muslims and 56% were the offsprings of consanguineous parents. The prevalence of splenomegaly beyond six years among SS and ST patients was 28.9% and 54.9%. Pain, acute chest syndrome, dactylitis and joint necrosis were seen in 33.5%, 13.7%, 18.8%, and 12% of SS patients and 24.6%, 18.3%, 13.2%, and 11.3% of ST patients. The prevalence rates of stroke, leg ulcers, priapism and death were 4.1%, 1.4%, and 0.8% and 6.8% in the total group. Cholecystectomy and splenectomy were the commonest surgical procedures reported in 12.8% and 16.1% of SS patients and 19.6% and 35.7% of ST patients. Comparing the SS and the ST patients, there were no statistically significant differences in the prevalence of clinical manifestations except for splenomegaly (SS: 28.9%, ST: 54.9%, p-value<0.001) and splenectomy (SS: 16.1%, ST: 35.7%, p-value<0.001). *Summary and Conclusions.* This first national data base, though preliminary and retrospective, has provided important information about distinguishing features of SCD in Lebanon on a large number of patients. In contrast to Northern American populations and similar to some Mediterranean populations, Lebanese SCD patients have a higher prevalence of persistent splenomegaly. The relatively low incidence of thrombotic complications deserves further investigation. .Preventive efforts to decrease the number of new off springs afflicted with this disease should target areas of high prevalence similar to what has been successfully achieved with Thalassemia, another hemoglobinopathy that is highly prevalent in the country.

0796

CARRIER SCREENING AND PRENATAL DIAGNOSIS FOR THALASSAEMIA AND HAEMOGLOBINOPATHIES IN COUPLES OVER THE LAST 5 YEARS IN NORTHERN GREECE

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Thalassaemia is the most frequent genetic disease in Greece with β-thalassaemia carrier frequency approximately 8%, while 1,5% of the population are *HbS* carriers. The risk of bearing a thalassaemic child depends on the region and varies from 1 among 150 to 1 among 24 couples in the general population. Through population screening and prenatal diagnosis program that is performed by the National Centre of Thalassaemia and the various Prevention Units throughout the country, natives and immigrants are screened and counseled, free of charge. One could claim that DNA based prenatal diagnosis was the most important contribution to thalassaemia research in the three decades that followed the mid -1970s. Since the introduction of chorionic villous biopsy, thousands of couples have children without the fear of giving birth to a sick thalassemic offspring. We report the data concerning prenatal diagnosis of thalassaemia and haemoglobinopathies in Northern Greece over a

5'year period (2002-2006). During the years 2002-2006, a total of 1375 couples were screened and counseled for haemoglobinopathies in our thalassemia prevention unit. In 811 couples (58,9%) both partners had no problem, in 416 (30,2%) only one partner was a carrier of thalassemia or other haemoglobinopathy and in 148 (10,7%) both partners carried an abnormal gene. The pregnancies at-risk involved 100 couples that were identified through preconception and prenatal carrier screening, while few had a positive family history or were identified during pregnancy. Prenatal diagnosis was mainly carried out by chorionic villus sampling (CVS) at 11-12 weeks of gestation, and in few cases by amniotic fluid sampling collected at 16-18 weeks. Very few late comers were tested by fetal blood sampling at 20-24 weeks of gestation. 77 couples were at risk for a β-thalassaemic child, 19 couples for sickle-cell/ β-thalassaemic child and 2 for sickle cell disease. 3 couples had more than one pregnancy. From 100 chorionic villous biopsies and amniocentesis and subsequent DNA analysis of the samples, 26 fetuses were found to be homozygous or double heterozygotes, and termination of the pregnancy was advised. 74 were either carriers (heterozygous) or non-carriers of either mutation that was found in both parents, and the pregnancies continued. In three cases of twin pregnancies selective abortion of the two affected fetuses was indicated. Among the couples screened various and rare combinations were detected. We found combinations of β-thalassaemic carriers with ‰β, Lepore, HbD, Hb O-Arab, HPFH, Hb Osu Christianborg and Hb E-Saskatoon. Combinations of HbS carriers with β-thalassemia, ‰β, HbD, Lepore, and E- Saskatoon were also encountered. In one couple in which both partners carried O-Arab mutations and in another where both carried HbE, prenatal diagnosis was not performed since homozygosity is not clinically severe. 5 couples were immigrants, Albania (2), Nigeria (1) Romania (1) and Bangladesh (1). The Greek population that was screened came from all over Northern Greece. The thalassaemia prevention program is successful and prenatal diagnosis is mandatory in at-risk couples. This Thalassemia prevention program has decreased effectively the incidence of severe thalassaemia in our country, and today the very rare cases of homozygous thalassemia that are born is due to couples that fail to use the National Thalassemia Prevention Program for one reason or other.

0797

HYDROXYUREA TREATMENT OF SICKLE CELL DISEASES CAUSES MEGALOBLASTIC TRANSFORMATION OF THE BONE MARROW THAT IS RESPONSIBLE FOR THE INCREASE OF THE MEAN CORPUSCULAR VOLUME

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Background. The positive impacts of hydroxyurea (HU) treatment on the clinical course and the survival of patients with sickle cell diseases are now well documented. Several clinical trials have demonstrated the efficacy of HU in reducing the frequency and intensity of the vaso-occlusive crises. An increase of the mean corpuscular volume (MCV) is the hematological parameter that correlates better with the clinical improvement and for this reason is most often used to monitor the therapeutic efficacy of HU. The mechanism whereby HU raises the MCV is still unclear. Aims.Our hypothesis is that HU increases the MCV because it causes megaloblastic transformation of the bone marrow cells by its effects on the ribunucleotide reduction. Materials and Methods. The study included 20 sickle cell anemia patients under regular follow-up (group 1: 10 patients taking 10-30 mg HU/kg/day; group 2: 10 patients not receiving HU), and 10 bone marrow donors as controls (group 3). All patients were taking folic acid supplementation. Samples of peripheral blood (1 mL) and bone marrow (1 mL) were collected from all participants for morphometrical evaluation of cells in bone marrow smears and measurements of adhesion molecules expression by flow cytometry. The study was approved by the institutional research review committee, and the patients signed an informed consent. *Results.* Bone marrow smears from the group 1 patients showed many signs of megaloblastic transformation, such as megaloblasts, red cells with open chromatin nuclei, hypersegmented neutrophils, giant metamyelocytes and hypersegmented megakaryocyte nuclei. Signs of megaloblastic transformation were rare in group 2 and absent in the control group. The morphometric data demonstrated an increase of the mean cell surface area of all cell types studied of group 1 compared with group 2 or group 3, especially of erythroid precursors (Table 1). Flow cytometry showed a statistically significant reduction ($p<0.01$) of the expression of CD64, CD71, CD36 and annexin V on the surface of sickle erythrocytes from the peripheral blood, and CD64, CD71 and CD36 of the bone marrow red cells of patients from group 1 compared to group 2. The reduction in the expression of adhesion molecules on the surface of peripheral red cells correlated negatively with the rise in MCV: CD64 x MCV (r=-0.71, $p=0.004$), CD71 x MCV (r=-0.81, $p<0.001$), CD36xMCV (r=-0.82, $p<0.001$), and annexin V x MCV (r = -0.46, $p=0.041$). *Conclusions.* HU causes megaloblastic transformation of the bone marrow of sickle cell patients, increases MCV and the cell surface area of all cell types studied, a mechanism that may contribute to reducing the polymerization of HbS molecules and cell adhesion to the endothelium. The strong correlation between the increase of MCV and the reduction of the adhesion molecules expression on the surface of sickle red cells indicates that MCV is a good marker to monitor cell adhesiveness during treatment.

Table 1. Cell surface area of erythroid cells (μm²).

Cell Type	Group 1	Group 2	Group 3
Proerythroblasts	318.2±7.5	265.7±4.9	202.6±5.8
Basophilic erythroblasts	248.8±2.2	197.1±2.8	134.5±4.5
Polychromatic erythroblasts	218.7±5.6	177.4±3.3	122.6±2.6

0798

THALASSEMIA: A COMPARATIVE ANALYSIS ACROSS EUROPE

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Background. People with thalassaemia major require lifelong access to a treatment regimen of regular blood transfusions. These transfusions must be coupled with iron chelation, as they can cause iron overload and damage organs such as the heart and the liver. The most available drugs for iron chelation are: Deferoxamine (DFO) which is parenterally administered, Deferiprone which is an oral iron chelator and Deferasirox which is another oral iron chelator. Even though blood supplied for transfusion should be extremely safe, and all thalassemia patients that are transfused should be concurrently receiving treatment for iron overload, the same standards are not adhered to across all Europe. Different countries have different systems. The goal standards of treatment are set out by the World Health Organization (WHO) and Thalassaemia International Federation published guidelines. *Aims.* This study aims to assess and compare the level of treatment amongst different European countries and to provide results and suggestions from the study of patients with Thalassemia across countries and treatment options. *Methods.* A survey of patients with Thalassemia major was conducted in the following 9 countries: Albania, Bulgaria, Cyprus, France, Germany, Greece, Romania, Spain and the United Kingdom. The information compiled through questionnaires distributed to Thalassemia centres includes blood transfusion policies, blood safety, transfusion associated infection rate, and iron chelation treatment patterns. The selected data and statistical analysis includes 26 centres and a total of 1534 patients in the aforementioned 9 European Countries. *Results.* In terms of blood safety only 4 of the countries have a national blood donation scheme that follows the guidelines of WHO for safe blood collection. A third of the countries in question allow for paid donation whilst more than half allow for replacement donation. Moreover, not all of the countries perform confirmation tests and there are patients under the age of 6 that have been infected with the Human Immunodeficiency Virus, Hepatitis B and Hepatitis C viruses. In the majority of the centres (20/26) patients receive 100% packed red blood cells. Even though European Union directives and other international and national guidelines exist in most countries, these are only applied in some. Only 85% of the total number of patients receives regular iron chelation. More than 50% of these patients are treated with DFO, while the rest are treated with Deferiprone (6%), a combination of DFO and Deferiprone (30%), and Deferasirox (8%). The mean percentage of patients treated for iron overload is 90% with a standard deviation of 11.14. Significant differences were found among the different countries and these differences show correlation with the Human Development Index. *Conclusions.* This is one of the first studies to assess the access and homogeneity of treatment for patients with Thalassemia major in Europe. Our findings show variation in blood transfusion standards and treatment. Therefore, a set of general guidelines that unites treatment of patients across Europe need to be established.

0799

INEFFECTIVE ERYTHROPOIESIS IN ADULT β THALASSEMIA MAJOR PATIENTS IS AFFECTED BY COMPLIANCE TO TREATMENT

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Background. Survival of β thalassemia major (BTM) patients has improved dramatically over the past few decades as better treatment became available. Consequently, the need for centers dedicated to the treatment of adult thalassemia patients has emerged. BTM is characterized by ineffective erythropoiesis leading to severe anemia and extensive erythropoietic expansion. One of the main purposes for keeping the hemoglobin at a relatively high level is to suppress erythropoietic hyperactivity, thus reducing the severity of the adverse effects associated with hyperactive as well as ineffective erythropoiesis. Improved methods for evaluating erythropoiesis including serum soluble transferrin receptor (sTfR) level, sTfR / log ferritin ratio (ferritin index) and reticulocyte count, allow the assessment of the optimal transfusion schedule which suppresses bone marrow erythropoiesis, hence reducing the iron overload to a minimum. *Aims.* Evaluation of erythropoietic activity and suppression among multi'transfused adult BTM patients. *Patients and Methods.* Nineteen chronically transfused adult BTM patients, all splenectomized, followed and treated in the recently opened comprehensive center of adult thalassemia and hemoglobinopathies, were included in this study. Laboratory parameters reflecting erythropoietic hyperactivity and data regarding patients' compliance to regularly scheduled transfusions and iron chelation were recorded over a period of three months and retrieved from patients' files. The data were comprised of hemoglobin and hematocrit levels, mean corpuscular volume, reticulocytes' absolute number and percentage, white blood cells and platelets count, serum ferritin, bilirubin, lactic dehydrogenase (LDH), vitamin B12, folic acid, sTfR and erythropoietin levels. In addition, number of days between transfusions, cumulative number of packed cells (PC) transfused and ferritin index were calculated. *Results.* The mean patient age was 30.5 years (range 20-47). The mean cumulative number of PC's transfused over the last three months was 10.5, and the mean interval between transfusions was 22.5 days (range 15-33). Highly statistically significant correlations ($p<0.01$) were found between levels of sTfR and ferritin index, sTfR and reticulocytes' percentage and absolute number, sTfR and bilirubin, reticulocytes number and bilirubin - all reflecting medullary as well as extramedullary erythropoietic hyperactivity. Highly statistically significant correlations ($p<0.01$) were also found between parameters reflecting erythropoietic hyperactivity and compliance to treatment: ferritin and number of days between transfusions, ferritin and sTfR, ferritin and bilirubin, ferritin and low level of folic acid, erythropoietin and cumulative number of transfused PC's, LDH and erythropoietin, LDH and lower levels of pre-transfusion hemoglobin and hematocrit. *Conclusions.* We assume that poor compliance, frequently associated with irregular transfusions and lower pre-transfusion hemoglobin and hematocrit levels, and neglect of iron chelation therapy resulting in high ferritin levels, has a significant contributing role in the failure to limit ineffective erythropoietic hyperactivity in adult β thalassemia major patients.

0800

HYDROXYUREA FOR SICKLE CELL DISEASE; A SUCCESS STORY OR CAUSE FOR CONCERN?

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Background. Hydroxyurea has beneficial effects in severely affected patients with Sickle Cell Disease (SCD). Its mechanism of action has not been fully understood but it increases HbF levels, reduces neutrophils & reticulocytes, improves red-cell hydration and deformability and generates NO (potent vasodilator). It also has been shown to reduce the frequency of painful crises, acute chest syndrome (ACS), blood transfusion and hospitalizations. Nevertheless questions remain regarding long-term effects such as predisposition to myelodysplasia & malignancy. *Method.* We retrospectively studied 42 children (21 male-21 female) with SCD on Hydroxyurea between 2002 to 2006. They were initially started on 15 mg/kg/day and gradually increased to maximum-tolerated-dose (MTD). These children were initially followed by biweekly clinical-examination, medical history, height, weight, HbS, HbF, Hb levels, reticulocytes, ANC, MCV platelet-counts & oxygen-saturation for the first 2-3 months and monthly thereafter. *Results.* Their median age was 7years (range 2-17). Median total duration of treatment was 68 weeks (range 7-235 weeks). Median MTD of Hydroxyurea was 24 mg/kg/day (range 15-42 mg/kg/day). Lab-based assessment: Their Hb improved from a median of 7.2 gm/dL (range 6-9 gm/dL) on week 0 to 9.2 -(range 7-11 gm/dL), 10 (range 8-11.3 gm/dL) and 10.8 (range 8-12.5 gm/dL) on week 24, 52, & 104 respectively. HbF level increased from median HbF level of 8% (range 1.8-15.8%) on week 0 to level of 22% (range 5-32%), 28% (range 6-38%) and 32% (range 9'52%) on week 24, 52, & 104 respectively. HbS level reduced from base-line median level of 79% (range 59-89%) to 61% (range 31-84%), 52% (range 28-82%) and 48% (range 26-78%) on week 24, 52 & 104 respectively. On week, 0 mean reticulocytes counts were 10% (range 4-28%). This reduced to 5%(range 2-13%), 4%(range 2-7%) and 3.5% (range 2-6%) on week 24, 52 & 104 respectively. We also noted that Neutrophils-counts were reduced from base-line of 6.6 $\times 10^9$ to 2.5, 2.4 and 2.1$\times 10^9$ on week 24, 52 & 104 respectively. Clinical assessment: The use of Hydroxyurea resulted in reduction in the number of painful crises and episodes of ACS by 64% i.e. from median frequency 5/year (range 3-16) to 1.3/year (range 1-4) and the overall number of hospitalisations related to SCD were reduced by 81%. Children with a median HbF level of less then 15% (Range 6-25%) had the highest number of SCD related complication with median frequency of 3/year and failure to respond was also related to poor compliance. In our study most commonly noted side-effects were neutropenia & thrombocytopenia which resolved with temporarily discontinuation. No ill effects of Hydroxyurea on growth and development were noted. At weeks 52 & 104 based on Growth-Chart, for height weight & sex: 85% of children were on 25th-75thcentile, 7% above 75th and 8% below 25thcentile. *Conclusions.* This retrospective study shows Hydroxyurea is both effective and safe in severely-affected patients with SCD. Among our patients no malignancy or death occurred and probably the benefits of Hydroxyurea may outweigh these possible long-term risks. The potential for using long-term Hydroxyurea therapy in all children with SCD requires additional & careful Investigation.

0801

CARDIAC FUNCTION IN THALASSAEMICS ON COMBINED DEFEROXAMINE AND DEFERIPRONE THERAPY

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Introduction. Improved survival of well chelated thalassaemia patients has been well documented the last decade. However transfusional myocardial disease remains the complication responsible for the majority of death in thalassaemia. It has been established that iron chelation therapy with adequate doses of Deferoxamine (DFO) and Deferiprone (DFP) reduces iron related complications and consequently increases survival. *Purpose.* This study aims to evaluate the cardiac function in multi transfused iron loaded thalassaemic patients on combination therapy with DFO and DFP for a prolonged period presented with established symptomatic myocardial disease or without any clinical symptom. *Methods.* Group A: Four patients (1 male, 3 female, median age 34 years) with established myocardial disease, treated with DFO 40 mg/Kg 24 hours subcutaneously (sb) 6-7 days per week and DFP 75mg/Kg/day for 6 years. Group B: Eight asymptomatic patients (3 male, 5 female median age 32 years) with severe ironload, treated with DFO 40 mg/Kg 24hours sb 3 days per week and DFP 75 mg/Kg/day for 6 years. Two patients of group A and four of B had medical history of diabetes mellitus type II. One of A and 4 of B had been infected with HCV, progressed to chronic active hepatitis in one case per group. Cardiac function was estimated with ECHO cardiography parameters such as Fraction Shortening (SF), Left Ventricular Ejection Fraction (LVEF) and End Systolic Diameter (ESD) markers showing myocardial contractility, and cardiac iron load with Magnetic Resonance Imaging (MRI T2,T2*). Serum ferritin and liver biochemical tests were measured. *Results.* Compliance with treatment was high in both groups, with no side effects during the study period .In group A clinical symptoms improved shortly after the commencement of chelation therapy (3 months). ESD decreased and SF and LVEF increased significantly (Table 1). In group B no significant change was observed in SF and ESD but a considerable increase in LVEF was noted (Table 2). In both groups cardiac T2 and T2* relaxation time increased significantly and ferritin levels fell dramatically. *Conclusions.* Combined therapy with DFO and DFP has beneficial results mainly on heavily haemochromatic myocardial cells as indicated by the improvement of

SF, ESD and LVEF. The stability of SF and ESD in asymptomatic patients may be attributed to inadequate dosage of DFO. Long term administration of both chelators had satisfactory effect on ferritin levels and cardiac T2 and T2*.

Table 1.

Median values	2001	2002	2003	2004	2005	2006
ESD mm	38,75	35,6	31,6	30,3	30,6	30
SF %	25,66	31,6	34	37,5	36,3	35
LVEF %	48,7	59	65	59	60	69
T2 msec -	27,5	30,52	36,17	36,8	39,27	
T2* msec -	-	-	-	15,15	19,18	
Ferritin ng/ml	5500	2700	1400	744	856	383

Table 2.

Median values	2001	2002	2003	2004	2005	2006
ESD mm	30	28,25	29	28,33	29,83	28,91
SF %	37,5	38,75	39,83	33,33	39	39,23
LVEF %	-	62,1	66,8	69,5	70,8	71,4
T2 msec -	37,01	45,91	41,95	40,82	44,04	
T2* msec -	-	-	-	21,9	26,36	
Ferritin ng/ml	7440	3670	3030	2100	2015	1900

0802

BONE MICROARCHITECTURE IN THALASSAEMIA

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Background. Due to improved blood transfusion and chelation therapy, survival has been increased in thalassaemia patients with the consequence of complications like osteoporosis not seen during childhood and adolescence. The diagnosis of osteoporosis or osteopenia is assessed by endocrinological parameters and bone mineral density (BMD) measurements. *Aims.* The obvious shortcomings of conventional BMD methods like dual energy x-ray absorptiometry (DXA), can be overcome by simultaneously assessing the microarchitecture of the bone using high-resolution peripheral quantitative computed tomography (HR-pQCT), which may improve the estimation of the fracture risk in patients with thalassaemia. *Patients and Methods.* In 17 regularly transfused patients (age: 13-43 y, 9/17 female) with β-thalassaemia major (n=10), -intermedia (n=6), and CDA-II (n=1), the BMD of lumbar spine (LS) and total hip was measured by DXA (Hologic QDR1000, Waltham, USA). Age related z-scores were calculated from BMD. In addition, we assessed the volumetric BMD and the trabecular architecture of the non-dominant distal radius and tibia by HR-pQCT (XtremeCT®, SCANCO Medical AG, Bassersdorf, Schweiz). Liver iron concentration and endocrinological parameters were also determined. Nonparametric statistical analysis was used. *Results.* In 15/17 patients low BMD values (LS z-score range: -1.1 to -3.1) were measured by DXA in significant correlation with total volumetric density (range: 91-388 mg/cm^2; Rs = 0.70, p=0.002) measured by HR-pQCT at the distal radius. Seven patients with LS z-scores <-1.0 had a relatively thick cortical bone (>570 μm). In 6/17 patients (>28 y), all with latent hypogonadism, the trabecular inhomogeneity parameter TbSp SD at the distal radius deviated by more than 100% from the upper normal value (Boutroy *et al.*, 2005) and their spongiosa was porous or nearly dissolved. Patients with hypogonadism (n=9) were significantly different from normals with respect to radial TbSp SD ((p=0.02), but not to LS z-score ((p=0.4). Patients with fractures (n = 5) had lower total densities (p=0.02) and trabecular TbSp SD ((p=0.02) at the tibia and started blood transfusions (Tx-age) at a higher age (p=0.023). However, z-scores did not reflect the fracture risk in this patient group ((p=0.11). Only the trabecular thickness of the tibia seems to be correlated with the Tx-age (Rs=0.62, (p=0.007), which was higher in the patients with thalassaemia intermedia and CDA-II (> 5 y). Liver iron was mainly correlated with tibial TbSp SD (Rs=0.54, (p=0.025). Summary. In diagnosing osteopenia or osteoporosis in patients with thalassaemia z- or T-scores seem to underestimate the fracture risk because a normal cortical thickness and density may conceal a porous trabecular structure. Endocrinological failures, especially hypogonadism, were responsible for the pathological microarchitecture of distal radius and tibia, while bone marrow expansion as in thalassaemia intermedia and liver iron concentration seem to play a minor role. These first results from bone microarchitecture measurements in thalassaemia have to be confirmed by larger patient numbers of different gender and age.

0803

COMPARISON OF EFFECTS OF DIFFERENT, LONG-TERM IRON CHELATION REGIMENS ON MYOCARDIAL AND HEPATIC IRON CONCENTRATIONS ASSESSED WITH T2* MRI IN PATIENTS WITH β-THALASSAEMIA MAJOR

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Background. Iron overload in patients with β-thalassaemia, develops insidiously and leads to multi-organ failure and premature death. Exceeded iron is initially accumulated in the reticuloendothelial system; however, iron-induced cardiomyopathy is the commonest cause of death among thalassaemic patients. Magnetic Resonance Imaging (MRI) has long been considered as a useful, noninvasive tool for estimating tissue iron overload. Additionally, as new chelation agents are being developed, MRI could represent a useful marker for comparing the efficacy of different chelation regimens in removing tissue iron overload. *Aims.* The aim of this study was to compare the efficacy of different iron chelation regimens in controlling myocardial and hepatic iron with the use of T2* MRI technique and to correlate these results to clinical and biochemical parameters. *Methods.* From a pool of 167 patients with β-thalassaemia major based on our Unit in this study we selected all patients aged ≥17 years that maintained unaltered iron chelation treatment and dosage for longer than 4 years. Sixty-four patients (28M and 36F) were finally enrolled in the study. Their mean age at the time of MR scanning was 26.49±5.8 years. Regarding their chelation therapy, patients were divided into three groups: the first group, was receiving deferiprone (DFP) at a dose of 75 mg/kg/day orally, the second group was receiving deferoxamine (DFO) at a dose of 30-50 mg/kg/day at least 5 times a week by a subcutaneous infusion overnight and the third group was chelated with combination of DFO (30-50 mg/kg/day, 3-4 days a week) and DFP (75 mg/kg/day, 7 days a week). Myocardial and Hepatic T2* measurements were acquired on a 1.5 Tesla Unit, based on the protocol developed by Prof. Pennell *et al.* Additionally, ventricular volumes and ejection fractions were measured by standard cardiovascular MR techniques. Means of serum ferritin concentrations and daily iron accumulation derived from total amount of transfused red blood cells were calculated for one year prior to MR scanning.

Table 1.

Parameters	DFP	DFO	Combined	Total
n	19	23	22	64
Sex (M / F)	11 / 8	7 / 16	10 / 12	28 / 36
Age (years)	27.78 ± 4.8	29.12 ± 5.2	22.62 ± 5.2	26.49 ± 5.8
Serum ferritin	1958 ± 1364	1470.7 ± 924	1330 ± 1103	1567± 1142
Tr. Iron (mg/kg/d)	0.36 ± 0.13	0.41 ± 0.07	0.42 ± 0.09	0.4 ± 0.09
Myocardial T2*				
Normal (≥20ms)	16/19 (84%)	14/23 (61%)	19/22 (86%)	49/64 (76.5%)
Excess (<20ms)	3/19 (16%)	9/23 (29%)*	3/22 (14%)§	15/64 (23.5%)
Heavy (<8ms)	0/19 (0%)	2/23 (8.5%)	0/22 (0%)	2/64 (3.25%)
T2* (mean ± SD)	35.77 ± 18.3	23.77 ± 13*	38.05 ± 15.3§	32.24 ± 16.6
Hepatic T2*				
Normal (≥6.3ms)	3/19 (16%)	11/23 (48%)*	10/22 (45%)*	24/64 (37.5%)
Excess (<6.3ms)	16/19 (84%)	12/23 (52%)	12/22 (55%)	40/64 (22%)
Heavy (<1.4 ms)	3/19 (16%)	0/23 (0%)	3/22 (14%)	6/64 (9.5%)
T2* (mean ± SD)	3.29 ± 2.5	8.16 ± 8.4*	11.3 ± 10.9*	7.79 ± 8.8

* Significant compared to DFP group, § Significant compared to DFO group.

Results. Demographic, laboratorial and MR characteristics of the three study groups are compare in Table 1. DFP group and combined group had significantly less myocardial iron than DFO group (mean T2*±S.D.: 35.77±18.3 and 38.05±15.3 vs 23.77±13, p=0.02 and p=0.001, respectively). DFO group and combined group had significantly less hepatic iron than DFP group (mean T2*±S.D.: 8.16±8.4 and 11.3±10.9 vs 3.29±2.5,

p=0.014 and p=0.003, respectively). In the totality of patients, myocardial T2* values were inversely correlated to age (r=-0.249, p=0.024) and positively correlated to both left and right ventricular ejection fraction (r=0.33, p=0.004 and r=0.279, p=0.014, respectively). Liver T2* was strongly inversely correlated with serum ferritin concentrations (r=0.465, p=0.001). Finally, no correlation was noted between myocardial and hepatic T2* values (r=-0.043, p=0.37). *Conclusions.* Our results indicated that DFP is more effective than DFO in removal myocardial iron, whereas DFO favours in removing hepatic iron compared to DFP. Combined chelation treatment with DFP and DFO seems to better control both myocardial and hepatic iron.

0804

HIGH DEGREE OF IRON BURDEN IN TRANSFUSION-NAIVE THALASSEMIA INTERMEDIA PATIENTS

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Background. Iron accumulation in Thalassemia intermedia (TI) patients has been estimated to occur at a rate of 2-5 gm/yr and occurs mostly as a result of increased gut iron absorption secondary to chronic anemia. Increased iron burden is partly responsible for the occurrence of complications that occur in this disease such as liver fibrosis, heart failure and pulmonary hypertension; therefore, close and accurate monitoring of total body iron is necessary for ultimate proper chelation therapy. To this day, serum ferritin (SF) has been the main tool used to follow up estimated iron overload. However the inaccuracy of this test due to confounding factors and its poor correlation with biopsy-determined liver iron concentration (LIC) (*the gold standard*) has prompted researchers to develop other non-invasive techniques such as R2 MRI which has recently gained FDA approval. The poor correlation between SF level increase and LIC has been shown to be particularly true in patients with TI. *Aim.* To determine the extent of iron overload in 80 randomly selected TI patients in Lebanon. *Methods.* Following informed consent and extensive relevant medical history review, LIC using R2 MRI, SF, and other Iron markers are measured. Also Doppler echocardiography is done to detect pulmonary hypertension and left ventricular ejection fraction. This abstract reports results for the sub-group of patients who have never received any transfusion in their lifetime. *Results.* Thirty-seven completely transfusion-naïve patients are included in this analysis. This subgroup comprised 16 males and 21 females with a mean age of 27.5 years (range 8 to 63 yrs). Of these patients, 27 were splenectomized and 10 were not. Overall LIC values ranged from 0.6 to 32.10 mg Fe/g of dry tissue liver with a mean LIC of 7.76±6.78. SF values ranged from 19 to 2030 ng/ml with a mean of 957.35±553.09 ng/mL. Overall, there a significant positive correlation between LIC and SF values (p-value 0.014) and this correlation was particularly true for the subset of splenectomized patients (p-value 0.005). Statistical analysis also revealed significant correlations between LIC and splenectomy on one side and SF and splenectomy on the other (p-value 0.05 and 0.009 respectively). In addition, echocardiographic analysis of the population has revealed a tricuspid regurgitant (TR) jet in 59.5% of the studied patients. The detection of TR represents a step toward the development of pulmonary hypertension as a complication. Other complications included one case of thrombosis, 2 cases of extramedullary hematopoiesis and leg ulcers, 3 patients had hypothyroidism and 14 had osteoporosis. *Conclusions*. Although non-transfused, this group of TI patients has evidence of significant iron burden as evident by the increased mean LIC and SF. There was good correlation between LIC and SF in splenectomized transfusion-naïve TI. This is contrary to previous reports that showed discrepancy between LIC and SF in the same population. Our analysis will gain more power as we enroll all 80 patients in the study in the coming few months. In addition, we will be able to extrapolate our results further and determine the need to address the issue of chelation therapy in this iron overloaded population.

0805

NEUTROPENIA AND AGRANULOCYTOSIS IN IRON OVERLOADED THALASSEMIA MAJOR PATIENTS, TREATED WITH COMBINATION OF DEFERRIOXAMINE AND DEFERIPRONE

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Background. Combination chelating therapy with Deferrioxamine (DFO) and Deferiprone (L1) has a specific protective effect mainly because adherence to treatment is improved and because L1 has a cardioprotective effect in iron overloaded thalassemia patients. Neutropenia and agranulocytosis are the most important side effects during L1 monotherapy. Neutropenia was also described in patients treated with DFO. Some reports showed that the frequency of these effects is higher with combination treatment, than with either drug alone. *Aims.* The aim of this study was to evaluate the frequency and incidence rate of neutropenia and agranulocytosis over a period of 6 years in patients receiving combination treatment. *Methods.* A total of 345 regularly transfused thalassemia major patients, from 4 thalassemia centers were assessed representing treatment with DFO/L1 from 3 months to 6 years. Patients started combination with L1 at a dose of 75 mg/kg daily divided into three doses, together with DFO 30-50 mg/kg/2-5 times/week mainly on s.c infusion. Assessment of the blood count with differential was performed every 7-10 days. A patient was considered to have neutropenia if the neutrophil count was confirmed to be $<1.5\times10^9$/L and agranulocytosis if $<0.5\times10^9$/L. Treatment was interrupted when agranulocytosis was occurred. The incidence rates and outcomes of these adverse reactions were evaluated. *Results.* Of the 345 patients, 127 had received combination for less than 2 years, 93 between 2 and 4 years, and 125 for a period between 4 and at least 6 years. The mean length of treatment with combination was 2.83 years for a cumulative total of 1014.1 patient-years of drug exposure. Agranulocytosis was observed in 15 (4.3%) patients and neutropenia in 35 (9.8%) patients. 5 patients experienced episodes of neutropenia prior to the onset of agranulocytosis. The incidence rate for agranulocytosis was 1.4 per 100 patient-years and for neutropenia 3.4 per 100 patient-years. The median time of therapy at the onset of agranulocytosis was 5 months (range 1-84). The median time for resolution of agranulocytosis was 10 days (range 4-27). Rechallenge was not attempted in any patient with agranulocytosis, but was attempted in 33 patients with neutropenia. 25 (75.8%) of these patients experienced a new episode. Netropenia was obserevd in 31 (15.8%) of 165 non splenectomized patients and in 4 (2.7%) of the 145 splenectomized patients. No fatal event occurred as a consequence of these reactions. *Summary.* Neutropenia and agranulocytosis are significant events and can happen any time during combination treatment, but the risk may not be higher than during L1 monotherapy. The outcome of these reactions can be favourable under contnuous and strict monitoring of patients.

0806

FORT AND FORD TWO NOVEL ASSAYS FOR THE ASSESSMENT OF OXIDATIVE STRESS IN PATIENTS WITH THALASSEMIA INTERMEDIA AND SICKLE CELL DISEASE

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Aims. To assess the levels of free oxygen radicals (free oxygen radicals test, FORT) and free oxygen radicals defense (FORD) in patients with thalassemia intermedia and sickle cell disease. These two diseases seem to be involved strongly in the production of reactive oxygen species (ROS) possibly by different pathways. *Patients and Methodology.* Twenty-five patients with thalassaemia intermedia (TI) and thirty patients with sickle cell disease (SCD) were included in the study. ROS were determined using the FORt test (Callegari, Parma, Italy), which is based on the Fenton reaction. When a 20 mL blood sample was dissolved in an acidic buffer, the hydroperoxides reacted with the transition metal ions liberated from the proteins in the acidic medium and were converted to alkoxy- and peroxy-radicals. The radical species produced by the reaction, which are directly proportional to the quantity of lipid peroxides present in the sample, interact with an additive (phenylendiamine derivative) that forms a radical molecule evaluable by spectrophotometer at 505 nm (Form CR 2000; Callegari, Parma, Italy). Results are expressed as FORT U (Fort units) whereby 1 FORT U corresponds to 0.26

mg/L H2O2. Similarly, the FORD test uses, preformed stable and colored radicals and determines the decrease in absorbance that is proportional to the blood antioxidant concentration of the sample according to the Lambert Beer's law. In the presence of an acidic buffer (pH=5.2) and a suitable oxidant (FeCl3) the chromogen, which contains 4-Amino-N,N-diethylaniline sulfate forms a stable and colored radical cation photometrically detectable at 505nm. Antioxidant compounds in the sample reduce the radical cation of the chromogen quenching the color and producing a decoloration of the solution, which is proportional to their concentration. The absorbance values obtained for the samples are compared with a standard curve obtained using Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a permeable cell derivative of vitamin E commonly employed as an antioxidant. *Results.* The main results of the study showed that: a) free oxygen radical levels were increased both in TI (2.71±0.68 mmol/LH2O2) and SCD patients (3.42±0.94 mmol/L H2O2), respectively and b) free oxygen radicals defense levels were normal both in TI (1.23±0.18 mmol/L Trolox) and SCD patients (1.22±0.20 mmol/L Trolox), respectively. The intraassay and interassay coefficients of variation were 3.7% and 6.2%, respectively for FORT, while The intraassay and interassay coefficients of variation were 4.2% and 6.6%, respectively for FORD. *Conclusions.* The determination of free oxygen radicals and free oxygen radicals defense seem to play an important role in the generation of oxidative stress, an imbalance between oxidants and antioxidants that can lead to oxidative damage and is involved in the pathogenesis of several diseases, such as thalassaemia intermedia and sickle cell disease. The methodology described above is simple, reliable, rapid and reproducible.

0807

FRAMESHIFT CD41/42 (-TTCT) MUTATION IDENTIFIED BY RT-LIGHT CYCLER AND MOLECULAR CHARACTERIZATION BY AUTOMATIC SEQUENCING. FIRST CASES DESCRIBED IN SPAIN

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Background. The thalassemias are hereditary anemias. In the β-thalassaemia (β-thal) there is deficient or absent synthesis of β-globin. A high incidence of β-thal occurs in populations of Mediterranean and African origin. Smaller, but significant concentrations of β-thal exist throughout the Middle-Eastern, India, Pakistan, and China, and sporadic cases have been described in most ethnic groups. More than 150 β-thal mutations have been described. However, each population group displays its own mutations. In Spain, as in other Mediterranean regions the most frequents mutations are CD 39 (C->T); IVS-1-nt1 (G->A); IVS-1-nt6 (T->C) and IVS-1-nt110 (G->A). However, a large number of uncommon alleles have been observed both in Spain and other populations. The frameshift mutation CD41/CD42 (-TTCT) is a very common allele in Chine population but is rare in the Mediterranean region although it has been recorded in East Asian population. *Aims*. We described the three first Spanish patients with the frameshift CD41/42 (-TTCT) mutation. This mutation has been detected in a RT-PCR Light Cycler and molecularly characterized by automatic sequencing. *Methods.* Three patients from Las Palmas de Gran Canarias (Canary Island, Spain) were sent us to molecular characterization, because in the screening of the most prevalent β-thal mutations of the Mediterranean area which were carry out by Real Time PCR (RT-PCR Light Cycler) with the probe CD37/39A the temperature of melting (TM) was different, did not correspond with usual TM. The molecular characterization was carrying out by automatic sequencing in an ABI-PRISM DNA Automated Sequencer. *Results.* The most relevant haematological data are. I1a [Hb 11.4 g/dL; MVC 64.7 fL; HB A2 5.3%; Hb F 0.9%; Iron normal]; I1b [Hb 12.3 g/dL; MVC 64.6 fL; HB A2 6.6%; Hb F 1.2%; Iron normal]; I2b [Hb 11.5 g/dL; MVC 60.6 fL; HB A2 5.8%; Hb F 0.9%; Iron normal]. In the RT-PCR Light Cycler with the probes CD37/39 S (CCCTTGGACCCAGAGGTTCTTTGAGTCCT-F) and CD37/39 A (LcRED640-TGGGGATCTGTCCACTCCTGATGCTGTTATG-P) the TM of normal sequence is 70.53°C±0.80 and CD39 mutation 62.67°C±0.92 (Br. J. Hematol 2002; 119: 554-557) in ours patients the TM of mutated allele was 57.45°C. Automatic sequencing of the exon 2 of β gene globin demonstrated the deletion of 4 bp (-TTCT) between CD 41/42 which originate a frameshift and a β°-thalassemia. *Summary and Conclusions.* This work once again demonstrates the heterogeneity of β-thal in Spain. To study the phenotype of our patients could confirm if this mutation is an example of genetic migration or whether it has arisen independently in our different ethnic group. Technologically the use of methods of screening in thalassemia is not sufficient, although they can orient in the identification of the molecular alterations, therefore in molecular biology all techniques are complementary.

0808

TREATMENT WITH THE ONCE-DAILY, ORAL IRON CHELATOR DEFERASIROX IS EFFECTIVE AND WELL TOLERATED IN β-THALASSAEMIA PATIENTS WITH A HIGH IRON BURDEN

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Background. Although it is well established that transfusional iron overload leads to progressive organ dysfunction and early death unless effectively controlled, many patients are currently inadequately treated due to poor compliance with deferoxamine infusions. The 1-year ESCALATOR trial evaluated deferasirox in heavily iron-overloaded β-thalassaemia patients, all of whom had previously received deferoxamine or deferiprone. *Aims.* To evaluate the efficacy and safety of, and compliance with, deferasirox in patients with high iron burden and history of inadequate chelation. *Methods.* Entry criteria included contraindications/unsatisfactory therapeutic response to, or unacceptable toxicity/non-compliance with, previous chelation therapy. All patients had baseline liver iron concentration (LIC) ≥2 mg/g dw, serum ferritin (SF) levels ≥500 ng/mL, and initially received deferasirox 20 mg/kg/day: based on results from another study the protocol was amended mid-study to allow dose adjustment according to monthly SF trends. LIC was measured at baseline and end-of-study (EOS). Primary endpoint was treatment success, defined as LIC reduction ≥3 if baseline LIC ≥10, or final LIC of 1-7 if baseline LIC 2'<7 or ≥7'<10. Safety was assessed monthly by evaluating adverse events (AEs) and measuring LVEF (by echocardiography) and laboratory parameters (including labile iron [LPI] in 14 patients). Compliance was assessed monthly by pill counts. *Results.* Of 252 enrolled patients (167 aged 2'<16 years), 247 previously received deferoxamine, 50 deferiprone and 45 deferoxamine/deferiprone combination; some had received >1 therapy. Overall median deferasirox dose was 23.0 mg/kg/day (range 12-30). Mean baseline LIC was 18.5±9.4, decreasing by 3.5±6.3 at EOS. In patients with baseline LIC >7 (therapeutic goal, LIC reduction), LIC decrease was 4.0±6.4 (p<0.001). In those with baseline LIC ″7 (therapeutic goal, LIC maintenance), LIC change was 0.9±3.8. Overall success rate was 56.3% (p=0.02) and 57.7% (p=0.007) for the intention-to-treat and per-protocol populations, respectively. Baseline SF was 4260±3079, falling by 458±1388 (p<0.001). A marked reduction in LPI was observed post versus pre-dose at baseline and week 4 (p<0.001). LPI levels were within normal parameters by week 16, which continued to EOS. Mean baseline LVEF was 65.0±6.9%, increasing by 1.7±7.8% (p<0.001). Per-the-protocol amendment, doses were escalated to 25/30mg/kg/day after a median 26 weeks in 192 patients (76.2%). Overall median compliance was 99.3% (range 72-120). 247 (98%) patients completed 1-year. The most common drug-related AEs were transient, mild/moderate vomiting (8.3%), nausea (7.1%) and skin rash (7.5%). Two deaths, unrelated to study medication, were reported. No clinically significant changes in median levels of renal or liver function markers were observed. There was no drug-induced agranulocytosis, neutropenia or arthralgia. *Conclusions.* Deferasirox, at a starting dose of 20 mg/kg/day, had a safety/tolerability profile compatible with long-term use and treatment compliance was high, leading to overall maintenance or reduction (depending on therapeutic goal) in iron burden. As patients had high iron burden due to previous inadequate chelation, dose escalation to 25/30 mg/kg/day was required in 76.2% patients not achieving target reduction in iron burden. This highlights the importance of timely dose adjustments to achieve therapeutic goals. The impact of higher doses on iron burden is being further evaluated in an ongoing extension trial.

0809

CURRENT APPROACH TO CHELATION THERAPY IN THALASSAEMIA MAJOR FOLLOWING 6 YEARS OF COMBINED CHELATION THERAPY

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Aims. To identify the golden standard in chelation therapy in order to reduce total body iron overload, reverse hemosiderosis complications and improve quality of life of Thalassaemia major patients. *Patients.* 50 patients (24 males, 26 females), 8-48 years, on combined chelation with oral Ferriprox® (75-9 0 mg/kg daily) and Desferal® (20-50 mg/kg, SC or IV 2-6 days/week), in a 6 year regimen strictly adjusted and closely monitored on individual needs. *Methods.* - Monthly ferritin. - 10d CBC with leukocyte differential & monthly biochemical evaluation. - Biannual cardiac function evaluation by ECG & Cardiac Echo. - Annual heart & hepatic iron quantification by MRI, T2 & T2*. - Annual endocrine evaluation included OGTT, IVGTT, Insulin, TSH, FSH, LH dynamic tests, FT3 and FT4. *Results.* 1) Mortality was eliminated after combined chelation. On Desferal® monotherapy mortality ranged from 13.3-14.3% in the last decade. 2) Currently, 35% of the highest compliers switched to Ferriprox® monotherapy (75-100 mg/kg daily). 3) Ferritin levels decreased dramatically: - In the highest compliers (35%) mean ferritin is 68 μg/L. -In the moderate compliers (54%) mean ferritin is 232 μg/L. - In the low compliers (11%) mean ferritin is 2.421 μg/L. 4) In 12/50 (24%) patients with pre-existing heart dysfunction, symptoms reversed and heart medications stopped. Ventricular dimensions and function in Echo normalized. LVEF increased from 54 to 72% (p<0.0001). None of the patients presented new onset or a worsening of cardiac function. 5) MRI analysis T2*Heart & T2*Liver revealed a significant reduction of iron overload over the time. Both mean T2*H and T2*L completely normalized. 6) Initially, 7/50 pts, mean age 38.7 years, had Insulin-dependent Diabetes and 22/50 (44%) had Impaired Glucose Tolerance (mean age 32.5 years). Following combined chelation, Glucose Metabolism improved. Insulin production increased and Insulin Resistance was reduced. An increment of insulin sensitivity ISIHOMA was manifested in most patients. 7) Initially, 14/50 pts had overt hypothyroidism and received thyroxin substitution. 13/50 pts had subclinical hypothyroidism (elevated basal TSH>5 IU/mL or increased TSH during TRH test. 23/50 pts were euthyroid. Following combined chelation, TSH quantitative secretion, calculated as the AUC, significantly decreased. 5/14 hypothyroid patients stopped treatment and 7/13 pts with subclinical hypothyroidism normalized. 8) 12/24 males were hypogonadal on testosterone every 20-30d. After combined chelation, FSH levels improved significantly at times: 0' & 30'. Testosterone remained unchanged. 4/12 hypogonadal patients showed normal pituitary response and normal testosterone levels after they stopped treatment for the dynamic test. 8) 19/26 females were hypogonadal and received hormone substitution. 2 became pregnant with IVF and 2 (eugonadal) with spontaneous ovulation. *Conclusions.* In our Unit combined chelation with Ferriprox® & Desferal® is the treatment of choice in thalassaemia major. The safety profile was acceptable. Cardiac function improved with reversal of cardiac complications. Abnormal glucose tolerance reversed. Thyroid function improved, particularly at early stages of hypothyroidism. Intensification of iron chelation might also be of benefit for the pituitary-gonadal axis. The quality of life of Thalassaemia patients has improved tremendously. Most importantly mortality was eliminated.

0810

INCREASED MATRIX METALLOPROTEINASE-9 LEVELS AND ACTIVITY IN SICKLE CELL DISEASE PATIENTS

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Sickle cell disease (SCD) is characterized by a chronic inflammatory state; however, the mechanisms underlying this inflammation are unclear. Zinc matrix metalloproteinases (MMPs) are members of a family of enzymes that cleave extracellular matrix (ECM) proteins. MMPs play an important role in physiological and pathological processes, including embryogenesis, wound healing, inflammation, arthritis, cardiovascular diseases, pulmonary diseases and cancer. MMP-9 is not limited to the ability to break down ECM but is also extended to the modulation of cytokines as well as to leukocyte migration. The aim of this study was to compare MMP-9 and TIMP-1 levels and the activity of MMP-9 in the plasma and mononuclear cells (MC) of healthy subjects (HS) and in SCD patients on or off HU therapy. Gelatin zymography was used to measure MMP-9 activity and ELISA was used for MMP-9 and TIMP-1 determination. The expressions of the MMP-9 and TIMP-1 gene were measure in MC by Real-Time PCR. Results are expressed as the fold change in gene expression as compared to the negative calibration control. After densitometric analysis of zymograms, a significant increase (p=0.03) in the activity of pro-MMP-9 was observed in the plasma of SCD patients (27.09±1.99 average pixel, n=30) compared with the HS (20.96±1.35 average pixel, n=29). Pro-MMP- 9 activity in the plasma of SCD patients on HU (SCDHU) was greater (27.2±1.35 average pixel, n=17); however this difference was not quite significant (p=0.07). MC-MMP-9 activity was significantly higher in SCD patients compared with HS (66.2±6.0 average pixel, n=11, 22.5±5.5 average pixel, n=13, respectively, p=0.0001) and HU therapy significantly reduced MMP-9 activity (31.5±6.3 average pixel, n=8, p=0.001). MMP-9 levels were significantly increased in the plasma of SCD patients (20.99±1.52, n=32), compared to HS (13.96±1.64, n=16, p=0.02), although no effect of HU therapy on these augmented levels was observed (SCDHU; 23.66±2.93, n=22, p>0.05). MMP-9 levels correlated significantly with total WBC counts (r=0.4221, p=0.01) in SCD patients (on and off HU), as well as with neutrophil counts (r=0.4436, p<0.001), but not with MC counts. TIMP-1 was significantly (p<0.0001) higher in SCD patients not on HU therapy (83.69±6.73, n=15; n=14) compared to HS (41.26±3.75, n=11; n=10,). HU therapy had no significant effect on increased TIMP-1 levels (84.14±4.69, n=12, p>0.05) when compared to SCD patients not on HU. Real-time PCR demonstrated that the MMP-9 and TIMP-1 mRNA expression in MC were not significantly different between control and SCD patients on or off HU therapy. Data demonstrate that the level and activity of plasma MMP-9 are significantly increased in SCD, and that these levels are not affected by HU therapy. Moreover, MMP-9 levels correlate with leukocyte counts in SCD. Interestingly, the MC-MMP-9 activity is higher in SCD and this increase was reserved by HU therapy. These are the first data suggesting that MMP-9 activity and levels are increased in SCD disease indicating that MMP-9 may be a useful marker for inflammation in SCD and may even have a role in manifestations of this disease.

0811

OUTCOME AND COMPLICATIONS OF SPLENECTOMY AND CHOLECYSTECTOMY IN A LEBANESE SCD POPULATION

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Background. SCD is one of the most prevalent hemoglobinathies in Lebanon. A significant number of patients with SCD develop cholelithiasis and possibly cholecystitis and affected individuals may experience acute splenic sequestration and hypersplenism. Splenectomy and cholecystectomy are the two most frequent surgeries performed in SCD patients and these surgeries can hold significant postoperative complications. *Aims.* 1. Identify outcome and complications of splenectomy and cholecystectomy in a Lebanese SCD population and 2. Delineate predictors of complications. *Methods.* A retrospective review of charts of 306 SCD patients followed in 3 centers specialized in inherited hemoglobin disorders was undergone. Data pertaining to demographics, clinical events, perioperative clinical status and care, and outcome were collected and analyzed. 65 splenectomies and 53 cholecystectomies were performed. Indications for splenectomy were recurrent acute splenic sequestration and/or hypersplenism, and for cholecystectomy were cholelithiasis and/or acute cholecystitis. Since 1995, laparoscopic surgery was the modality used in both types of surgeries. 83% received transfusions preoperatively and all patients were given adequate hydration, high oxygen tension intra operatively and incentive spirometry postoperatively. Fisher's Exact test was employed to study the association of binary data with the two surgeries. Logistic regression analysis was used to model probability of surgery as a function of multiple fixed covariates. The SAS v8.2 (Cary, N.C.) was used to analyze the data. A p-value less than 0.05 was considered significant. *Results.* Of patients who underwent splenectomy, 43% were females and 57% males; 61.5% had sickle cell anemia (SCA) and 38.5% sickle-β thalassemia (ST). As for those who underwent cholecystectomy, 37.25% were females and 62.75% males; 84.3% had SCA and 15.7% ST. Patients undergoing splenectomy were younger than those undergoing cholecystectomy [mean age 9.82 years versus 16.62 years, (p=0.0001)]. Median operative

time was 50 minutes, and median hospitalization duration was 2 1/2 days. Postoperative complications were minimal and included acute chest syndrome in 4 SCA patients after cholecystectomy. No fatalities or other postoperative complications were noted. Bivariate analysis showed that patients with regular blood transfusions and joint necrosis were more likely to undergo splenectomy and/or cholecystectomy ($p<0.05$). Pain and osteomyelitis were associated with cholecystectomy only ($p<0.05$). Borderline significance was found for the association of splenectomy and/or cholecystectomy with acute chest syndrome. Death, stroke, dactylitis and age at diagnosis were not associated with either splenectomy or cholecystectomy ($p>0.05$). Multivariate analysis showed that there are no predictors of splenectomy in the data studied, however predictors of cholecystectomy were osteomyelitis (OR: 4 [95%] CI: 1.14-14.2) and joint necrosis (OR: 3.7 [95%] CI: 1.55-9.17). Predictors of postoperative complications could not be studied due to the small number of complications. *Conclusions*. Splenectomy rate in the Lebanese SCD population is higher than that seen in the North American populations. That may be attributed to persistent splenomegaly in the former population. The current incidence (3.4%) of post surgical ACS is lower than previously reported and may be due to strict adherence to meticulous perioperative care. Splenectomy and cholecystectomy can be performed in patients with SCD with minimal morbidity and no mortality.

0812

HEREDITARY AND SECONDARY HEMOCHROMATOSIS MAY BE SAFELY TREATED, IN SELECTED CASES, BY THERAPEUTIC ERYTHROCYTAPHERESIS

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B*ackground.* Therapeutic erythrocytapheresis (EA) is an alternative therapy to phlebotomy, effective in removing iron excess in patients with hereditary (HH) and secondary hemochromatosis (SH). This technique is useful in order to quickly remove a large volume of erythrocytes, saving plasma, proteins and clotting factors. EA may be combined with erythropoietin to obtain more benefits. *Aims*. In order to evaluate the efficiency and safety of this technique in these pathologies we reported a retrospective analysis of our experience with 24 patients treated by EA. *Methods*. In Caserta's Hospital and in University, 24 patients (aging 39-66 years) have been studied: 4 affected by C282Y homozygous mutation, 8 H63D heterozygous mutation and chronic hepatitis (5 HBV-related and 3 HCV-related), 12 patients with chronic hepatitis HCV-related without HFE mutation. Clinical and biochemical data indicated marked iron overload and hepatic failure; all patients underwent liver biopsy that showed cirrhosis and confirmed severe iron overload, moreover the portal hypertension and splenomegaly were echo-graphically evident. The C282Y homozygous patients showed the most severe clinical conditions, in fact all of them were also affected by multiorgan failure: diabetes, hypogonadism and hypokinetic cardiomyopathy. The remaining 20 patients showed high serum levels of Ferritin associated with a high Transferrin's saturation, but however lower than the 4 subjects homozygous with HH. All patients were referred to us for counseling since the phlebotomy was contraindicated: in fact some of these patients showed hypotension, causing dizziness and faintness, during phlebotomy or prolonged fatigue after venesection, while other patients cannot undergo to this treatment because of low levels of plasmatic proteins (probably due to liver failure). EA was performed using a computer-guided discontinuous flow cell separator. Our protocol of treatment consists in an erythrocytapheresis every ten days until iron depletion; for every apheretic procedure 210±20 mL of packed RBC were removed, while plasma with 250 mL of saline solution were re-infused to the patient. For the patients' safety, procedure parameters were set to achieve a final hematocrit not lower than 34%. *Results*. The baseline laboratory evaluations was (mean±SD): Transferrin Saturation=77?7%, Ferritin=978±403 ng/mL, Alanine Transferase=75±33 IU/L. At the end of treatment all patients achieved iron depletion, showing a Transferrin Saturation lower than 40%, a Ferritin lower than 100 ng/mL, and a normalization of Alanine Transferase serum levels. *Conclusions*. EA is effective in removing iron excess such as phlebotomy, but, in our opinion, it presents three advantages: 1) hypovolemia is absent because the collected blood volume is compensated by autologous plasma and saline solution; 2) the frequency of apheretic procedures is decreased respect to (weekly) phlebotomy, because for every EA the amount of red blood cell removed is higher than phlebotomy; 3) in these cirrhotic patients, EA permits the saving of platelets, plasma proteins and clotting factors. EA, compared to phlebotomy, presents two disadvantage: 1) it requires adequate equipment and trained staff; 2) it is more expensive. In conclusion, for these last reasons, we suggest that this procedure should be proposed for selected patients whose clinical conditions do not permit the execution of the phlebotomy.

0813

CARRIER SCREENING OF β-THALASSEMIA AND HEMOGLOBIN VARIANTS IN THE CENTRAL PART OF PORTUGAL

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B*ackground.* Hemoglobinopathies are the most common monogenic hereditary disorders worldwide, it has been estimated that ~7% of the world population are Hemoglobinopathie carriers. Although these conditions occur at their highest frequency in populations with an history of endemic malaria, migration movements have ensure that they are now encountered in most countries, with a wider genetic variability. In Portugal, the study of hemoglobinopathies is recommended for pregnant women and young adults with anaemia and/or MCV <80 fL and/or MCH<27 pg and for those who, despite having normal haematological parameters, are included in the at-risk populations (Portuguese from the south of the country or immigrants from parts of Africa, Indian subcontinent, south-east Asia and Este Europe). *Aims.* In order to evaluate whether it is sufficient or not to restrict this study to the population who follow the criteria mentioned above, we initiated in 2005 a screening program, supported by the national program Saúde XXI, to identify the carriers of hemoglobinopathies among the population of the central part of Portugal. We present the results obtained after two years of screening. *Methods.* The target population was young adults and pregnant women until 18 weeks of gestation attending the primary care medical centres. Since there are no facilities to collect venous blood in these medical centers, we establish a methodology to identify Hb variants and to quantify Hb A2 and Hb F accurately based on the study of capillary blood samples. This new procedure exploits the Kit Hb A1c Capillary Collection System from BioRad, followed by HPLC analysis in the Variant II Hemoglobin Testing System with the β thal Short Program-Biorad. *Results*. Among the 17.281 capillary blood samples studied, 367 were carriers of an hemoglobinopathy: 249 β-thalassemia minor, 74 HbS, 22 Hb D-Punjab, 4 HbC, 14 Hb Lepore and 4 with less common variants: Hb N-Baltimor, Hb Shenyang, Hb Banbury and Hb Cocody. Among the 74 HbS carriers, 20 are Caucasians from the Portuguese ancestry, 43 are Africans and 18 Brazilians. *Conclusions*. The frequency of the β-chain hemoglobinopathies carriers found was of 2%. 20/74 of the HbS carriers are of Portuguese origin although HbS is usually found in Africans. We found also 22 Portuguese individuals with Hb D-Punjab, the identification of these 42 carriers alert to the importance of not disregard the search of this two types of variants in the Caucasian population, since both are clinically silent but result in sickle cell disease when combined with HbS. We found six couples at risk of having an affected baby (3 for β-thal major and 3 for sickle cell disease), two of these required pre-natal diagnosis. Giving the percentage of carriers detected we think that this study should be applied in the overall fertile Portuguese population. The methodology used in this program is efficient and simple and allows large population screening of carriers in primary care medical canters with no facilities to collect venous blood.

Thrombosis II

0814

UNSUSPECTED PULMONARY EMBOLISM: IMPACT ON CANCER PATIENTS' SURVIVAL

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Background. The association between cancer and thrombosis is well established. The development of a thrombotic episode, either deep venous thrombosis (DVT) or pulmonary embolism (PE), significantly relates with worse cancer prognosis and shorter survival. With the use of multidetector computed tomography (MDCT) for the staging and follow-up of cancer patients, the diagnosis of incidental PE is increasing, but its clinical relevance in cancer patients is yet unknown. *Aims.* To describe the clinical features and outcomes of adult cancer patients diagnosed with unsuspected PE using MDCT, comparing completely asymptomatic patients and patients with non-specific symptoms subsequently attributed to PE. *Methods*. Retrospective analysis of consecutive cancer patients diagnosed with unsuspected PE by MDCT performed for disease staging or follow-up. Clinical data and D-dimer levels were assessed at diagnosis. Statistical analysis was performed using Chi-square tests with SPSS v11.0 software. *Results.* Between February 2002 and September 2006, 63 cancer patients (mean age 58±12 years, 47.6% males and 52.4% females) were diagnosed with incidental PE. The most frequent histologic type of cancer was adenocarcinoma (58.7%), especially gastro-intestinal. Most patients (66.7%) had metastatic disease at the time of the PE diagnosis. 58.7% of unsuspected PE were found at main pulmonary arteries, 27% at lobar arteries, while 14.3% were at segmental or subsegmental level. Clinical records showed that 58.7% of patients presented clinical symptoms that could be attributed to PE. During follow-up (median 17 months), 27 patients (42.9%) died, most of them due to cancer progression. Mortality was significantly lower in completely asymptomatic patients than in patients with non-specific symptoms subsequently attributed to PE (29% vs 57%; p=0.032). In adition, mortality was related to higher D-dimer levels at diagnosis (607 ng/mL vs 474 ng/mL; p=0.048). No significant association between mortality and age, sex, cancer stage or subtype or PE location was found. *Conclusions*. The absence of clinical symptoms of PE in cancer patients with unsuspected PE diagnosed by MDCT seems to be associated with a better prognosis of the neoplastic disease than patients with symptoms later attributed to PE. pecial attention to signs and symptoms suggestive of PE in this population is required, in order to achieve an early diagnosis and treatment. D-Dimer levels could be prospectively evaluated as prognostic markers after diagnosis of unsuspected PE.

0815

COINHERITANCE OF FV-LEIDEN MUTATION AND FV-HR2

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The activated protein C resistance (APCr) phenotype is found approximately in 40% of thrombophilic patients. The factor V gene Leiden (Arg506Gln) mutation is considered interesting, looking for other factor V gene mutations associated to thrombophilia: The HR2 haplotype, the factor V Cambridge (Arg306Thr), the factor V Hong Kong (Arg306Gly) and the FV Liverpool (Ile359Thr). Because both factor V R506Q and the HR2 haplotype are very frequent, the effect of their coinheritance on the risk of venous thromboembolism may present a clinically relevant issue, and screening for HR2 in carriers of factor V R506Q should be considered. For this purpose we studied the presence of mutations in a group of 90 symptomatic patients (who suffered from one or more thromboembolic events). DNA was extracted from whole blood using the Qiagen extraction kit and polymorphism was determined by PCR method. Using the CVD strip assay, we found out that 22 patients were carriers of the FV Leiden mutation, 7 of the HR2, 10 of the G20210A polymorphism of prothrombin, 19 of the L34 mutation of XIII, 8 of the b-Fib 455G>A of fibrogen and 12 were homozygote (4G/4G) of PAI-1. Among these patients, a young individual was found to be double heterozygote, carrying FV Leiden and FV HR2. This, 21 year-old patient, suffered an episode of pulmonary embolism. A gene mutation test took place, because his mother in the age of 47 presented a thrombosis of the left hand and she was found to be heterozygote for the FV Leiden mutation. His grandmother passed away in the age of 68 because of ischemic stroke. Haemostasis control was normal, except from the pathological value of aPCR (39 sec, normal values >120 sec). The patient was prescript antivitamin-k for a long-term treatment. Double heterozygosity for factor V R506Q and HR2 conferred a 3- to 4-fold increase in the relative risk of venous thromboembolism compared with factor V R506Q alone. No increase in risk of venous thromboembolism could be demonstrated when the HR2 haplotype was associated with inherited thrombophilic defects other than factor V R506Q. The median age at first event was lower when the 2 defects were associated (46 v 52 years).

0816

RISK FACTORS AND SEQUELAE OF CEREBRAL VEIN THROMBOSIS: A FIVE YEAR RETROSPECTIVE AUDIT

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Background. Cerebral vein thrombosis (CVT) is a rare cause of neurological dysfunction that has, in recent years been diagnosed more frequently, in part due to improved non-invasive diagnostic techniques. A recent meta-analysis that re-evaluated the true natural history of CVT has identified a lower mortality rate and long-term prognosis than previously reported. The annual incidence is approximately 3-4 cases per million[1] however it can be associated with significant morbidity. A prothrombotic risk factor or direct cause is found in 85% of patients. The risk of sinus thrombosis is increased in the last trimester of pregnancy and after delivery[2] and the frequency of peripartum and postpartum sinus thrombosis is 12 cases per 100,000.[3] A study by Cantu et al. concluded that CVT during pregnancy and puerperium, while of more acute onset with a progressive course is often benign[2] Little data exists on the incidence of recurrent sinus thrombosis in women who have had a first event. In one series of 68 cases, no recurrent events occurred.[5] A systematic review by Dentali et al. showed the incidence of recurrence to be 2.8% in a combined cohort of almost 1100 patients. Almost ninety percent of surviving patients either recover fully or have only a mild functional or cognitive deficit.[6] *Objectives.* In the light of this recent meta-analysis, we evaluated risk factors, morbidity and mortality associated with CVT in a single Irish centre. *Patient and methods.* A retrospective audit was performed of patients diagnosed with CVT in an Irish hospital during January 2001 to July 2006, evaluating presenting symptoms, risk factors, survival and neurological sequelae. *Results.* Eighteen patients were identified, with a median age of 31 years. The commonest presenting symptoms were headache, vomiting and visual disturbance. 77% had at least one risk factor. 39% of events were associated with oral contraceptive use, pregnancy or postpartum state. 11% were associated with a local infection. 6% experienced a recurrent CVT. 6% died as a direct result of the event. 23% developed permanent neurologic deficit. *Conclusions*. Our study identified similar risk factors and rates of morbidity and mortality to those in recent literature, demonstrating that CVT has a more benign natural history than previously suspected.

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0817

THROMBOPHILIC RISK FACTORS AMONG 16 LEBANESE PATIENTS WITH CEREBRAL VENOUS AND SINUS THROMBOSIS

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Background. Cerebral venous and sinus thrombosis (CVST) is a multifaceted disorder. It has multiple predisposing factors including pregnancy and postpartum state, surgery, head trauma, arterio-venous malformations, infection, paraneoplastic and autoimmune disease, and particularly the use of oral contraceptives. In addition, inherited blood coagulation disorders play an important role in the development of CVST. *Aims.* In the present study, the frequency of inherited thrombophilic risk factors among 16 CVST patients has been evaluated. *Methods.* Sixteen patients, diagnosed with CVST at the American University of Beirut Medical Center, were identified by medical chart review and thrombophilia screening. We have tested these patients for the prothrombotic mutations G20210A of the Factor II gene and G1691A of the factor V Leiden gene, and the C677T methylenetetrahydrofolate reductase (MTHFR) gene. We also assessed the presence of acquires thrombophilia (lupus anticoagulant and anticardiolipin antibodies) and other risk factors (pregnancy and postpartum state, surgery, paraneoplastic disease, and the use of oral contraceptives). *Results.* Of the 16 CVST patients, half were female and the mean age was 22.9 years (range: 1-46 years). Five out of the 16 CVST patients (31.2%) showed the G1691A mutation of factor V (4 were heterozygous and 1 was homozygous). The frequency of the C677T MTHFR genotype was 50% (8/16) in patients (2 of them were homozygous). Four of the patients (25%) had both factor V Leiden and MTHFR mutation. None of the patients expressed the G20210A Factor II allele variant. Three of the patients had positive antiphospholipid antibodies; one of them was heterozygous for factor V Leiden and MTHFR mutations. At the time of CVST, 2 females were taking oral contraceptives; one of them was homozygous for factor V Leiden (this was the only patient in this series to have a positive family history of venous thromboembolism). Four patients were known to have a malignancy; 2 had non-Hodgkin's lymphoma, 1 had acute lymphoblastic leukemia and the last had breast cancer. *Conclusions.* Despite the limitation of sample size, we identified an inherited coagulopathy at high rate in our patients. Combined inherited thrombophilia was also present in 25% of patients. In addition, the prevalence of acquired thrombophilia and other contributing factors was also high. This finding supports the impression of a multifactorial process leading to CVST in Lebanese patients.

0818

GENETIC POLYMORPHISM OF HEMOCOAGULATION FACTORS IN PATIENTS WITH PORTAL THROMBOSIS

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Background. the prehepatic portal hypertension due to portal thrombosis was belived to be a rare condition (about 10% of all cases of portal hypertension). Chronic myeloproliferative disorders (MPD) were considered to be the main cause of thrombotic complications in adult patients (pts), and thrombophilia - to be a predisposing factor. *Aims.* To study genetic polymorphism of hemocoagulation factors in patients with portal thrombosis. *Materials and Methods.* 54 patients (21 males, 33 females, Median age - 43 years) with portal thrombosis confirmed by Doppler sonography were included into this study. The period from the first manifestation of portal hypertension (splenomegaly, varicose dilatation of esophageal veins) to examination in our Center varied from 4 to 480 months (Median 60 months). Only 25 (46%) patients had bone marrow morphology of myeloproliferative disorders. The other patients had normal pattern of bone marrow, normal blood picture or cytopenias. All patients were screened for polymorphism of 10 genes of hemocoagulation system. *Results.* Polymorphisms of genes hemocoagulation system were found in 67% pts, including mutation in genes of methylenetetrahydrofolatereductase in 26 (48%) pts (5 - homozygous), plasminogen activator inhibitor-1 (PAI-1) in 22 (41%) pts (8 -homozygous), β-Fibrinogen-455 G-A heterozygous - in 12 (22%) pts, integrin α II - in 14 (26%) pts (3 - homozygous). Heterozygous mutations in genes factor V Leiden were found in 2 cases, prothrombin - in 1 pt., factor VII - 4 pts, P-selectin (CD 162) - in 4 pts. The majority of patients (35%) had combination of 3 and more polymorphisms. Combination of MPD and polymorphisms of genes hemocoagulation system was found in 23 (43%) pts. *Conclusions.* The use of molecular diagnostic methods reveals the high frequency of genetic polymorphism of hemocoagulation factors in patients with portal thrombosis The presence of the hereditary thrombophilia proves the necessity of prescribing anticoagulant/antiaggregant therapy in patients with portal thrombosis.

0819

PROTHROMBOTIC RISK FACTORS IN CHILDREN WITH HENOCH-SCHONLEIN PURPURA

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Background. Henoch-Shönlein purpura (HSP) is an acute immunoglobulin A (IgA)'mediated leukocytoclastic vasculitis that affects primarily children. The dominant clinical features of HSP are cutaneous purpura, arthritis, abdominal pain, gastrointestinal (GI) bleeding due to bowel infarction with or without perforation, orchitis, and nephritis. Stroke may occur in some of the cases. The precise etiology of the disease is not clear, but HSP typically follows an upper respiratory tract infection. Yilmaz *et al.* (2005) reported that in patients with HSP, fibrinogen, D-dimer, TAT complex, PF-1, PF-2, vWAg, and RiC of levels were significantly higher during the acute phase were than during recovery phase and significantly higher than those of control subjects. The severity of disease was significantly correlated with TAT, PF-1, PF-2, vWAg, and D-dimer levels. These factors may be risks for thrombosis seen in vasculitis. Several hereditary factors have been associated with increased risk of thrombosis. Of these, protein C (PC), protein S (PS), anti-thrombin (AT) and thrombophilic gene mutations [factor V Leiden (FVL), prothrombin G20210A, and C677T variant of methlenetetrahydrofolate reductase (MTHFR)] were well described risk factors for thrombosis. Herein, we report the results of prothrombotic risk factor analysis of patients with HSP. *Methods.* Twenty-four patients (16 girls, 8 boys) with HSP and 48 (32 girls and 16 boys) healthy age- and sex-matched children were investigated for risk factors for thrombosis (PC, PS, AT, factor VIII, factor IX, vWF, FVL, prothrombin 20210A, and MTHFR C677T) and its relation with clinical signs. Blood samples were drawn minimum three months elapsed from the diagnosis. *Results.* The rates for skin, joint, GI, and renal involvement were 100%, 50%, 8%, and 4,1%, respectively. Plasma PC, PS, AT, factor VIII, factor IX, and vWF levels were within normal limits. The percentages of FVL and MTHFR mutations were 4.1% and 54.1%, respectively. Factor V Leiden was found in 10.4% and MTHFR mutation in 54.1% of the controls. Prothrombin 20210A was not found in the patient group whereas it was shown in 2% of the controls. There was no relationship between the prothrombotic risk factors and clinical manifestations. *Conclusions.* Well-known prothrombotic risk factors possibly do not play a role in clinical manifestations of Henoch-Schonlein purpura.

0820

THE IG.G ANTI-PROTHROMBIN ANTIBODIES ASSOCIATE WITH THE VENOUS THROMBOEMBOLISM IN WOMEN

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Background. The association of antiphospholipid antibodies with either vascular thrombosis or fetal morbidity is known as the antiphospholipid syndrome (APS). Although the pathogenic role in the thrombotic complications of the APS seems clear for the lupus anticoagulant the association between thrombosis and other related auto-antibodies is not obvious. Data of the clinical association between the venous thromboembolism (VTE) and anti-prothrombin (aPT) antibodies are scarce and inconclusive. We aim to know any possible association between these antibodies (using methods for both isotypes) and the VTE. Population and methods. We studied 79 unrelated patients with an episode of VTE (objectively diagnosed) and 79 healthy controls of similar gender and age [in overall 62.0 (12.8) y, 48.7% males]. Among the patients, 25 (32%) have suffered a pulmonary embolism (PE) with or without a related DVT and 24 (30%) a recurrent episode of VTE. We performed a commercial enzyme linked immunoassay (ELISA) for both isotypes (IgG and IgM) of the aPT antibodies in serum seven months after the acute thrombotic episode. *Results.* We tested several arbitraries and statistical cut-off values obtaining a weak positive association with

the 97.5th percentile (5.2 U/mL) generating an OR=4.3 (1.0-18.7) (p<0.05). Seven patients and one control have serum concentrations of the IgG isotype of aPT antibodies higher than 7.0 U/mL (99th percentile) equivalent to an OR of 7.2 (1.2-48.5) (p<0.05). Stratifying by sex the association increases in females [OR= 8.0(1.2-52.8) and 11.1 (1.0-119.9) respectively (p<0.05)] and disappears among the males. When we excluded the 23 carriers (16 patients and seven controls) of a thrombophilic mutation (FV Leiden or prothrombin mutation) the association even increases. By contrary, the IgM isotype of aPT antibodies did not show any association with the VTE. *Conclusions.* If further prospective studies using larger cohorts could confirm these results, high titers of the isotype IgG of the aPT antibodies might have a significant utility in the evaluation of the VTE risk.

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0821

GENETIC POLYMORPHISMS IN SELECTINS AND TISSUE FACTOR IN YOUNG PATIENTS WITH VENOUS THROMBOEMBOLISM

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Background. Microparticles play an important role in thrombus formation and propagation. Genetic variation in proteins related to microparticle function, such as selectins and tissue factor, may influence the thrombotic expression in young patients. *Aims.* We aimed to analyze the role of functional polymorphisms on P-selectin (Thr715Pro), P-selectin glycoprotein ligand PSGL-1 (VNTR and M62I) and tissue factor (TF) (-1208I/D) and its relationship with venous thrombosis in young patients. *Methods.* Eighty-two patients with VTE younger than 50 years were included. Forty-one patients had idiopathic VTE (Group 1) (mean age 34.4, SD 9.61 years; female gender 51%) and 41 had VTE associated with the common thrombophilia polymorphisms factor V Leiden or prothrombin G20210A (Group 2) (mean age 34.2, SD 11.7 years; female gender 63%). Deficiencies on protein C, protein S, antithrombin and antiphospholipid antibodies were excluded. In addition, eighty-two age and sex matched healthy controls were also included. P-selectin Thr715Pro, PSGL-1 VNTR and M62I and TF -1208I/D polymorphisms were determined by PCR. *Results.* The genotype distribution observed in controls was: P-selectin Thr715Pro (Thr/Thr 84%, Thr/Pro 15%, Pro/Pro 1%), TF -1208I/D (I/I 24%, I/D 57%, D/D 19%), PSGL-1 VNTR (AA 69%, AB 29%, BB 2%), and PSGL-1 M62I (M/M 90%, M/I 10%). There were no differences in genotype distribution among controls and Group 1 or Group 2 patients for P-selectin Thr715Pro, TF -1208I/D, and PSGL-1 VNTR polymorphisms. For PSGL-1 M62I polymorphism, the prevalence of heterozygous (M/I) carriers was significantly higher in patients of Group 2 (25%) than in patients of Group 1 (15%) or in controls (10%) (p<0.05). *Conclusions.* M62I polymorphism on PSGL-1 may contribute to the thrombotic expression in young patients carriers of factor V Leiden or prothrombin G20210A polymorphisms.

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0822

P-SELECTIN, P-SELECTIN GLYCOPROTEIN LIGAND AND TISSUE FACTOR POLYMORPHISMS IN CANCER-RELATED THROMBOEMBOLISM

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Background. Patients with cancer frequently develop venous thromboembolism (VTE), even though its pathogenesis is not well understood. Microparticles play an important role in thrombus formation and genetic variation in proteins related to their function, such as selectins and tissue factor, may influence thrombus development. *Aims.* The aim of the study was to analyze the role of functional polymorphisms on P-selectin (Thr715Pro), P-selectin glycoprotein ligand PSGL-1 (VNTR and M62I) and tissue factor (TF) (-1208I/D) in patients with cancer and VTE. *Methods.* We included 80 patients with cancer who presented VTE episodes related to the tumour (42 male gender; mean age 62.8, SD 11.1 years) and 160 sex and age matched healthy controls. Fifty-six patients had deep venous thrombosis in lower extremities, 18 in upper extremities, 1 in visceral veins, and 20 pulmonary embolism. One patient was heterozygous for factor V Leiden and no prothrombin G20210A mutation, or deficiencies on protein C, protein S, antithrombin or antiphospholipid antibodies were observed. Plasma microparticles were quantified by an ELISA. P-selectin Thr715Pro, PSGL-1 VNTR and M62I and TF -1208I/D polymorphisms were determined by PCR. Soluble P-selectin (s-PSel) and soluble TF (s-TF) were determined by ELISA in platelet poor plasma. *Results.* Cancer patients had significantly elevated microparticles, s-Psel an s-TF in comparison with controls (p<0.001 in each). The prevalence of heterozygous carriers of M62I polymorphism was significantly higher in patients with cancer and VTE (22.5%) than in controls (8,7%) (p=0.006; OR= 3,02, 95% confidence interval: 1,42-6,47). There were no differences in genotype distribution between controls and cancer patients for TF -1208I/D, P-selectin Thr715Pro and PSGL-1 VNTR polymorphisms. *Conclusions.* M62I polymorphism on PSGL-1 may contribute to the development of VTE in cancer patients.

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0823

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 hemolitic 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 microvascolature. 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 CD45 charactherized EPCs. Methods. We investigated 14 TTP patients with complete remission of disease, evaluating plasma ADAMTS-13 activity, EPCs plasmatic level and colony formation and CECs plasmatic level. Patients were diagnosed from 1985 to 2005 and were M/F 3/11, with a median age of 35.5 years (range 24-60). Two of the cases were trauma-induced, one partuum-induced, one pregnancy-induced, while the remaining cases had no evident cause. At diagnosis nine patients were treated with plasma-exchange and prednisone, one with plasma infusion and prednisone, one with only plasma-exchange, one with plasma-exchange, vincristine and prociclide, one with plasma-exchange, vincristine and prednisone, one with plasma-exchange ciclophosphamide, vincristine, immunoglobulines, prednisone and rituximab. Three out of 14 patients relapsed once, one relapsed twice and one relapsed three times. At the moment of the study, six of them were on antiplatelet therapy, one was taking azathioprine, one was taking both. *Results.* Among five relapsed patients, three (60%) had a level of ADAMTS activity less than 20% (15%, 10% and 8% respectively, the first of them relapsed three times), while only three of nine non-relapsed patients (33%) had a level of ADAMTS activity less than 20%. No differences were evidenced between the groups relapsed/ non relapsed as regards the plasma levels of EPCs, CECs and endothelial colonies. Two out of three patients under azathioprine threatment (66.7%) had undectectable levels of CECs, while the same datuum was evidenced only in two out of eleven patients who were not receving immunosoppressive therapy (18.2%). A positive correlation between numbers of EPCs and the levels of VWF was evidenced, while we didn't find any positive correlation between numbers of CECs and VWF. *Conclusions.* Studies are under way to clarify the issue of VWF abnormalities as related to EPCs and CECs in TTP.

0824

PROPOSAL FOR THERAPEUTICAL STRATEGIES TO PREVENT THE RECURRENT ABORTIONS, REPEATED FETAL LOSS OR THE INTRAUTERINE FETAL GROWTH RETARDATION IN WOMEN WITH COAGULATION ABNORMALITIES AND/OR GENETIC THROMBOPHILIC MUTATIONS

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To date, several therapeutical programmes have been performed in healthy women with idiopathic recurrent abortions (ARI), repeated fetal loss (RFL) or intrauterine fetal growth retardation (IFGR). In this sce-

nario, many drugs in varied combinations have been employed to achieve the completion of the pregnancy. Nevertheless, the results are often unsuccessful. 46 consecutive healthy women, age ranging 18-52 years (median 39.2 yrs), with body mass index in normal average, with ARI from 2 to 9 in each subject and/or RFL at 2nd trimester of pregnancy were treated early at he onset of their umpteenth gestation. 11 of them have had one birth in the list of their ARI and/or RFL. 8/47 had venous thromboembolism in juvenile age. In 40 pregnant females we documented a hypercoagulation proneness and/or several genetic thrombophilia mutations: ATIII deficit (n=1), PC reduction (n=3), PS deficiency (n=6), LAC (n=7), ACA (n=11), APA (n=8), A beta2 -GP-I (n=9), increased PAI-1 activity (n=13), elevated D-dimer (n=33), Prothrombin mutation (n=15 etherozygousity and n=2 homozygous state), FV Leiden (n=10 etherozygousity and n=1 homozygous condition), combined FII and FV Leiden (n=5), MTHFR (n=30 etherozygous pattern and n=10 homozygous state). Hyperomocysteinemia (> 14 μg/mL) was found in 9 subjects. The following therapeutical strategy was performed: oral prednisone (10 mg/die) until the 2nd trimester of the pregnancy and 5 mg/die till the eight month, aspirin (50-100 mg/die), and subcutaneous low molecular weight heparin (LWMH at mean dosage 4.000 IU/die) till the eight month, intramuscular progesterone until the fourth month. Folic acid and vitamin B complex were orally administered until the completion of the pregnancy. If necessary, oral iron preparations were also given. Body mass index and diet style were monitored. 41 females successfully completed their pregnancy; 2 of them had eclampsy at the sixth month with miscarriage. From our observations we suggest that the reported therapeutical combination may prevent ARI or RFL and IFGR even in women with personal documented plasma hypercoagulation and genetic thrombophilic risk factors.

0825

PROTHROMBIN ACTIVITY AND ANTIGEN IN CARRIERS OF PROTHROMBIN G20210A MUTATION

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Background. Prothrombin (FII) G20210A mutation and elevated plasma prothrombin activity are known risk factors for thromboembolic diseases. *Aims.* The aims of our study are 1) the evaluation of prothrombin activity (FII:C), antigen (FII:Ag) in patients affected by heterozygous prothrombin G20210A mutation compared to a normal population, 2) the evaluation of prothrombin antigen and activity in heterozygous prothrombin G20210A muatation in patients with and without thromboembolic events. *Methods.* We studied 63 subjects (14 men and 49 women, median age = 42 years, range =24-72 years) with the prothrombin G20210A polymorfism (Group A) and a control population (14 men and 49 women, median age = 40 years, range = 20-76 years) (Group B). In the Group A 34/63 (54%) patients presented previous thromboembolic events. Normal ranges of FII:C and FII:Ag were 80-126 U.I./dL (Mean=103, SD=11.5) and 50-150 U.I./dL (Mean = 100, SD= 25) respectively. *Results.* In the Group A, mean value of FII:C was 122.6 U.I./dL (SD=18.9) and 105.7 U.I./dL (SD= 12) in the Group B (p<0.0001). In the Group A, mean value of FII:Ag was 141.8 U.I./dL (SD=48.2) and 98.6 U.I./dl (SD=22) in the Group B (p<0.0001). Mean value of FII:C was 124.9 U.I./dL in patients with previous thromboembolic events and 119.8 U.I./dL in patients without previous thromboembolic events (p=0.2847). Mean value of FII:Ag was 142.2 U.I./dL in patients with previous thromboembolic events and 141.4 U.I./dL in patients without previous a thromboembolic events (p=0.9462). *Conclusions.* In our patients FII G20210A polymorfism is associated with higher levels of prothrombin antigen and activity compared to normal population. Patients with the FII G20210A polymorfism and previous thromboembolic events do not show higher levels of prothrombin antigen and activity compared to those without thromboembolic events.

0826

EVIDENCE FOR THE SIGNIFICANCE OF THE PLASMA COAGULATION TESTS AND GENETIC THROMBOPHILIC MUTATION STUDIES TO PREVENT THE IDIOPATHIC RECURRENT ABORTIONS OR REPEATED FETAL LOSS IN HEALTHY WOMEN

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Recently plasma coagulation abnormalities and inheritable genetic thrombophilic mutations have been considered as potential risk factors in the etiopathogenesis of the idiopathic recurrent abortion(ARI)and repeated fetal loss(RFL)in apparently healthy women.In this regard, several methodological approaches have been proposed with inconclusive and/or conflicting. *Results.* 46 consecutive healthy women,age ranging 18-52 yrs (median 39.2 yrs), with body mass index in normal average,with ARI from 2 to 9 in each subject and/or RFL at 2nd trimester of pregnancy were evaluated at the onset of their umpteenth gestation.11 of them have had one birth in the list of their ARI and/or RFL.8/47 had venous thromboembolism in juvenile age.Plasma coagulation tests, LAC, fibrinolytic status (PAI-1,D-dimer)were assayed by standard methods. Anticardiolipin antibodies (ACA IgG and IgM), antiphospholipid antibodies (APA IgG and IgM) and anti ʔ2-glycoprotein-I antibodies (Aʔ2-GP-I) were determined. Plasma homocysteine was also measured.Genetic thrombophilic mutations (13 tests) were performed by Reverse Dot Blot-Real Time PCR. Our results showed in 39 females hypercoagulation proneness and/or several genetic thrombophilia mutations:ATIII deficit (n=1),PC reduction(n=3), PS deficiency(n=15), LAC(n=7), ACA(n=11), APA(n=8), Aʔ2-GP-I(n=9),increased PAI-1 activity(n=11), elevated D-dimer(n=31), Prothrombin mutation(n=15 etherozygousity and n=2 homozygous state), FV Leiden(n=10 etherozygousity and n=1 homozygous condition), combined FII and FV Leiden(n=5), MTHFR(n=30 etherozygous pattern and n=10 homozygous state). Hyperomocysteinemia (>14 μg/mL) was found in 9 subjects. Our data strongly suggest that an accurate clinical evaluation in conjuction with a well-reasoned screening for hypercoagulability and/or genetic thrombophilia profile must be considered in women with ARI or RFL.So,therapeutical approaches can be established to preventing the idiopathic recurrent miscarriages in healthy women.

0827

PROGNOSTIC SCORE INDEX AND D-DIMER IN PULMONARY TROMBOEMBOLISM

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Background. Pulmonary tromboembolism (PE) is a clinicopathological entity with elevated mortality within 3 months from diagnosis. D-dimer is widely used as an initial test in the management of suspected PE and elevated troponin levels are an indirect marker of ventricular dysfunction. Right ventricular dysfunction is a very important prognostic factor related to a higher mortality ratio. *Aims.* 1) To analize clinical and biological parameters in patients diagnosed of PE and possible correlations with troponin levels at diagnosis. 2) To investigate two different D-dimer assays and its association clinical severity of PE and biological features. 3) To evaluate the feasibility of an assessment for clinical diagnosis of suspected PE. *Patients and Methods.* We reviewed clinical files of patients diagnosed of PE in our centre between January 2005 to December 2006. Demographic parameters, D-dimer, PaO2, oxygen saturation (OS), troponin levels and pulmonary hypertension (measured by echocardiography) were evaluated. Diagnosis was made on clinical suspect and computed tomography. We developed a 6-parameter score based on the next features with a numeric assignment: Signs or symptoms of deep vein thrombosis (DVT) (4), immobilization or surgery in the previous 4 weeks (3), previous DVT/PE (3), active cancer (2), pregnancy (1), oral anticonceptives (OA) intake (1). A score of 3 or less indicates PE as unlikely (low risk); score from 4 to 5 was classified as intermediate risk, a score more than 6 identified with high probability of PE. *Results.* 67 patients were evaluated and divided in two groups according to D-dimer assay employed: Group A corresponding to D-Dimer PLUS (Dade Behringʔ) (normal: 0-192 μg/L) and Group B D-Dimer HS (HemosILTM) (normal: 0-230 μg/L). Group A characteristics: 38 patients (21 male/17 female). Median age: 72.5 years (29-98). Median D-dimer at diagnosis 520 μg/L (184-4999). 20 patients (52.63%) showed signs or

symptoms of DVT, 3 (7.89%) previous DVT, 6 (15.73%) surgery within 4 weeks before, 7 (18.42%) active cancer, 1 (2.63%) refered OA intake. 5 patients (13.15%) died. Group B characteristics: 29 patients (7 male/22 female). Median age: 69 years (30-92). Median D-dimer at diagnosis 2096 μg/L (237-7198). 14 patients (48.27%) showed signs or symptoms of DVT, 7 (24.13%) required immobilization in the previous 4 weeks, 2 (6.89%) surgery within 4 weeks before, 5 (17.24%) active cancer, 1 (3.44%) was pregnant. 2 patients (6.89%) died due to complications of acute event. In group A we found a statistically significant between score of clinical assessment and OS. We found a positive correlation coefficient (r 0.508) statistically significant (p=0.005) between clinical score and troponin levels in group B. There were no differences between the two D-dimer assays with regard of severity of PE, PaO2, troponin, pulmonary hypertension and other features. *Conclusions.* a) The score of clinical assessment employed in our series may be useful as a right ventricular dysfunction marker; neverthless the prognostic value of this score need to be evaluated in properly designed pospective studies. b) Results of the two D-dimer assays are not related to the severity of PE assessed by various markers in our serie.

0828

ORAL ANTICOAGULATION REVERSAL: FRESH FROZEN PLASMA OR PROTHROMBIN COMPLEX CONCENTRATE?

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Background. Oral anticoagulation (OA) reversal may be required due to bleeding, asymptomatic over anticoagulation or surgical procedures. The anticoagulant effect may be reversed by a variety of *Methods.* vitamin K, fresh frozen plasma (FFP) and prothrombin complex concentrates (PCCs). *Aims.* To analize the characteristics of patients that have required rapid OA reversal and to evaluate the results of the aplication of an OA reversal guidelines. *Patients and Methods.* We reviewed the clinical files, demographic and clinical parameters, and complications of patientes who requiered OA reversal during a period of twelve months in our center. According to our guidelines all patients received vitamin K and FFP (rapid but no immediate reversal required) or PCC (immediate reversal). *Results.* 73277 samples from 6464 anticoagulated patients were processed. 75 pacientes (1.16%) (33 male / 42 female), median age 76 years (range 38-86) received PCC; 40 patients (0.63%) (14 male / 26 female), median age 79 years (range 49-92) received FFP. All patients were under acenocumarol treatment but 3 (2 in PCC group and 1 in FFP taking warfarin). OA indications in PCC patients: atrial fibrillation (FA) 45 (60%), prosthetic heart valve (PV) 19 (25.3%), deep venous thrombosis (DVT)/pulmonary embolism (PE) 8 (10.6%), myocardiopathy 3 (4%), stroke 1 (1.3%), acute arterial ischemia (AAI) 1 (1.3%). OA indications in FFP patients: FA 22 (55%), PV 9 (22.5%), DVT/PE 5 (12.5%), stroke 2 (5%) and mitral stenosis (MS) 2 (5%). In PCC group 8 (10.6%) patients pressented a time from starting OA to PCC administration less than 4 weeks, in the remaining 67 patients median of OA treatment was 37.5 months (range 2-168). In FFP median time of OA was 47 months (0-168). Median INR at the moment of PCC administration 3.18 (range 1.44-9.8) and 3.93 (range 1.55-12) in FFP group. 22 patients in FFP group and 29 in PCC group were over therapeutic anticoagulation levels.

Table 1.

FFP		PCC	
Indication/Number	OA	Indication/Number	OA
Gastrointestinal Bleeding/16	10 AF, 4 PV, 1 DVT, 1 MS	Intracraneal Bleeding/19	11 FA, 7 PV 1 DVT
Urgent surgery/11	4 FA, 3 DVT, 1 PE, 1 MS	Gastrointestinal Bleeding/17	10 FA, 5 PV, 1 DVT, 1 AAI
Mucous Bleeding/6	3 AF, 2 Stroke, 1 PV	Urgent surgery/28	16 FA, 7 PV, 2 DVT, 1 PE 2 DM
Hemoptysis/2	1 AF, 1 PV	Hemoptysis/2	2 FA
Intestinal obstruction	1 PV	Hematuria/2	1 FA, 1 PV
Intracraneal Bleeding/1	1 FA	Invasive procedure 7	4 FA, 2 PV, 1 DVT
Retroperitoneal Bleeding/1	1 FA		
DIC/1	1 PV		
Thigh hematoma/1	1 FA		

38 (50.6%) patients received PCC and 18 (45%) received FFP due to major bleeding. Incidence of major bleeding in our serie 0.86%. After PCC all patients normalized INR whereas 7 (17%) patients treated with FFP pressented INR over 1.5 after administration. Thrombotic complications after PCC administration were not observed. 3 (7.5%) patiens treated with FFP presented complications due to volume overload (cardiogenic shock, congestive heart failure and pulmonary edema). In the group of PCC 6 (8%) patients died due to complications of the acute event and 4 (10%) in the group of FFP. *Conclusions.* a) Anual incidence of major bleeding in OA patients in our serie is slightly less than described in literature. b) Administration of PCC is a safety and effective treatment, able to achieve immediate reversal of OA. c) According our results FFP takes more time to correct anticoagulation; at the same time we must consider volume overload complications due FFP administration.

0829

ELUSIVE ASSOCIATION BETWEEN THE ANTICARDIOLIPIN ANTIBODIES AND THE VENOUS THROMBOEMBOLISM

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Background. The association of some antiphospholipid antibodies and the venous thromboembolism (VTE) seems categorically established for the lupus anticoagulant but is less conclusive for the other related autoantibodies. Among them, the possible thrombogenic role attributed to the anticardiolipin antibodies (ACA) is one of the most controversial mainly due to methodological issues. In fact, the available clinical data are influenced by the absence of reference material for calibration and by discrepancies about the cut-off values used for the interpretation of *Results.* We aim to know any possible association between anticardiolipin antibodies (using methods for IgG and IgM isotypes) and the VTE with regards to the influence of different cut-off levels. *Population and methods.* We studied 574 subjects, 297 consecutive patients suffering an episode of VTE objectively diagnosed (105 with pulmonary embolism and 192 with deep venous thrombosis) and 277 healthy controls of similar gender and age [in overall 61.9(13.9) y, 50.8% males]. The 21.5% of the thrombotic episodes were recurrences. The blood sample of patients was obtained several weeks after the oral anticoagulation withdraw (around seven months after the acute thrombotic episode). We performed a commercial enzyme-linked immunoabsorbant assay (ELISA) for both isotypes (IgG and IgM) of the anticardiolipin antibodies (ACA) in serum and a molecular identification of the main thrombophilic mutations (FV Leiden or prothrombin 20210A). *Results.* ACA of IgG isotype. Using the manufacturer criteria the 5% of samples (19 patients and 10 controls) were considered as positives (>15 U/mL) (non-significant difference). We tested several arbitraries and statistical cut-off values (90th, 95th, 97.5th, 99th percentiles) obtaining a non-significant association with the exception of a very weak one using the value of 20 U/mL [OR= 3.1 (1.1-9.2) (p<0.05)]. Stratifying by sex this association persisted among females alone. When we excluded the 70 carriers (53 patients and 17 controls) of a thrombophilic mutation (FV Leiden or prothrombin variation) this association even increases [OR=6.5 (1.0-41.8) (p<0.05)]. ACA of IgM isotype. Only five samples (less than 1%) were considered as positives following manufacturer criteria (>15 U/mL). The observed differences on titers of anticardiolipin antibodies of the IgM isotype between patients and controls did not reach statistical significance with the several considered cut-offs. *Conclusions.* The assessment of the anticardiolipin antibodies in a late phase of the VTE could have a minor significance with the possible exception of those directed against the Ig.G isotype in women without thrombophilic mutations. Furthermore, the utilization of in-house cut-off values (using local normal populations following the contemporary advice) did not contribute to reveal the association between the ACA and the VTE.

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0830

FACTOR V HR2 HAPLOTYPE: A RISK FACTOR FOR VENOUS THROMBOEMBOLISM IN INDIVIDUALS WITH ABSENCE OF FACTOR V LEIDEN

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Background. Venous thromboembolism (VTE), including deep venous thrombosis (DVT) and pulmonary embolism (PE), occurs secondary to a number of hereditary and acquired disorders of hemostasis. A recent-

ly recognized polymorphism in Factor V (FV) gene (His1299Arg; also named HR2) has been reported to be a possible risk factor for the development of VTE. The significance of HR2 has not yet been tested in VTE patients in Lebanon. *Aims.* The aim of this study is to evaluate the role of HR2 polymorphism in VTE in a group of Lebanese patients. *Methods.* Seventy-three VTE patients (40 males and 33 females) and 125 healthy subjects (72 males and 53 females), were examined for HR2. The patients were admitted to our medical center between March 2003 and December 2005. The average ages for the patients and controls were 45.0±19.1 years and 35.4±18.6 years, respectively. The DNA was extracted and stored at -80°C for later use and the CVD StripAssay (ViennaLab, Austria) was used. The thermocycler program consists of an initial step of 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 3 minutes. The amplification products are selectively hybridized to a test strip which contains allele-specific (Wild type and Mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. *Results.* Thirty-six patients (82.2%) had DVT, 8 patients (11%) had PE, and 5 patients (6.8%) had both. There was significant association between FV Leiden and VTE (p<0.005). HR2 haplotype was present in 12 patients (all heterozygous) and 13 healthy subjects (11 heterozygous and 2 homozygous), with a prevalence of 16.4% and 10.4%, respectively (p=0.159). VTE patients with normal FV were 2.7 times more likely to have the HR2 haplotype as compared to controls with normal FV (p=0.036, 95% CI=1.04-7.06). Among the patients who had the HR2 haplotype, 3 patients had the Factor V G1691A mutation and 1 had the prothrombin G20210A mutation. The distribution of patients and controls with respect to FV and FV HR2 is shown in Table 1. *Conclusions.* We conclude that the FV HR2 haplotype significantly affects the risk of VTE in subjects with normal FV. This finding entails that screening for the HR2 haplotype should be done in VTE patients with normal FV. Moreover, this haplotype may coexist with other thrombophilic mutations. Further larger studies are needed to be conducted in the Lebanese population in order to confirm the importance screening patients with VTE for the HR2 haplotype.

Table 1. The distribution of patients and controls with respect to FV Leiden and FV HR2.

	FV Leiden - /FV HR2 +	FV Leiden - /FV HR2	FV Leiden + /FV HR2 +	FV Leiden + /FV HR2	Tota
Patients (%)	3 (4.1%)	36 (49.32%)	8 (10.96%)	26 (34.25%)	73
Controls (%)	0 (0%)	14 (11.2%)	13 (10.4%)	98 (78.4)	125

0831

LACK OF ASSOCIATION BETWEEN ANTI-Β-2 GLYCOPROTEIN I ANTIBODIES AND VENOUS THROMBOEMBOLISM

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The antiphospholipid syndrome (APS) is defined as the occurrence of thrombosis and/or recurrent fetal loses in association with a confirmed positive result for some kind of antiphospholipid antibodies such as lupus anticoagulant or anticardiolipin antibodies (Sapporo criteria). Some investigators postulate that β-2 glycoprotein I (B2GPI) is the most relevant autoantigen for this syndrome and recently, the inclusion of the anti-B2GPI antibodies among their diagnostic criteria was proposed by an international consensus (Miyakis S et al, JTH 2006: 295). However, the independent role of these antibodies in the venous thromboembolism (VTE) remains controversial probably due to methodological issues. Our purpose was to know the possible association between anti-B2GPI antibodies (using assays for IgG and IgM isotypes) and the VTE jointly with the possible influence of different cut-off points. *Population and methods.* We studied 584 subjects, 307 consecutive patients suffering an episode of VTE objectively diagnosed (112 with pulmonary embolism and 195 with deep venous thrombosis) and 277 healthy controls of similar gender and age [in overall 61.8(14.0) y, 50.8% males]. The 21.8% of the thrombotic episodes were recurrences. The patients were sampled several weeks after the oral anticoagulation withdraw (around seven months after the acute thrombotic episode). We performed a commercial enzyme-linked immunoabsorbant assay (ELISA) for both isotypes (IgG and IgM) of the anti-B2GPI antibodies in serum and the molecular identification of the main thrombophilic mutations (FV Leiden or prothrombin 20210A). *Results.* Anti-B2GPI (IgG isotype). Using the manufacturer criteria (positive >15 U/mL) we found high titters of antibodies in 41 subjects: 24 patients (7.8%) and 17 controls (6.1%) (non-significant difference). Considering the requirement for internal cut-off points we tested the 90th, 95th, 97.5th and 99th percentiles of our normal population obtaining a non-significant association in every case. Likewise occurs following the gender stratification or the exclusion of 73 carriers (56 patients and 17 controls) of thrombophilic mutations. The prevalence was similar among the recurrent cases. Anti-B2GPI (IgM isotype). We only found 20 positive samples (3.4%) using the manufacturer criteria (>15 U/mL) with similar prevalence in patients and controls. Considering the same internal cut-off points, we observed a borderline excess of women (p=0.03) among the patients, using the 95th percentile (10 U/mL) that declines at P97.5th and disappears at P99th. *Conclusions.* Our study did not allow associate the VTE and high levels of the IgG or IgM anti-B2GPI antibodies even by means of in-house ranges of positivity. The recommended utilization of a different cut-off (calculated for each laboratory with the 99th percentile) did not increase the potential utility of this assay. Why these upper ranges of antibodies could work apparently better when they are considered as diagnostic criteria for the APS (among lupus patients) than as independent related factors in a random population with thrombosis is unclear (although might be an issue of pre-test probability).

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Transfusion medicine and vascular biology

0832

AN AUDIT OF RED CELL CONCENTRATE, FRESH FROZEN PLASMA AND CRYOPRECIPITATE USE WITHIN THE WEST OF SCOTLAND: A 5-YEAR REPEAT ANALYSIS

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B*ackground.* The Regional Haematology Audit Group covers a population of 3 million (the area of the regional transfusion centre). An audit was undertaken in 2000 analysing use of plasma products and was published: discrepancies in use were identified between hospitals and the following represents a repeat analysis over the interim 5-year period. *Aims.* The main aim was to show a change in FFP and cryoprecipitate use between the 2 study periods in order to assess the effectiveness of modifications introduced following the last audit. Additional areas evaluated were the assessment of Rhesus status on prescription of FFP and the laboratory systems in place to recognise those requiring methylene blue-treated FFP (MBTFFP). *Methods*. A questionnaire was distributed to all 15 regional hospitals with an 87% response rate. This compared April 2004-March 2005 with the same period 1999-2000. *Results.* The results showed a decline in blood product use during this period despite a 2.9% increase in bed numbers within the region. Since 2000, there was a 9.1% reduction in red cell transfusions, but an even greater reduction in FFP (17%) and cryoprecipitate (20%) used. Using bed numbers as a surrogate reflection of activity, a mean of 13.65 red cell units were issued per bed (range 7.68-21.32, SD 4.51) in 2000, compared to11.99 in 2005 (range 5.15-17.91, SD 3.34) p=0.046. Repeat analysis identified a substantial change in practice in previously outlying hospitals in terms of blood product use; two hospitals, shown to have excessive use of both FFP and cryoprecipitate in the initial study (presumed due to automatic issue in 'crash-packs') now compare favourably to the rest of the region This practice has now been discontinued at both institutions and as a result they are mainly responsible for the observed reduction in use within the area. Resultant savings of around £250,000 per annum have been estimated due to this alteration in practice. A marked difference in blood product use between the two cardiac surgery centres was observed, which was thought to be attributable to regular use of thromboelastography in one. Interestingly, 15% of centres still continue to take Rhesus D status into consideration on FFP issue whilst 23% consider it in females of childbearing age, contrary to recent guidance from the BCSH. It was also observed that no standard laboratory procedures were in place for the issue of MBTFFP and only 50% of the hospitals with potential use of this product had it in stock. *Conclusions*. At a time when national FFP use is rising, we have shown a reduction in red cell, FFP and cryoprecipitate use. The report demonstrates that effective audit can improve and change clinical practice - something that is often questioned.

0833

TRANSFUSION-RELATED ACUTE LUNG INJURY (TRALI) FOLLOWING INTRAVENOUS IMMUNOGLOBULIN (IVIG) INFUSION

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Background. Transfusion related acute lung injury is an uncommon life threatening complication of blood product transfusion. It is defined in the recent Canadian consensus conference on TRALI as a new episode of acute lung injury (ALI) that occurs during or within 6 hours of completed transfusion, and which is not temporally related to a competing aetiology of ALI. The Serious Hazards of Transfusion (SHOT) data from the United Kingdom showed an incidence of 70 confirmed cases per 16 to 17 million-blood components transfused. However, the true incidence of TRALI remains unknown mainly due to the previous lack of standardisation of its definition and under recognition. Virtually all plasma-containing blood products have been implicated, particularly when they are sourced from female donors. Despite the ample amount of leucocyte antibodies in IVIG, this blood product has rarely been reported to cause TRALI. *Aim*. To report an adverse event to IVIG that has rarely been reported on literature review. We also highlight that TRALI is a clinical rather than a laboratory diagnosis. *Methods.* A 55-year-old patient with angioimmunoblastic T-cell lymphoma (AITL) was admitted 3 weeks after his first cycle of oral fludarabine and cyclophosphamide with severe pneumonia. Three days following his clinical and radiological recovery from pneumonia, he was given prophylactic IVIG as he had secondary hypogammaglobulinemia, which is an unusual finding in AITL (polyclonal increase in immunoglobulins is commonly found). He developed non-cardiogenic pulmonary oedema 40 minutes into the IVIG infusion. This manifested itself by a drop in O2 saturation to 77% and bilateral pulmonary infiltrates on a chest X-ray. His O2 saturation improved to 91% on 15 L/min oxygen and he made a complete recovery within 12 hours of onset. *Results.* Testing in our regional Blood Transfusion Centre revealed a positive direct granulocyte immunofluorescence (GIF), which persisted for 11 weeks following the TRALI event, and a negative indirect GIF assay. This suggests the presence of granulocyte-bound rather than free antibodies. A crossmatch between the patient's granulocytes and IVIG was positive. No anti-HLA Class I or II antibodies were detected on standard complement-dependent cytotoxicity (CDC) and ELISA assays respectively, in either the patient or the residual IVIG infused. S*ummaryand Conclusions.* TRALI still remains a challenging diagnosis despite the proposed guidelines for defining it. The same consensus panel that proposed the definition agrees that TRALI is a clinical and radiographic diagnosis rather than a laboratory one. Based on this, our patient fulfilled the clinical diagnostic criteria. AITL is commonly associated with autoimmune phenomena secondary to marked immune activation evidenced by increased expression of an array of cytokines. A significant proportion of patients have circulating autoantibodies. There is at least 1 case report in which antigranulocyte antibodies were noted in association with AITL. Such phenomena can 'prime' neutrophils, which upon exposure to the IVIG infused are activated resulting in endothelial damage and capillary leak. IVIG has rarely been reported to cause TRALI. We postulate that the immune dysregulation associated with AITL predisposed to the development of TRALI following IVIG in this case.

0834

CONTRIBUTION OF STORAGE-INDUCED PLATELET MICROPARTICLES TO THROMBIN GENERATION

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Background. Procoagulant activity of platelet microparticles (MPs) has been extensively described. The number of platelet-derived MPs increases significantly with time during platelet storage. The understanding of storage-induced MPs' contribution to thrombin generation (TG) could be important *in situ*ations when additional procoagulant effect of platelet transfusions is undesirable (e.g. disseminated intravascular coagulation). *Aims.* To assess the contribution of storage-induced platelet MPs to thrombin generation at the beginning and the end of platelet concentrate shelf life. *Methods.* Platelet concentrates used were leucodepleted pooled preparations, sampled initially at Day 2 and at the end of shelf life (6 to 8 days, mean 7.25 days, n=13). Platelet concentrates were stored under standard blood bank conditions, at 22°C on a lateral shaking device. After sampling, platelet concentrates were diluted with autologous platelet-free plasma (PFP) and mixed 1:2 with freshly prepared donors' PFP containing corn trypsin inhibitor (CTI). This step was introduced in order to prevent contact activation as well as to replenish plasma clotting factors that may have been lost during platelet storage. Thrombin generation was triggered with 0.5 pM TF and recorded using automated calibrated thrombography. Parameters such as lag, peak thrombin, initial and maximal velocity of TG were studied in platelet-rich (PRP) and PFP, as well as in PFP filtered through 0.1 μm filter. Initial velocity of TG (Vini) was defined as the slope of the TG curve between 1 and 10 nM/min. Vmax was calculated as the maximal velocity of TG. *Results.* Using low-dose TF trigger in presence of CTI, we observed minimal or no TG in freshly prepared PFP obtained from normal donors. However, in PFP obtained by centrifugation of platelet concentrates, we demonstrated significant procoagulant activity both at early and late stages of storage. All of the TG parameters tested in MP-containing PFP of stored platelets were not significantly different from those produced by PRP with a platelet concentration of 50×10^9/L. Further increase in platelet concentration to 100×10^9/L did not lead to a significant rise in procoagulant activity. Removal of MPs by filtration of PFP, derived from Day 2 platelet concentrates, led to a significant prolongation of lag time and reduction in thrombin peak, Vini and Vmax as compared with unfiltered PFP. TG was completely abolished when PFP from 7 day-old platelets was subjected to filtration. We observed a significant loss of both platelet-dependent and MP-dependent procoagulant activity in platelet concentrates stored for 7 days as compared with ones stored for 2 days. Although all of the tested parameters pointed to a significant loss of thrombin generation potential with platelet storage, most noticeable dif-

ferences were observed in the propagation phase of TG as indicated by a reduction in Vmax (3.9-fold for PRP, p=0.008 and 3.7-fold, p=0.006 for PFP) by the end of shelf life. *Conclusions.* MPs contained in platelet concentrates make a significant contribution to TG potential, that is comparable with procoagulant activity supported by platelet concentration of 50×10^9/L. MP-dependent procoagulant activity does not increase with platelet storage, but decreases in parallel with platelet function.

0835

POLYETHYLENE GLYCOL PRECIPITATION AND ION EXCHANGE CHROMATOGRAPHY ELIMINATE A TSE-MODEL AGENT SPIKED INTO THE INTRAVENOUS IMMUNOGLOBULIN PRODUCTION PROCESS

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Introduction. The variant Creutzfeldt-Jakob disease (vCJD) is a transmissible spongiform encephalopathy (TSE) mainly present in United Kingdom (UK). The agent (PrPSc) is a prion essentially found in the Central Nervous System, however it can also be detected in the lymphatic system and non-neural tissues at much lower concentration. The vCJD agent has never been detected in blood with methods capable of detecting it in other tissues, so if present, the concentration must be very low. No case of vCJD has ever been reported by plasma-derived products, despite the four cases of possible transmission of the vCJD agent related with blood cell transfusions. There is no evidence of vCJD transmission by plasma products in spite of active surveillance specially among haemophilic patients. Many different published studies (Foster P.R. Vox Sang., 2004; 87 (suppl.2), S7-S10) indicate that the production processes of plasma-derived products remove TSE-model agents. *Aims.* Grifols has studied the capacity of the 8% PEG (polyethylene glycol) precipitation step and the DEAE Sephadex treatment followed by several filtrations of Flebogamma's production process to eliminate an experimental TSE agent. *Methods.* PEG precipitation and DEAE Sephadex treatment followed by filtrations were studied on a laboratory scale model. Hamster adapted Scrapie strain 263K was spiked into intermediate process material to determine PrPSc removal through the purification process, for each step studied. Two different spikes were used, clarified hamster brain homogenate and detergent treated hamster brain homogenate. Two runs were performed for each type of spike and process step. PrPSc was detected by western blot. *Results.* PEG precipitation removed an average of 3.6 log10/mL PrPSc, 3.40 log10/mL PrPSc when clarified hamster brain homogenate was used and 3.70 log10/mL PrPSc when detergent treated hamster brain homogenate was used. DEAE Sephadex treatment followed by several filtrations removed an average > 4.8 log10/mL PrPSc, > 4.98 log10/mL PrPSc when clarified hamster brain homogenate was used and > 4.62 log10/mL PrPSc when detergent treated hamster brain homogenate was used, with the absence of any detectable PrPSc in the resultant material. *Conclusions.* The results obtained show a great removal capacity of PEG precipitation and DEAE Sephadex treatment followed by several filtrations for the TSE-model studied. The overall clearance would be > 8.4 log10/mL PrPSc for the manufacturing process. These results indicate that the Flebogamma production process has a high potential to eliminate TSE agents, in case they were present.

0836

FATAL TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE IN GOODPASTURES SYNDROME TREATED WITH CYCLOPHOSPHAMIDE

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Introduction. Transfusion associated graft versus host disease (TA-GVHD) is a rare but commonly fatal complication of transfusion of cellular blood products. It may even arise in apparently immuno-competent individuals, if viable donor-derived T-lymphocytes escape the recipient's immune system due to complete or incomplete HLA match and attack his organs. *Case Report.* A 54-year-old man with newly diagnosed Goodpasture's syndrome was transfused with twenty-four leukocyte depleted but not irradiated units of red blood cells. He was considered immunocompetent at admission but received cyclophosphamide pulse therapy during the hospitalisation. Two weeks after the first transfusion he developed severe persisting watery diarrhea, fever, generalised erythema and jaundice. Blood counts became increasingly leuco-, lympho- and neutropenic with moderate anemia and thrombopenia. The patient died of cardiovascular failure in septic shock on day 47 after admission. *Results.* Sigmoidoscopy on the 31st day after the first transfusion showed diffuse colitis with multiple ulcerations. Biopsies showed lymphocytic infiltration in the crypts and disappearance of crypts consistent with a diagnosis of graft versus host disease. There was no evidence of viral or parasitic infections. Gastroduodenoscopy several days later revealed severe hemorrhagic jejunitis, duodenitis and gastritis with multiple small ulcerations. The patient and 13 blood donors were typed for HLA A*B*Cw*DRB1*3*4*5* and DQB1* using low resolution PCR SSP. Two donors were found to be matched for 5 respectively 8 out of 12 tested HLA antigens with the patient, one female donor turned out HLA haploidentical with him. PCR HLA typing however revealed no evidence for chimerism in the patient`s peripheral blood (Figure 1). *Conclusions.* The therapy with cyclophosphamide might have critically contributed to the patient's susceptibility. As there is no causative therapy for TA-GvHD, the indication for preventive irradiation of cellular blood products in high risk groups is out of question. Additionally, the indication for patients at questionable risk, for example those who are receiving potent immunocompromising drugs or suffer from a potentially predisposing disease must be considered. In doubt, irradiation should be undertaken to prevent this serious condition.

Figure 1. Course of hemoglobin, leukocyte and bilirubin.

0837

REPORTING ADVERSE REACTIONS ASSOCIATED WITH BLOOD TRANSFUSION

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Background. One of the objectives of SKAE and its six regional bases (PEDIA) is the collection and analysis of side effects and incidents associated with blood transfusion. SKAE is submitting recommendations to the National Authorities for the improvement of safety and quality of transfusion. *Methods.* Reporting is voluntary and codified using standard forms for individual cases and aggregate hospital data. The type, severity and imputability of adverse reactions are analysed in relation to the associated blood product and the degree of morbidity along the lines of the European Directive 2005/61/EC, the Guide of the Council of Europe and European Haemovigilance Network standards. Reports are initially gathered by PEDIA and analysed by SKAE. For further information SKAE may communicate with local or regional haemovigilance officers.

Table 1. Haemovigilance data: 1997-2005.

Year	1997-2000*	2001	2002	2003	2004	2005	Total
Blood units	375530	234183	206317	274563	449400	485362	2,026,345
Components	508190	334882	296396	371456	674100	727900	2,919,944
Adverse reactions							
All	330	212	316	435	651	662	2606
Serious	49	231	21	21	44	31	189
	15%	11%	7%	5%	7%	5%	7.3%
Fatalities	1	0	0	0	1	2	1.2
Incorrect blood product	1	2	1	1	3	8	5.0
TRALI	0	0	0	1	4	5	3.0

*Pilot study

Results. Hospitals reporting represented 17-18% of national totals of blood units and omponents in 1997-2000, increasing to 83% in 2005 (45-50% overall). All adverse reactions 9:10,000 blood products, serious 0.65:10,000. Near misses not included. Associated with RBCs 72%, plasma 9%, platelets 2.5%. Infectious factors caused 12% of serious reac-

tions (most bacterial, 2 malaria, 6 viral - 4 HIV, 1 HCV and 1 HBV). Long term morbidity in 13% of serious adverse reactions. 2 fatalities (1 ABO incompatibility, 1 hyperhaemolysis syndrome), 6 TRALI, 12 incorrect blood products. Allergic (41%) and NHFTR (54%) in 2005 were mostly in multitransfused patients. *Conclusions/Recommendations.* Reporting improved and hospitals' participation increased to 83% in 2005. The reports of incorrect blood component transfused and TRALI increased after 2004 in part because of increased awareness of these transfusion side effects. Allergic reactions and NHFTR remain high. Improved patient identification, leukoreduction, phenocompatibility and strategy for bacterial sepsis reduction. Continuous training for better reporting and cooperation.

0838

COMPARISON OF VON WILLEBRAND FACTOR, FACTOR VIII AND ADAMTS13 IN PLASMA PRODUCTS USED IN THE TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA

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Introduction. Attention has recently turned to the composition of plasma products used in the treatment of thrombotic thrombocytopenic purpura (TTP). In Belgium, two virus inactivated fresh-frozen plasma (FFP) products are available: solvent/detergent (SD) treated pooled plasma (SD-FFP) and methylene blue/light (MB) treated single plasma units (MB-FFP). The use of MB-FFP in TTP patients is only anecdotal and seems less effective. *Materials and Methods.* Plasma levels of factor VIII, von Willebrand factor antigen and activity (VWF:act) were measured with routinely used methods. ADAMTS13 antigen (ADAMTS13ag) was measured with a commercial polyclonal and a non-commercial monoclonal antibody-based ELISA. ADAMTS13 activity (ADAMTS13act) was measured by proteolysis of FRETS-VWF73, a fluorogenic substrate for the metalloprotease. Five production batches of SD-FFP (Octaplas, Octapharma, Vienna, Austria) and 198 single donor units of MB-FFP (Red Cross-Flanders Blood Services, Brugge, Belgium) were analysed. The plasma of 40 healthy volunteers was analysed in parallel. The pooled plasma of these 40 healthy volunteers was used as reference for selected activity assays, whereas standard human plasma, as provided by the manufacturer, was referred to when using the commercial ADAMTS13 ELISA kit. *Results.* MB treatment alters levels of all measured plasma proteins. Although statistically significant, the differences were, however, minor. SD-FFP contains less VWF:act, ADAMTS13act and ADAMTS13ag (only with the monoclonal-based ELISA) compared to the normal volunteers and MB-FFP. Interestingly, ADAMTS13ag measured with the polyclonal ELISA shows higher levels. *Conclusions.* The quantitative differences in ADAMTS13ag levels with both methods give rise to further investigations regarding the protein structure of ADAMTS13 in both plasma products. Although the ADAMTS13 activity in SD-FFP is lower than in MB-FFP, the clinical outcome in TTP patients has been shown to be better with SD-FFP. Hitherto unidentified plasma proteins may contribute to the pathophysiology of TTP and explain the reported difference in clinical efficacy. A randomized clinical trial should be conducted to find out which plasma product is best suited to treat TTP patients.

0839

A CASE OF TRALI AFTER THE INFUSION OF CRYOPRESERVED UMBILICAL CORD BLOOD CELLS

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Introduction. Transfusion-related acute lung injury (TRALI) is an uncommon complication reported after the infusion of most blood components. We describe a case of TRALI following the infusion of cryopreserved umbilical cord blood (UCB) cells. *Case report.* A 34-year-old woman with an AML in second complete remission was admitted for a cord blood transplant. Cardiac and pulmonary tests were normal. The day of infusion she was afebrile with a blood pressure of 120/85 mmHg, SpO2 98%, a cardiac frequency of 78 bpm and a central venous pressure (CVP) of 6 cm. She was pancytopenic and biochemistry was normal. A chest-X-ray two days before the infusion was normal. No blood products had been given in the previous 48 hours. She received 100 mg of hydrocortisone and 2 mg of dexchlorphenylamine IV pre-infusion, according to our protocol. The UCB unit had been fractionated before cryopreservation (final volume, 27 mL) and diluted post-thawing with 30 mL of 5% human albumin in normal saline. The product was infused over 15 minutes through a central line. Total volume: 57 mL, DMSO 2.7 mL, red blood cells 12.5 mL and total nucleated cell count 1.524×10^9. The patient complained of thoracic discomfort but oxygen saturation remained normal, as well as blood pressure, cardiac frequency or CVP. One hour later she referred shortness of breath. Crepitants could be heard in both lung bases. Blood pressure had increased to 150/100 mm Hg; CVP was 9 cm, cardiac frequency 72 bpm and oxygen saturation dropped to 90%. A chest-X-ray showed bilateral fluffy perihiliar infiltrates. She was treated with supplementary oxygen, diuretics and enalapril. In the following hours, blood pressure returned to normal but she required supplementary oxygen to keep a normal saturation. Temperature rose to 37.4° C and she complained of pain in the wrists and ankles with local edema. Twenty hours later she was afebrile, with normal blood pressure, CVP, oxygen saturation on room air; a chest-X-ray showed no infiltrates. Further course of the transplantation was uneventful. Blood cultures taken the day before, the afternoon of the infusion and from the UCB unit were negative. *Discussions*. TRALI is a leading cause of transfusion- related morbidity and mortality. It has been reported after the infusion of any blood product but usually with more than 50 ml of plasma and exceptionally after cryopreserved bone marrow or peripheral blood stem cells. A multi-factorial mechanism has been proposed involving antineutrophil antibodies or a previous lung damage and the infusion of biologic response modifiers (the two-hit hypothesis). Our case fulfils the criteria of TRALI: acute onset, hypoxemia, bilaterial infiltrates and no evidence of circulatory overload, within 6 hours of the infusion and no temporal relationship to an alternative risk factor. Bacterial contamination can be ruled out. The volume of DMSO and red blood cells in this unit was small, but UCB units contain usually mature granulocytes. We have observed no cases of acute lung injury after any of the 64 UCB units infused in our centre.

0840

REGULATION OF DLL4 EXPRESSION IN BM-EPCS CONTROLS TUMOUR NEOANGIOGENESIS

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Bone marrow-derived endothelial progenitor cells (BM-EPCs) have been implicated in adult neoangiogenesis and consequently used as biomarkers for human pathologies with endothelial damage. The administration of these cells in human patients temporally improves endothelial function, although the engraftment of these cells in newly formed vessels is inefficient. The mechanisms by which these cells promote vessel regeneration remain largely unclear.In this work, we analysed the role of the Notch/Delta signalling pathway in EPC function during tumour neoangiogenesis, by regulating the expression of Notch ligand, delta-like 4 (Dll4) in these cells. Sublethally irradiated NOD-SCID mice received either WT or Dll4±BM-EPCs and were subcutaneously inoculated with tumour cell lines (leukemias and breast cancer). Tumours generated in Dll4±EPCs transplanted mice presented increased microvessel density when compared with WT EPCs transplanted mice or non-transplanted controls, regardless of VEGF expression. Although with increased vessel number, tumours of Dll4±EPC transplanted mice presented more hypoxic cells and decreased tumour cell proliferation, revealing impairment in vessel function. In addition, these tumours present a diminished expression of PDGF, a vessel stabilizing factor, and increased expression of Ang2, known as a vessel destabilizing factor. These results showed that EPCs have a major role in vessel stabilization in sites of active neoangiogenesis by the regulation of Dll4 expression. We propose that targeting the Notch/Dll4 pathway on EPCs, modulating vessel stability, may have therapeutic potential.

0841

CHALLENGES IN TRANSFUSION MEDICINE: NEED OF TRANSFUSION DEPENDENT PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. With the increasing proportion of elderly individuals in the population, the prevalence of Myelodysplastic syndromes (MDS) will likely rise further in the future. For rational resource allocation information on resource consumption and costs of potential complications (e.g.

anemia), therapeutic treatments (e.g. transplantations) and supportive care (e.g. transfusion support) will be requested in the future. Since most of the MDS patients are not eligible for transplantation it can be assumed that the economic burden of MDS is determined predominantly by anemia and transfusions. *Aims.* To describe the economic burden of transfusion and anemia in MDS on the basis of published literature. To make a first estimate of the economic impact for Germany. *Methods.* Literature search via PUBMED was systematically conducted (covering 1/96 to 12/06) using the search terms: Myelodysplastic Syndrome AND Transfusion AND Cost and cost analysis OR cost of illness OR health care costs OR epidemiology; anemia AND cancer AND cost OR transfusion. Further desk-top researches were conducted to evaluate the economic burden of transfusion-dependency of MDS patients from the German transfusion medicine's perspective. *Results.* Three MDS cost-analyses focus on transfusion costs and present direct costs from the payers' perspective. Regarding anemia in cancer patients 4 cost-of-illness analyses, 4 cost-effectiveness-analyses, 3 cost-minimisation analyses and 4 other cost analyses were identified. Most studies analyse the US-American situation from the payers' perspective. Prevalence of MDS in Germany is estimated to range between 7,300 and 10,500 patients. Assuming a need of 24 erythrocytes per transfusion dependent MDS patient per year in average, MDS transfusions add up to 2 to 3 per cent of whole erythrocyte production in Germany in 2004. Depending on the current unit price of erythrocytes and the numbers of transfusion dependent MDS patients, the total costs of transfusion vary between 8 and 23.5 million Euro per year. The number of transfusion dependent MDS patients will grow by approximately 1,760 up to approx. 7,200 patients in Germany in 2010 resulting in transfusion costs between 15 and 30 million Euro. *Summary and Conclusions.* There is a lack of information on the economic burden of MDS. To evaluate the economic consequences of innovative therapeutics in MDS it is important to provide information of resource consumption and the respective costs on the basis of real life data. It is important to present the results by disease severity and treatment options. The treatment of transfusion dependent MDS is of special public health interest. Blood is an increasingly scarce and expensive resource and unnecessary transfusions may cause a shortage of blood products for patients in real need. Therefore the value of blood conserving strategies should be evaluated from a broader perspective - the societal perspective.

0842

PHARMACOGENOMIC APPROACH OF SICKLE CELL DISEASE TREATMENT: GLOBAL ANALYSIS OF HYDROXYUREA EFFECT ON HUMAN ENDOTHELIAL CELLS TRANSCRIPTOME

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Background. The clinical hallmarks of sickle cell disease (SCD) are recurring painful vaso-occlusive episodes, chronic anaemia and multiple organ damages. Although the polymerisation of Haemoglobin S (HbS) is the base of the pathophysiology, the vascular endothelium seems to play an essential role in vascular occlusion. Hydroxyurea (HU) is the only drug with a measurable clinical benefit for SCD patients by reducing the frequency of vaso-occlusive crisis. Initially, HU was administrated to induce HbF expression but there is no link between the observed clinical benefit and the expected increase of HbF expression. Due to the crucial role of ECs in SCD, our laboratory investigates the effect of HU therapy on ECs. Indeed, we have shown that HU affects the expression of specific endothelial genes. These genes seem to be implicated in the pathobiology since they encode for adhesion molecules (VCAM-1), vaso-modulators (endothelin-1) and cytokines (Il-6, Il-8). *Aims.* We decided to pursue analysis of HU effect on endothelial cells in the way to identify HU targets genes and downstream pathways by establishment of differential transcriptome profiling of human ECs (cells exposed to HU vs unexposed). *Methods.* Our analysis was performed by a systematic screening of HU target genes based on the use of a micro-array system which allows to evalute the expression level of the whole human transcriptome since the array contains probes for 29,198 identified genes. We have analysed the effect of HU 24h treatment period on the transcriptome profile of a human endothelial cell line derived from bone marrow microcirculation (TrHBMEC). *Results.* The subtraction profile (HU vs non-treated) identifies 2448 potential novel target genes. RQ-PCR experiments were carried out to validate the method applied and these differentially expressed HU target genes. Among them, thrombospondin-1 (TSP-1), von Willebrand factor (vWF), PECAM-1, AXL mRNA levels are decreased and interleukin-1 α and β mRNA are increased. All these factors seem to be relevant for the pathobiology and endothelium functions. In fact, TSP-1 and vWF are implicated in adherence and coagulation events and high levels of these two proteins are detected in SCD patients. PECAM-1 modulates vascular permeability and lymphocytes extravasation, AXL is a new vascular system regulator and is implicated in adhesion phenomenon. IL-1 α and β are pro-inflammatory cytokines; their up-regulation by HU is paradoxical with the improved clinical features. Experiments at the protein level are thus carried out to validate the mRNA data. *Conclusion.* Presently, we find, by an exhaustive transcriptome analysis, new relevant endothelial target genes of HU, however some of our results seem to be inconsistent with clinical benefits suggesting that SCD and its treatment are complex and require more experiments. The same type of microarrays experiments has also been carried out combining pro-inflammatory cytokines and hydroxyurea treatment in a way to simulate SCD inflammatory context. A global signaling pathway analysis of the data is performed to identify the biological processes and the molecular pathways affected by HU. A better understanding of HU molecular effects and downstream pathways should lead to the emergence of improved and more targeted therapies.

0843

ANGIOGENESIS AND PERYCYTE MARKERS IN SEVERE HEMOPHILIACS WITH DIFFERENT CLINICAL PHENOTYPES

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Background. Haemophilia is an inherited hemorrhagic disorder, characterized by spontaneous bleeding episodes. However, some severe haemophilic patients exhibit a mild bleeding tendency. It is possible to hypothesize that the vascular and perycyte component of haemostasis can influence the capacity of repairing vascular damage in these individuals. *Aims.* to investigate different surrogate markers of angiogenesis and vasculogenesis in haemophilic patients. *Methods.* Two groups of patients with severe haemophilia were enrolled: 11 mild bleeders (MB: ≥2 spontaneous bleeding episodes/year; concentrate use <120U/Kg/year) and 9 severe bleeders (SB: ≥25 spontaneous bleeding episodes/year; concentrate use ≥3000 U/Kg/year). A group of healthy controls was also evaluated. Using 4-color flow cytometry, circulating endothelial cells (CECs) were defined as negative for CD45 and CD133, positive for CD146 and CD31. Circulating endothelial progenitors (CEPs) were depicted by CD133 expression. 7AAD was used to identify apoptotic and necrotic CECs and CEPs. Circulating levels of VE-cadherin, RGS5, Nanog, VEGFR2 and VEGFR3 mRNA were investigated by real time PCR. The amount of m-RNA was given as arbitrary units using as housekeeping gene as a reference. Healthy controls were used as a relative calibrator, therefore the genes expression levels in controls were assigned the value of 1. Soluble VEGFR2 and VEGF-C levels were also evaluated in plasma samples using a ELISA test. *Results.* CECs were 13.8±8.7/μL (59±10.5% apoptotic) in controls vs 15.9±11.3 CECs/μL in patients. In particular MB had 16.7±11.9 CECs/μL (35±17% apoptotic) while SB had 14.8±11.2 CECs/μL (41±14% apoptotic). CEPs were 1.12±0.7/μL in controls and 1.45±1/μL in patients (1.84±1.2/μL in MB, 0.97±0.57/μL in SB). After adjusting for age, sex and white blood cell count, a statistically significant difference was found between all patients and controls for CECs (p=0.023), percentage of apoptotic CECs (p=0.04) and CEPs (p=0.001). A separate analysis for MB and SB vs controls confirmed a significant difference in the percentage of apoptotic cells (p=0.01 for MB and 0.048 for SB). Another statistically significant difference was found between SB and controls for CECs (p=0.032) and between MB and controls for CEPs (p= 0.01). RGS5 mRNA levels were 0.73 vs 0.39 (p=0.03) in SB and MB, respectively. *Conclusions.* Haemophiliacs had slightly higher CECs (but with a decreased percentage of apoptotic cells) compared to controls. MB had a significantly higher number of CEPs, suggesting a better capacity of re-endothelization after vascular injury. The difference in RGS5 mRNA expression in patients compared to controls suggest a possible involvement of pericytes, because RGS5 is considered a perycyte-specific gene.

0844

INCREASED LEUKOCYTE-PLATELET INTERACTIONS ASSOCIATED WITH HIGH RISK HEART SURGERY

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The role of circulating leukocytes and their interaction with platelets play an important role the hemostasis and inflammation. Leukocyte-platelet complex formation depends on the interaction between platelet P-selectin and leukocyte ligand P-selectin glycoprotein ligand-1 (PSGL-1). Implantation of a left ventricular assist device (LVAD) is used to provide hemodynamic support in patients with severe heart failure. Bleeding, thromboembolism, and infections are common complications associated with LVAD. Cell activation and leukocyte-platelet interactions during LVAD implantation is reported in a limited number of studies. We measured circulating levels of monocyte-platelet complexes (MPC), granulocyte-platelet complexes (GPC), and lymphocyte-platelet complexes (LPC) in 11 patients who received LVAD support. Blood samples were collected before LVAD implantation, and at days 3, 7, 14, 21, 30, 60, 90, and 180. Flow cytometry was used to measure circulating heterotypic cell aggregates. The following antibodies were used: CD14, CD162 (PSGL-1), CD41(GPIIb), and CD62P (P-selectin). Monocytes were identified by specific staining with CD14 antibody, and lymphocytes and granulocytes were identified by their forward and side light scatter properties. The percentage of the leukocyte-platelet complexes was defined by their CD41 positivity. Baseline levels of PSGL-1 on monocytes were significantly higher than that on granulocytes and lymphocytes (160.6±21.5, 91.6±12.3, and 77.2±8.4), respectively. After transient increase on postoperative day 3, PSGL-1 surface expression showed persistent decreasing trends thereafter. The average percentages of the three types of leukocyte-platelet complexes were within normal range before implantation (9.1±2.5, 8.3±2.6, and 8.6±2.2, respectively). MPC and GPC increased significantly on day 7 (16.5±7.6 and 14.4±7.2), peaked on day 21 (26.9±11.7 and 25.4±8.8), and remained significantly elevated up to 60 days of the post implantation period. The average percentage of the LPC increased by day 14 and remained elevated thereafter. A significant inverse correlation was found between MPC and monocyte PSGL-1 (R=-0.84), LPC and lymphocyte PSGL-1 (R=-0.78) and GPC and granulocyte PSGL-1 (R=-0.69), indicating increased levels of leukocyte and platelet activation. A significant positive correlation between MPC and CD14 (R= 0.6), further confirmed ongoing monocyte activation. The preliminary results of this pilot study documented an increased cellular activation and heterotypic cell interactions in patients undergoing high risk heart surgery. Circulating leukocyte-platelet complexes along with other markers of cell activation require correlation with longer follow-up of larger groups of patients and subsequent clinical events in order to understand its clinical significance.

0845

TRANSFUSION RATES AND PLATELET ACTIVATION IN PATIENTS UNDERGOING HIGH RISK CARDIAC SURGERY

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Introduction. Left ventricular assist device (LVAD) implantation is associated with a high incidence of hemorrhagic complications, high-volume transfusion and reexploration rates for bleeding. Increased platelet activation in these patients is reported in a limited number of studies. We sought to determine the effect of preoperative platelet activation on bleeding and transfusion rates in this surgical procedure. *Methods.* We used flow cytometry methods to measure expression of the platelet activation markers in 11 patients (7 men, 4 women) with end stage heart failure who underwent LVAD implantation. P selectin (CD62P) and thrombospondin (TSP) levels were analyzed and compared to transfusion rates and bleeding. Data collected include: age, body weight, sex, intraaortic balloon pump insertion, previous cardiac surgery, creatinine, BUN, total bilirubin, and hemoglobin levels, PT, INR, PTT, platelet count, WBC count, and antifibrinolytic use. *Results.* Preoperatively, the average percentage of positive platelets was 26±13% for CD62P and 9.4±5.4% for TSP. Six patients with percent of positive platelets equal or greater than the median value for CD62P (23.7%) and TSP (9.5%) required more platelet transfusions (9.2±3.1 vs. 3.8± 2.9 units, p=0.015) and bled more (74±23 vs. 43±26 mL/kg, p=0.037). They also required more red blood cell units (11.3±4.4 vs. 6.8±3.9, p=0.052) and fresh frozen plasma units (14.7±5.6 vs. 6.2±4.1, p=0.035) than patients who had less activated platelets. *Conclusions.* These results on a limited number of patients suggest correlation of platelet activation with increased bleeding and transfusion requirements during LVAD implantation. Further study is warranted to confirm this finding.

0846

IMMUNE CELLS MIMIC THE MORPHOLOGY OF ENDOTHELIAL PROGENITOR COLONIES *IN VITRO*

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Background. Endothelial progenitor cells (EPC) are considered powerful biologic markers for vascular function and cardiovascular risk predicting events and death from cardiovascular causes. Colony-forming units of endothelial progenitor cells (*CFU-EC*) are used to quantify EPC circulating in human peripheral blood. The mechanisms underlying colony formation and the nature of the contributing cells are not clear. *Methods.* We performed subtractive *CFU-EC* analyses to determine the impact of various blood cell types and kinetics of protein and gene expression during colony formation. *Results.* We found that *CFU-EC* mainly comprise T cells and monocytes admixed with B cells and NK cells. The combination of purified T cells and monocytes formed *CFU-EC* structures. The lack of colonies after depletion or functional ablation of T cells or monocytes was contrasted with effective *CFU-EC* formation in the absence of $CD34^+$ cells. Microarray analyses revealed activation of immune function-related biological processes without changes in angiogenesis-related processes during colony formation. In concordance with a regenerative function, soluble factors derived from *CFU-EC* cultures supported vascular network formation *in vitro*. *Conclusions.* Recognizing *CFU-EC* formation as the result of a functional cross between T cells and monocytes shifts expectations of vascular regenerative medicine. Our data support the move from a view of circulating EPC towards models that include a role for immune cells in vascular regeneration.

0847

EVALUATION OF RELEASED FACTORS, WITH A POTENTIAL POST-TRANSFUSION IMMUNOMODULATORY ROLE, DURING THE STORAGE OF PLATELET CONCENTRATES

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Background. Platelets have a specialized role in haemostatic responses, in spite of this, new functions have been recently identified. In fact, the thrombocytes also participate in the process of inflammation by releasing different substances capable of modulating the whole inflammatory response by interactions with both endothelial and white blood cells. Recent studies have demonstrated that the platelets take part in immunity and inflammation mainly by means of the CD40/CD40L pathway. *Aims.* Our objective has been to evaluate the accumulation of cytokines, during the storage, in platelet concentrates obtained from platelet-rich plasma. *Methods.* Fifty platelet concentrates have been analyzed in Caserta's Hospital; a plasma sample has been taken from every unit just after production of the hemocomponent (T.0) and after 1 (T.1), 3 (T.3), and 5 (T.5) days of storage (at 22±2°C in continuous agitation). IL-6, IL-8, PDGF-AA, sCD40L and leptin levels have been assayed by specific ELISA commercial kits (R&D Systems). *Results.* IL-6 (T.0= 6.3±1.9 pg/mL, T.1=7.1±2.6, T.3=9.3±3.1, T.5=10.2±3.9, p>0.05) and IL-8 (T.0=8.9±3.3 pg/mL, T.1=10.1±3.6, T.3=12.3±4.1, T.5=16.8±6.4, p>0.05) levels have been stable during all the storage with no statistical significant variation. On the other hand, PDGF-AA (T.0=210±20 pg/mL, T.1=298±36, T.3=463±29, T.5=690±47, p<0.001), sCD40L (T.0=14.9±2.0 pg/mL, T.1=21.1±2.9, T.3=26.3±3.6, T.5=44.8±5.1, p<0.01) and leptin (T.0=3.1±2.0 pg/mL, T.1=4.4±2.6, T.3=5.3±1.9, T.5=7.1±4.0, p<0.05) levels have consistently and significantly increased. *Conclusions.* The platelets contain molecules with known immunomodulatory competence and, on the basis of our data, we can affirm that these are differentially released in a platelet concentrate during its storage. In fact cytokine/chemokine levels have been demonstrate to be generally higher in platelet concentrate supernatants and/or platelets lysates in com-

parison to platelet-free plasma. This observation is notable because, when a platelet unit is transfused to a patient, a large quantity of cytokines and chemokines is additionally infused. These biologically active molecules may be possible mediators of post-transfusion immune disorders, moreover a strong correlation between cytokines' plasma levels and transfusion reactions seems to exist. In fact, for example, *ex vivo* production of sCD40L was quantified at levels sufficient to induce B cell activation and differentiation. The inhibition of cytokine releasing during the PLT storage could decrease the related transfusion reactions. In order to increase transfusion safety, two procedures could be actually and immediately carried out: the first one is represented by distributing PLT concentrated as soon as possible after preparation (with only 1-2 days of storage); the second one consists in eliminating the residual WBCs (and their cytokine secretion).

0848

ANTI-D ALLOIMMUNIZATION AFTER D-MISMATCHED ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWING REDUCED-INTENSITY CONDITIONING

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Background. Anti-D alloimmunization develops in up to 20% of RhD-negative patients on chemotherapy following exposure to RhD antigen, but is reported to be rare in recipients of haematopoietic stem cell transplants (HSCT), especially following myeloablative conditioning. After HSCT following reduced-intensity conditioning (RIC) rapid isohemagglutinin production of donor lymphocytes have been observed in the minor ABO-incompatible setting resulting in severe hemolysis. *Aims.* The objective of this study was to evaluate the incidence of anti-D alloimmunization after D-mismatched HSCT following RIC. *Methods.* From 112 consecutive patients receiving RIC-HSCT between April 1999 and March 2006, 26 patients had a D-mismatched donor. Twelve RhD-positive patients had an RhD-negative donor, 14 RhD-negative patients received a RhD-positive graft. RIC consisted of the Seatle protocol (fludarabine and 2 Gy total body irradiation;TBI) or the FLAMSA protocol (amsacrine, fludarabine, cytarabine, cyclophosphamide, ATG and 4 Gy TBI). For graft-versus-host disease (GvHD) prophylaxis cyclosporin A (CsA) and mycophenolate mofetil (MMF) were given. From the day of HSCT, red blood cell support consisted of donor Rh-type RBCs. *Results.* After a median follow-up of 30 months, 16 of 26 patients with a D-mismatch donor were alive. Two RhD-negative patients died within 10 days after HSCT and were not evaluable for anti-D alloimmunization. Eight patients developed acute GvHD between days 7 and 52 and 11 patients chronic GvHD between days 75 and 274 after HSCT, respectively. RhD-positive patients with RhD-negative donors received a median of 11 (range, 0-92) RhD-negative RBC units during a median of 8 months (range, 0-38) after HSCT. RhD-negative patients with RhD-positive donors were transfused with a median of 11 (range, 0-44) RhD-positive RBC units during a median of 2 months (range, 0-54). There was no difference in transfusion requirements between D-mismatched vs. D-matched patients. After a median of 13 months (range, 0-73) only in 1 RhD-positive patient with an RhD-negative donor an anti-D antibody was detected. This patient received 2 units of RhD-negative RBCs 5 days after HSCT and never experienced acute or chronic GvHD. *Conclusions.* Fludarabine based RIC and CsA with MMF given post transplant prevents anti-D formation in RhD-negative recipients of an RhD-positive graft. However, anti-D developed in an RhD-positive recipient of an RhD-negative graft who never was exposed to RhD-positive blood products after HSCT. This may be caused by the relative high amount of residual RhD-positive RBCs at the time of transplant and an immunosuppressive regimen with CsA and MMF were donor B-cells can escape T-cell control as seen in the minor ABO-incompatible setting. Therefore, patients with an RhD-mismatched donor should routinely be tested for RhD alloimmunization in the post-transplant course.

UCB transplantation & mesenchymal stem cells

0849

CELLULAR RECOVERY AFTER MANIPULATION IN 27 UNITS OF UMBILICAL CORD

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Background. The success of an allogenic graft with placental blood is directly linked to the cellular dose, both of nucleated cells (NC) and $CD34^+$ ($CD34^+$) cells, infused. It is therefore essential that the thawing method allow for recovery of as much NC and $CD34^+$ as possible. *Aims.* Our study examines the results our Center obtained when assessing recovery of NC, $CD34^+$, and cellular viability. *Methods.* From January 2000 to October 2006 our Center performed 27 allogenic grafts with units of placental blood from several different umbilical cord banks. Thirteen patients had ALL, 4 had AML, 3 had myelodysplasia, 3 had histiocytosis, 2 had NHL, 1 had Fanconi's Anemia and 1 had Chediak-Higashi Syndrome. Median weight for these patients was 22.5 Kg (range 6-72). The Rubinstein protocol was used for thawing. Nucleate cell count was performed using an electronic cell counter while $CD34^+$ and viability was assessed with flow cytofluorometry. *Results.* (All values given are median) The volume of the units before thawing was 120 mL. (25-278), following manipulation of 127 mL (35-385). The NC went from 161×10^7 (75.6×10^7- 290×10^7) to 123×10^7 (50×10^7-230×10^7), with a 77.8% recovery rate (56.4-96.7). Recovery rate for CD34 was 91.4% (25.1-121.4) inasmuch as before manipulation the units contained 5.9×10^6 (1.3×10^6-19.9×10^6) while after thawing the recovered $CD34^+$ were 5.1×10^6 (1.2×10^6-10×10^6); in one case recovery was higher than 100%, due to the fact that the first measurement of the $CD34^+$ was performed at a different center that uses different instruments and a different protocol. Post-thaw viability was 85% (60-98). Patients received 5.3×10^7/Kg (2.1×10^7-14.8×10^7) NC and 0.2×10^6/Kg (0.1×10^6-1.7×10^6). *Conclusions.* Our study showed that the Rubenstein protocol is the best method for manual thawing; in all the cases cellular recovery obtained an optimal cellular dose with which to perform a graft and cellular viability was always high.

0850

MESENCHYMAL STEM CELL MEDIATED IMMUNOSUPPRESSION IS NOT CONFINED TO PROGENITOR STATUS

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Although it has been widely demonstrated that mesenchymal stem cells (MSC) exert potent immunosuppressive effects, there is little information as to whether more mature mesenchymal stromal cells (SC) share the same property. Accordingly, we set out to test the ability of SC from different tissues to inhibit the proliferation of peripheral blood mononuclear cells (PBMC) exposed to polyclonal stimuli. Chondrocytes, along with fibroblasts from synovial joints, lung and skin were used as a source of SC. Irrespective of their differentiation potential and/or content of progenitor cells, SC from all tissues exhibited powerful anti-proliferative functions. This was in marked contrast to the parenchymal cells tested. Although SC did not interfere with early T lymphocyte activation, they arrested T cells in the G0/G1 phase of the cell cycle after stimulation and rescued them from apoptosis. In addition, IFNγ and TNFα production was reduced by the presence of SC in stimulated T cell cultures. We observed that the inhibitory effect is ultimately mediated by soluble factors, the production of which requires SC to be *licensed* in an inflammatory environment by cell contact. We conclude that the immunosuppressive effect of mesenchymal cells is not confined to multipotent stem cells but is a fundamental characteristic of all stroma. Our data suggests that SC, appropriately licensed, regulate T cell homeostasis.

0851

CORD BLOOD DERIVED MESENCHYMAL STEM CELLS ARE EFFECTIVE AT PREVENTING GRAFT-VERSUS-HOST DISEASE

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Background. Evidence has emerged that Mesenchymal stem cells (MSC) represent a promising population for cellular therapy and their immunosuppressive properties make them particularly attractive to manipulate graft-versus-host disease (GvHD). So far, the experience of using MSC to treat GvHD is limited to a few cases and controversial results come from preclinical models. *Aim.* The present studies were designed to address these questions in a xenogenic model testing the ability of Umbilical Cord Blood derived MSC (CB-MSC) to prevent and /or treat GvHD. *Methods*. Subletally irradiatiated NOD/SCID mice transplanted with human peripheral mononuclear cells (PBMC) selected for their ability to engraft, showed extensive human T cells proliferation in the peripheral blood, lymphoid and non lymphoid tissues, which evolved in extensive GvHD (wasting, ruffled hair and hunched back). *Results.* The chimeric-mice treated with a single dose of MSC did not behave differently form the controls. However, when MSC were given at weekly intervals, there was a marked decrease in human T cells proliferation and none of the mice developed GvHD. No therapeutic effect was obtained if MSC were administered at onset of GvHD. *Conclusions*.

This work supports the clinical use of MSC in SCT as a prophylaxis rather than treatment of GvHD.

0852

FLOW-SORTED HUMAN HAEMATOPOIETIC STEM CELLS DO NOT TRANSDIFFERENTIATE INTO FUNCTIONAL CARDIOMYOCYTES

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Background. Several clinical trials showed that purified $CD133^+$ or $CD34^+$ cells injected in patiënts with myocardial infarction can contribute to the repair of ischemic myocardium and improve heart function. The mechanism responsible for this improvement in not clear yet and contradictory reports have been published. Some groups declare that haematopoietic stem cells (HSCs) are able to transdifferentiate into cardiomyocytes (CMs), while others could not reproduce these findings. Therefore further thorough investigations remain. *Aims.* This study aims to examine haematopoietic stem cells when they are incorporated in a cardiac environment. To mimic the microenvironment of the heart *in vitro*, a co-culture system was developed in which flow-sorted human haematopoietic stem cells were cultured in the presence of neonatal rat cardiomyocytes (NRCMs). *Methods.* Mononuclear cells (MNC) were isolated from human bone marrow (BM) samples. And incubated with CD34-Pe-cy7 and CD133-PE antibodies. Subsequently, $CD133^+/CD34^+$ double positive cells were isolated under stringent purity conditions on a FACSAria®. Co-culture experiments were performed using celltracker green (5-chloromethylfluorescein diacetate) labelled HSCs and celltracker red (5-(and6)-(((4-chloromethyl)benzoyl) amino)tetramethylrhodamin) labelled NRCMs. HSCs and NRCMs were plated at different ratios and cultured in X-Vivo15 medium containing 2% fetal bovine serum (FBS) or 2% autologous serum (AS) of the patient respectively. Since several reports indicate that dimethylsulfoxide (DMSO) and 5-azacytidin (5-aza) induce myocardial differentiation, 1% DMSO for 48h or 3 µM 5-aza for 24h was added and compared to conditions without additives. After 3 weeks of incubation, green and red cell populations were separated by flow-sorting and expression of cardiac specific genes, including b-actin, Kv4.3, a-actinin, Connexin43, Troponin T, Troponin I, a1c, Myosine Heavy Chain, GATA-4 Nkx2.5, were analysed by reverse transcriptase polymerase chain reaction (RT-PCR). *Results.* Co-culturing human HSCs with NRCM induced the expression of Troponin T while expression of Connexin43 and Nkx2.5 was detected in both co-cultured and freshly isolated HSCs. However, there was no expression of a-actinin, Myosin Heavy Chain, Kv4.3, a1C, Troponin I and GATA-4. Adding DMSO or 5-aza had no influence on the differentiation of these cells. *Conclusions.* Our results show no convincing evidence for transdifferentiation of HSCs after 3 weeks of co-culture with NRCM. Even so, no cell fusion between HSCs and NRCM could be detected. Probably, other mechanisms like improved angiogenesis or paracrine effects stimulated by HSCs can contribute to an improved heart function.

0853

PLASMACYTOID DENDRITIC CELLS AND TOLERANCE INDUCTION: *IN VITRO* ASSAYS ON UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS

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Background. The tolerizing function of both classic myeloid and more recently identified plasmacytoid dendritic (pDC) cell subsets has become obvious. They may be used as tools and targets to promote transplant tolerance. There is growing understanding of the role of pDC in immune tolerance *in vitro* by promoting differentiation of T regulatory (Treg) cells and *in vivo* their administration may be effective in promoting T cell tolerance in autoimmunity and transplantation. *Aims.* The aim of our study is to produce and expand plasmacytoid dendritic cells from umbilical cord blood (UCB) and to test their functional properties to promote *in vitro* differentiation of T regulatory cells. *Methods.* $CD34^+$ hematopoietic stem cells (HSC) were isolated from fresh human UCB by positive selection with CD34 mAb coated beads and cultured with IL-3, Flt3-L and SCF in *ex vivo* 20 medium (n=6) for 14 days. Phenotypical and morphological analysis were performed at days 0, 3, 6, 9, 12 and 14 by MGG staining and flow cytometry. The following markers were tested: CD2, CD11c, CD19, CD22, CD33, CD34, CD45, CD56, CD64, CD123 and CD304, specific markers for pDC. *Results.* Our culture system allows to obtain until a 100 fold cell expansion at day 14. On MGG staining, cells displayed a typical plasmacytoid cell morphology, characterized by an excentric nucleus, a blue basophilic cytoplasm and pale Golgi zone. Cell surface phenotype was analyzed by flow cytometry: the cells do not express some lineages specific markers: CD19, CD22 (B cells), CD56 (natural killer cells), CD14 (monocyte), CD64 (FcgRI). At day 0, more than 95% of cells were $CD45^+CD34^+$ and $CD33^+$. At day 14, the number of cells expressing CD34 decreased, a sign of cell culture differentiation. A fraction of these cells expressed CD11c (23.55±10.2%), CD2 (10.1±6.4%), CD304 (35.5±9.5%) and CD40 (32.5±9.5%) but remained CD123 negative. In two experiments, we activated the cultured cells with soluble CD40L at day 14 and analysed the cells 24 h later. No significant phenotypical differences were observed between activated and non activated cells. However, soluble CD40L induced the development of dendrites on plasmacytoid cells, a pDC characteristic described by others. The observation that a fraction of cells generated from UCB CD34+ cells in our culture system possessed morphological but not all phenotypical characteristics of pDC, led us to assess their potential regulatory function after activation by CD40L or CpG ODN A on proliferation of allogenic naïve $CD45RA^+$ T cell. We evaluate by co-culture the potential differentiation of naïve allogenic T cells into $CD4^+CD25^+$ Treg cells and their suppressor function on autologous and allogenic T cell proliferation *in vitro*. These experiments are still in progress. *Conclusions.* We were able to produce and expand plasmacytoid-like dendritic cells by culturing UCB HSC *in vitro* with growth factors. These cells are morphologically similar to pDC but they lack some markers in their phenotypic profile. Co-cultures to test their potential functional immune regulatory properties are in progress.

0854

A NOVEL SYSTEM FOR HIGHLY EFFICIENT CLINICAL SCALE PROPAGATION OF HUMAN MESENCHYMAL STEM CELLS WITH HUMAN PLATELET LYSATE

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Background. Human multipotent mesenchymal stromal cells (MSC) are promising candidates for a growing spectrum of regenerative and immune modulating cellular therapies. Translation of experimental results into clinical applications has been limited by the dependence of MSC propagation from fetal bovine serum (FBS). *Aims.* We analyzed the capacity of human platelet lysate (HPL) to replace FBS for clinical scale MSC propagation from human bone marrow (BM). *Materials and Methods.* MSC expansion was performed under good manufacturing practice conditions. Multiplex profiling was used to measure cytokines and growth factors in HPL and compared to factor profiles derived from expanded MSC. MSC function was further tested in potency assays for clonality and differentiation. Genetic stability was determined using conventional cytogenetics. Potential tumorigenicity of *ex vivo* expanded MSC was studied *in vivo* by injecting graded MSC numbers into immune incompetent mice. *Results.* HPL could be efficiently produced from normal buffy coats. Multiplex analyses allowed delineating a distinct HPL as compared to MSC-derived growth factor profile. Based on a previous-

ly established two-step clinical scale procedure, HPL was reproducibly more efficient than FBS in supporting MSC outgrowth. Using only 3×10^5 MSC derived primary culture of less than 10 mL human BM we obtained mean $4.36\pm0.51\times10^8$ MSC within a single secondary 11-13 day culture step. Although morphologically distinct, HPL-MSC and FBS-MSC did not differ significantly regarding immunophenotype, differentiation potential *in vitro* and lack of tumorigenicity in nude mice *in vivo*. *Conclusions.* A clinical quantity of functional human MSC for adult patients could be reproducibly obtained from minute volumes of starting BM under completely FBS-free conditions within less than four weeks. Replacing FBS with HPL excludes bovine prion, viral and zoonose contamination of the stem cell product. This new efficient FBS-free procedure for clinical scale MSC propagation will largely facilitate rational clinical testing of MSC based therapies.

0855

CLINICAL SCALE PROPAGATION OF FUNCTIONAL CORD BLOOD STEM CELLS IN A HUMANIZED SYSTEM

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Background. Umbilical cord blood (UCB) is an easily accessible alternative source for human hematopoietic stem cells (HSC) and mesenchymal stromal cells (MSC). Limitations in UCB stem cell (SC) number have so far hampered clinical applications of UCB for adult patients. Strict dependence of SC expansion procedures from fetal bovine serum (FBS) and inefficient expansion of HSC further limited clinical progress. *Aims.* Expansion of cord blood stem cells in a FBS-free system for clinical applications. *Methods.* We analyzed isolation and proliferation potential of human UCB-MSC as compared to BM-MSC under optimized *ex vivo* culture conditions. We further investigated the impact of human platelet lysate (HPL) as an alternative to replace FBS for clinical scale expansion of MSC. Progenitor cell function was determined by CFU-F assays and correlated to proliferative senescence. MSC functions were tested in hematopoiesis support, vascular regenerative and immune modulation potency assays. *Results.* MSC cultures could be initiated from UCB with and without FBS. MSC propagation was effective in 46% of UCB samples compared to 100% of BM samples. Once established, the proliferation kinetics of UCB-MSC did not differ significantly from that of BM-MSC under optimized culture conditions resulting in > 50 population doublings after only 10 weeks. A clinical quantity of 100 million MSC could be obtained from UCB-MSC despite minute primary cell amounts. Immune suppression and vascular regenerative function *in vitro* could be shown for UCB-MSC propagated under both culture conditions. *ex vivo* expansion of UCB-derived $CD34^+/CD38^+$ hematopoietic progenitors and $CD34^+/CD38^-$ HSC was more efficient with HPL compared to FBS-cultured UCB-MSC. *Conclusions.* We demonstrate for the first time that human MSC can be obtained and propagated to a clinical quantity within reasonable time from UCB in a completely FBS-free system. The efficient propagation of UCB-derived $CD34^+/CD38^-$ HSC encourages further studies to develop effective strategies for UCB-SC expansion for adult patients.

0856

THE ROLE OF WNT SIGNALING IN DIFFERENTIATION OF CORD BLOOD UNRESTRICTED SOMATIC STEM CELLS INTO DOPAMINERGIC NEURONS

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Wnts are family of signaling glycoproteins that act through Wnt intracellular transduction pathway transferring β-catenin, as a transcription factor, from the cytoplasm into nucleus. There are large amounts of evidence for the involvement of this pathway in early developmental processes. Recent evidence shows that molecules in this pathway are also expressed in mesenchymal stem cells and their niches. In this study, we sought to examine the role of Wnt pathway in differentiation of stem cells into dopaminergic neurons. Pluripotent cord blood stem cells, known as unrestricted somatic stem cells (USSCs), were isolated from mononuclear cells of the umbilical cord and recognized by specific markers such asCD45, CD34. Neural differentiation factors such as basic fibroblast growth factor and retinoic acid and a Wnt pathway inducer, 6-bromoindirubin-3-oxime (BIO), were used. Different dopaminergic and neuronal markers such as TH, Nurr1 were considered for dopaminergic neuron differentiation status of cells. Our results showed that in the presence of both neural differentiation and Wnt inducer factors in culture, more differentiated cells with morphological and molecular characteristics of dopaminergic neurons were seen in comparison with control cells. Moreover, there was a significant increase in the expression of β-catenin in BIO treated cells, confirming that Wnt pathway is activated in these cells. Our evidence on the involvement of Wnt signaling in increasing dopaminergic differentiation of stem cells together with its neuroprotective effect may have potential impact on therapy of neurodegenerative diseases

0857

HUMAN PLATELET-DERIVED FACTORS INDUCE DIFFERENTIAL GENE EXPRESSION IN BONE MARROW MESENCHYMAL STEM CELLS

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Background. The use of animal-derived products during human stem cell processing bears the evident risk of xenogeneic prion, virus, or zoonose contamination. Human platelet lysate (HPL) has recently been recognized as a rich source of cytokines and growth factors with the potential to replace fetal bovine serum (FBS) during *ex vivo* stem cell manipulation. *Aims.* This study was performed to compare the gene expression profile of human multipotent mesenchymal stromal/stem cells (MSC) during *ex vivo* expansion for clinical applications under the aegis of either FBS or HPL. *Methods.* The Applied Biosystems 1700 Expression Array System was used for full genome expression profiling of MSC after a 12-14 day expansion period in a previously optimized low density expansion system. Data have been obtained from biological replicates. A starting amount of 40 µg total RNA was directly labeled and DIG-labeled cDNA was hybridized to Human Genome Survey Microarray V2.0. Attribution of regulated genes to biological processes and pathways was done using the PANTHER® db analysis software. *Results.* Gene expression profiling identified a unique signature of human MSC including 19 genes which discriminate MSC from mature fibroblasts under both culture conditions. Interestingly, we identified 45 additional genes that were more than two fold upregulated upon culture of MSC in the humanized system compared to FBS supplemented media ($p<0.01$). Biological processes specifically activated in HPL culture include mesoderm development and immunological processes which interestingly correspond to a considerable proportion of the regenerative function of MSC. In contrast, processes related to cell adhesion, adhesion-mediated signaling and cell communication are significantly upregulated in MSC stimulated by FBS. *Conclusions.* Replacing FBS with HPL definitively avoids bovine prion, viral and zoonose contamination of MSC for clinical use. New insights into the tightly regulated gene expression under the aegis of human growth factors and cytokines provided by HPL may even help to develop new therapeutic strategies.

SIMULTANEOUS SESSION II

Chronic myeloid leukemia - Clinical II

0858

COMPARABLE OUTCOME OF MATCHED RELATED AND UNRELATED DONOR STEM CELL TRANSPLANTATION FOR FIRST CHRONIC PHASE CHRONIC MYELOID LEUKEMIA IN THE PROSPECTIVE GERMAN CML III STUDY

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Background. To date, allogeneic hematopoietic stem cell transplantation (HSCT) is considered the only curative treatment option for patients with chronic myeloid leukemia (CML). HSCT from a matched unrelated donor (MUD) has been associated with poorer outcome as compared to matched related donor transplantation (MRD) in retrospective studies. We compared the outcome between MRD and MUD HSCT in patients who received an HSCT in first chronic phase within the prospective German CML study III. *Methods*. In CML study III, patients with newly diagnosed CML in chronic phase were assessed for their eligibility for HSCT and randomized according to availability of a related donor. Patients with a related donor were transplanted as soon as possible. 91% of patients with a MRD indeed received an HSCT. Patients lacking a MRD received best available drug treatment and were transplanted with a MUD if they did not achieve or lost major cytogenetic response after 18 months. Between 1995 and 2004, 113 and 97 patients received a MRD HSCT or a MUD HSCT in first chronic phase, respectively. Patients' characteristics at baseline and transplant characteristics were descriptively compared. Survival times were compared by Kaplan-Meier estimation. *Results*. Median age at HSCT was 40 yrs for MRD HSCT and 36 yrs for MUD HSCT patients (p=0.015). The median time from diagnosis to transplantation was longer in the MUD group (17 vs 10 mo). 164 patients had received prior Interferon alpha (IFN) therapy. IFN therapy was stopped at a median of 77 and 138 days before HSCT in the MRD and MUD groups, respectively. The percentage of male recipients with female donors was not significantly different in both groups. Median observation time was 95 (41-123) months. At that time point, 133/210 (63%) patients who had undergone HSCT were alive, 66/113 (58%) after sibling transplantation and 67/97 (69%) after MUD transplantation. Probabilities of 5-year survival were 65% after MRD HSCT and 69% after MUD HSCT. *Conclusion*. MRD and MUD HSCT offer high cure rates for patients with CML in first chronic phase. Both donor sources produced equivalent long-term outcome in the prospective CML III study. Thus, the prognostic impact of the donor source in the pretransplant risk assessment, which has been derived from retrospective analyses, has not been confirmed in a prospective study setting and needs to be re-evaluated.

0859

DASATINIB 140 MG QD VS 70 MG BID IN ADVANCED-PHASE CML OR PH+ ALL RESISTANT OR INTOLERANT TO IMATINIB: RESULTS FROM A RANDOMIZED, PHASE-III TRIAL (CA180035)

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Background. Dasatinib, a potent inhibitor of BCR-ABL (325-times more potent than imatinib and 16-20-times more potent than nilotinib *in vitro*) and other tyrosine kinases, has been shown to be both safe and effective at doses of 70 mg BID in accelerated and blast-crisis CML and Ph+ ALL resistant or intolerant to imatinib. QD and BID schedules were equipotent in Phase I for patients with chronic-phase CML (both were associated with significant hematologic and cytogenetic responses) and in turn led to this dose-optimization study. *Aims.* This study was designed as a non-inferiority trial to compare the major hematologic response (HR) rate between the two regimens. *Methods.* In this Phase-III, open-label, prospective study, patients with imatinib-resistant or -intolerant accelerated or blast-crisis CML or Ph+ ALL were randomized to dasatinib 140 mg QD or 70 mg BID. Dose escalation to 180 mg QD or 90 mg BID was allowed for inadequate response, and dose reduction to 100 mg or 80 mg QD or 50 mg or 40 mg BID for adverse events. All patients provided written informed consent. *Results.* From June 2005 through March 2006, 609 patients (median age 55 years; 56% male) were randomized and received treatment per schedule. 42% of patients had previously received imatinib at doses >600 mg/day and 37% were treated for >3 years. Response rates, with a median follow-up of 6.5 months (range 1-17 months), were equivalent for the two schedules and are summarized in the Table1. Median durations of HR and progression-free survival were 10.2 and 7.9 months for the 140 mg QD regimen vs 12.3 and 11.7 months in the 70 mg BID arm. Significantly lower incidences of pleural effusions (p=0.024), peripheral edema (p=0.004), pericardial effusion (p=0.012), and gastrointestinal bleeding (p=0.025) were noted on the QD schedule. Minimal differences were noted between treatment groups for other AEs, including cytopenias (Table 1). Dose reductions (24% vs 36%, p=0.002) and interruptions (47% vs 54%, p=0.105) were required less frequently for the 140-mg QD regimen, whereas dose escalations were more prevalent (33% vs 22%, p=0.005). *Summary/Conclusions.* Dasatinib 140 mg QD shows comparable hematologic and cytogenetic response (pre-defined non-inferiority criteria were met) and a trend towards improved tolerability in relation to dasatinib 70 mg BID with a median follow-up of 6.5 months. Further follow-up is ongoing to assess the long-term benefit of these two schedules in patients with advanced-phase CML or Ph+ ALL; 1-year follow-up data will be presented.

Table 1.

Rate (%)	Accelerated (N=315)		Myeloid blast (N=149)		Lymphoid blast (N=61)		Ph(+) ALL (N=84)	
	QD	BID	QD	BID	QD	BID	QD	BID
Major HR	63	66	27	25	39	32	38	32
MCyR	31	40	26	30	54	47	68	55
CCyR	27	27	16	20	42	43	50	37

Side effects (%)	140 mg QD (N=304)		70 mg BID (N=305)	
	All grades	Grade 3-4	All grades	Grade 3-4
Pleural effusion	16	5	23	6
Neutropenia	85	65	87	70
Thrombocytopenia	89	68	92	70

0860

NILOTINIB IS ASSOCIATED WITH MINIMAL CROSS INTOLERANCE TO IMATINIB IN PATIENTS WITH IMATINIB-INTOLERANT PHILADELPHIA-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA IN EITHER CHRONIC PHASE OR ACCELERATED PHASE

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Background. Nilotinib is a highly selective BCR-ABL tyrosine kinase inhibitor that is 30-fold more potent than imatinib and is an important therapeutic option for patients (pts) who have either imatinib-resistant or

-intolerant Philadelphia-positive (Ph^+) chronic myelogenous leukemia (CML). Results of a subset of pts with Ph^+ CML-chronic phase (CP) or -accelerated phase (AP) who received nilotinib for imatinib-intolerance are reported. All pts signed an informed consent and received nilotinib for an unapproved indication. *Methods*. Pts were part of a phase II open-label study evaluating the safety and efficacy of nilotinib in imatinib-resistant or -intolerant Ph^+ CML-CP. Imatinib resistance was defined using standard criteria. Imatinib intolerance was defined as the absence of a prior major cytogenetic response (MCyR) and discontinuation of imatinib due to grade 3/4 adverse event (AE) or persistent (>1 mo) or recurrent grade 2 AE (recurred >3 times) despite optimal supportive care. The proportion of pts achieving MCyR was the primary endpoint; safety and toxicity were secondary endpoints. Planned starting dose was nilotinib 400 mg BID, but could be escalated to 600 mg BID for lack of response. Nilotinib cross-intolerance was defined as pts who experienced any grade 3/4 AE with the same preferred term that was identified for their imatinib-intolerance. All pts signed an informed consent and received nilotinib for an unapproved indication. *Results*. Of 318 pts with CML-CP enrolled (median duration of exposure 245 days), 95 pts were enrolled for imatinib-intolerance for either nonhematologic and/or hematologic AEs. Of the 120 pts with CML-AP enrolled (median duration of exposure 137.5 days), 22 pts were enrolled for imatinib-intolerance. Some pts had >1 AE satisfying the criteria for intolerance. The frequency of grade 3/4 AEs in both CP and AP pts for imatinib and nilotinib is shown (Table 1). Only 2/109 (3%) pts with nonhematologic imatinib-intolerance experienced a recurrence of similar grade 3/4 AEs during nilotinib therapy. 42 pts were enrolled with hematologic intolerance to imatinib (neutropenia, thrombocytopenia) and only 7/42 (17%) pts developed similar events during nilotinib therapy and discontinued nilotinib. All 7 pts experienced a recurrence of grade 3/4 thrombocytopenia and discontinued nilotinib. Of 117 pts with imatinib intolerance, 103 had at least 6 mos of follow-up and 50% of these pts achieved MCyR, which is similar to that reported for imatinib-resistant pts (45%), presumably because these pts also had imatinib-resistance. *Conclusions*. These results demonstrate that nilotinib has a very low rate of cross-intolerance with imatinib and can be used effectively in both CML-CP and -AP pts with imatinib-intolerance. Thrombocytopenia appears to be the only intolerant AE that may recur with nilotinib. Although imatinib and nilotinib are chemically similar with an identical target and mechanism of action in CML, there is minimal clinical overlap in terms of intolerant symptoms. Overall, these results support the excellent tolerability of nilotinib.

Table 1. Occurrence of Nilotinib Intolerance in Imatinib-Intolerant Pts.

	No. of Pts With Intolerance (n=117*)	
Grade 3/4 AEs	Imatinib	Nilotinib
Nonhematologic		
Rash/skin	30	0
Fluid retention	22	0
GI	16	1 (6%)
Liver toxicity	13	1 (8%)
Myalgia/arthralgia	10	0
Hematologic		
Thrombocytopenia	29	7 (24%)
Neutropenia	10	0

*Includes 95 CML-CP and 22 CML-AP pts.

0861

IN PATIENTS WITH CHRONIC MYELOGENEOUS LEUKEMIA (CML), RESPONSE DYNAMICS TO NILOTINIB AFTER IMATINIB FAILURE DEPEND ON THE TYPE OF BCR-ABL MUTATIONS

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Background. Nilotinib (AMN107) is an orally bioavailable inhibitor of the BCR-ABL tyrosine kinase with improved potency and specificity over imatinib. In preclinical models, activity of nilotinib was also demonstrated in 32/33 imatinib-resistant mutant cell lines. *Aims.* We sought to investigate the efficacy of nilotinib in patients (pts) according to the type of preexisting BCR-ABL mutations associated with imatinib resistance. *Methods.* We have investigated peripheral blood samples from 183 chronic phase (CP) and 51 accelerated phase (AP) CML pts who had been enrolled in a phase II study investigating the efficacy and safety of 400 mg nilotinib BID after imatinib failure. Screening for BCR-ABL mutations was performed by direct sequencing of the BCR-ABL tyrosine kinase domain ranging from amino acid 230 to 490 (GenBank accession no. M14752) and its surrounding regions. *Results.* Prior to nilotinib, 31 different BCR-ABL mutations involving 23 amino acids were detected affecting 43% of CP and 57% of AP pts. After 6 months of therapy, complete hematologic response (CHR) was achieved in 57%, major cytogenetic response (MCyR) in 42%, and complete cytogenetic response (CCyR) in 24% of pts with mutations vs 79%, 57%, and 39% of pts without mutations, respectively. Response dynamics were associated with preclinical activity of nilotinib: MCyR was achieved in 17/32 pts with mutations associated with preclinical IC50 to nilotinib of <100 nM, 8/27 pts with IC50 of 100-1000 nM, and 0/4 pts with mutation T315I demonstrating complete resistance to imatinib and nilotinib. In AP pts, hematologic response was achieved in 48% vs 46%, MCyR in 27% vs 21%, and CCyR in 17% vs 14% of pts with or without mutations, respectively. *Summary/Conclusions.* Nilotinib is efficacious in pts with BCR-ABL mutations, except T315I, as well as in patients with BCR-ABL'independent resistance. Time to response and rate of response may depend on the individual type of the mutation and correlates with the IC50 to nilotinib. Thus, nilotinib may have an important therapeutic role in imatinib resistance as well as in frontline CML therapy to prevent emergence of resistant clones.

0862

COMPARISON OF DASATINIB TO HIGH-DOSE IMATINIB IN PATIENTS W320HO EXPERIENCE IMATINIB FAILURE: RESULTS FROM A RANDOMIZED, PHASE-II TRIAL (CA180017, START-R)

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Background. Relapses on imatinib at doses of 400-600 mg/d are a well-recognized problem occurring in approximately 13% of newly-diagnosed chronic-phase (CP) CML patients with 5 years of follow-up. Effective therapeutic options for these patients are limited. Escalating imatinib doses to 800 mg/d may overcome resistance in some cases and dasatinib, a novel BCR-ABL kinase inhibitor, has also been shown to be safe and effective in this population. Unlike imatinib (and its analog nilotinib), dasatinib binds to the active, oncogenic conformation of BCR-ABL. *Aims.* To assess the relative efficacy and safety of dasatinib and high-dose imatinib in patients with imatinib-resistant CP-CML. *Methods.* Patients with CP-CML with primary or acquired resistance to conventional doses of imatinib (400-600 mg/d) were randomized on a 2:1 basis to dasatinib 140 mg (70 mg bid) (N=101) or imatinib 800 mg (400 mg bid) (N=49) as part of this international, multicenter study. Primary resistance was defined as a lack of complete hematologic response (CHR) at 3 months, lack of any cytogenetic response (CyR) at 6 months, or lack of major CyR (MCyR) at 12 months. Relapse after a HR or MCyR was considered as acquired resistance. Crossover to the alternate regimen was permitted following confirmed progression, lack of MCyR at Week 12, or intolerance (Grade 3-4 non-hematologic toxicity or hematologic toxicity requiring multiple dose modifications). Dose escalation of dasatinib to 90 mg bid or reduction to 50 mg bid or 40 mg bid were allowed for inadequate response or adverse events, respectively. A 600-mg dose of imatinib was permitted for toxicity for patients who had not previously received imatinib 600 mg. *Results.* Patient characteristics were well balanced at baseline (median age 51 years; median time form diagnosis 59 months), with one exception; BCR-ABL mutations were 2-fold more frequent for the dasatinib treatment group (45% vs. 22%). With follow-up now extending to 21 months, results are consistently in favor of dasatinib over high-dose imatinib in terms of: CHR (93% vs. 82%; p=0.034), MCyR (52% vs. 33%; p=0.023), complete CyR (CCyR) (40% vs. 16%; p=0.004), major molecular response (16% vs. 4%; p=0.038), time to treatment failure (hazard ratio [HR], 0.16; p<0.0001), and progression-free survival (HR, 0.14; p<0.0001). Rate of MCyR favored dasatinib for both mutation-positive (46% vs. 27%) and mutation-negative patients (55% vs. 34%). MCyRs for patients with difficult-to-treat P-loop mutations were only attained by those receiving dasatinib therapy (50% vs. 0%), although numbers were limited. Post-crossover results also favored dasatinib in terms of MCyR (45% vs. 15%; p=0.063) and CCyR (29% vs. 0%; p=0.031). The safety and tolerability of dasatinib are consistent with previous studies and are acceptable. Superficial edema (15% vs. 43%) and fluid retention (30% vs. 45%) were both more prevalent with imatinib, whereas pleural effusion was more common with dasatinib (17% vs. 0%). Cytopenia was more frequent and severe with dasatinib. *Summary/Conclusions.* Dasatinib represents a safe and effective therapy for CP-CML resistant to conventional doses of imatinib. Study CA180017 provides evidence that dasatinib is superior to high-dose imatinib for patients who experience imatinib failure.

Non-Hodgkin's lymphoma - Clinical II

0863

PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL) ARISING IN DISTINCT SITES OF HEAD AND NECK HAVE DIFFERENT CLINICAL CHARACTERISTICS AND OUTCOME

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Clinical outcome of patients with primary extranodal head and neck lymphoma (PEHNL) is not only influenced by histology and stage, but also by the site of presentation. However, it has not yet been clarified whether the reason for this difference is intrinsic to the location or depends on distribution of histopathology in different sites of disease. Therefore, the aim of this retrospective study was to evaluate the clinical outcome and prognostic factors of PEHNL patients with DLBCL according to the sites of presentation. From December 1990 to June 2004, 478 patients were referred to 14 cancer centres. This series included 253 males and 225 females, with a median age of 60 years (range, 14-93 years). Waldeyer's ring (WR) was the most common site of presentation (n=297), followed by nose and paranasal sinuses (NPS) (n=48), thyroid (n=48) (T), salivary glands (SG) (n=38), combined sites (CS) (n=25) and oral cavity (n=22) (OC). The frequency of adverse features varied in different locations: WR had more stage II patients, while in thyroid cases advanced age (>60 yrs), female sex, bulky disease, poor ECOG-PS, elevated LDH and >1 adverse factors according to stage-modified IPI (MIPI) were more frequent. The commonest treatment was a short course of anthracycline-based chemotherapy (CHT) + involved field radiotherapy (IFRT). Forty-two percent of T patients also underwent surgery. Only 28/428 (6.5%) received CNS prophylaxis. The CR rate ranged from 79% (CS) to 95% (OC), while relapse was most common in SG (31%) and prevailed in distant sites (60%). Four patients (1 WR, 1 SG, 2 NPS), without CNS prophylaxis, relapsed in CNS (0.8%) and 4 WR patients (0.8%) in GI tract. After a median follow-up of 49 months (range 1-219 months), 5-yr OS, EFS and DFS were 72%, 59% and 74%, respectively. OS varied among different locations from 51% (T) to 89% (OC). It is noteworthy that CS patients did not fare worse than those with disease presenting in single sites. In all presentations with the exclusion of T patients, 5-yr EFS differed according to MIPI (MIPI 0-1, 68% vs. MIPI >1, 49%; p=0.0001) and CHT+IFRT (combined treatment) (CHT 43% vs. CHT+IFRT 71%; p=0.0001) as confirmed by Cox multivariate analysis (MIPI, p=0.012; combined treatment, p=0.0001). However, in thyroid involvement MIPI seems to be not predictive of survival due to a high mortality unrelated to disease. Moreover, T patients seem to benefit more from surgery in combination with chemotherapy and/or IFRT than from other treatments not including partial or complete thyroid resection (p=0.04). *Conclusions*. patients with DLBCL of different head and neck sites represent a heterogeneous group regarding clinical characteristics, prognostic factors and outcome. A low MIPI and a combined treatment with addition of radiotherapy influence the outcome favorably. Moreover, a very low rate of CNS recurrence suggests that CNS prophylaxis may not be mandatory for these patients.

0864

THE CHARACTERISTICS AND CLINICAL COURSE OF MALT-LYMPHOMA: THE UNIVERSITY OF VIENNA EXPERIENCE

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Background. A prospective assessment of clinical characteristics and potential risk factors affecting outcome in patients with MALT lymphoma was performed at our institution. *Patients and Methods.* Between 1997-February 2007, a total of 173 consecutive patients with histologically verified MALT lymphoma (101 female / 72 male) underwent uniform staging and assessment of clinical characteristics including assessment of genetic changes, presence of autoimmune disease (AD), Helicobacter pylori (HP)-

status, monoclonal immunoglobulins (MG) and plasmacytic differentiation (PD). All patients with follow-up at our institution were analysed, and influence of various parameters on dissemination and outcome were evaluated. Sixty-five patients (38%) had gastric MALT-lymphoma (GML) and 108 (62%) extragastric MALT-lymphoma (EGML). *Results.* 68/172 patients (39%) had multiorgan-involvement upon presentation, while only 3 patients had bone marrow involvement. Patients with EGML (51/108, 47%) were at significantly higher risk for multiorgan-disease than patients with GML (17/65, 27%, p=0.013). 58 patients had genetic aberrations, with t(11;18) being more common in GML (p=0.002) and trisomy 3 (p=0.03) and trisomy 18 (p=0.02) in EGML. Among GML, translocation t(11;18) was significantly associated with multiorgan-disease, as was trisomy18 in EGML (p=0.038). After a median follow-up time of 42 months, 142 patients (86%) are alive.58 patients (39%) have relapsed, with the median to relapse being 60 months. Overall, multifocality and extragastric origin (p<0.001) were significantly associcated with relapse, while patients with t(11;18) had a significantly longer time to relapse (131 vs 49 months). Survival and relapse were not affected by the form of therapy applied (i.e. local vs systemic). An underlying AD was found in 67 patients (39%), and was significantly associated with EGML (p=0.007) and multifocality (p=0.04), but did not influence relapse and survival. HP-positivity was significantly associated with GML (p=0.001), and patients with EGML did not respond to HP-eradication. *Conslusions*. Our data suggest dissemination and (late) relapse to be relatively common in MALT-lymphoma. Based on our findings, however, an individualized staging approach and risk-assessment is possible.

0865

IDENTIFICATION OF NEOPLASTIC INFILTRATION OF THE CEREBROSPINAL FLUID IN AGGRESSIVE B-NHL: A MULTICENTER STUDY COMPARING THE UTILITY OF FLOW CYTOMETRY VS CONVENTIONAL CYTOLOGY

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Background. Infiltration of the central nervous system (CNS) is a relatively common finding in specific subtypes of B-NHL, such as Burkitt's lymphoma (BL) and diffuse large B cell lymphoma (DLBCL). CC analysis of the CSF is considered as the reference method to diagnose meningeal disease in B-NHL; however, recent studies suggest that B-NHL patients who are at high risk of CNS relapse, FCM could be more sensitive than CC for detecting meningeal disease in CSF. *Aim.* To evaluate the sensitivity and specificity of a standardized FCM immunophenotypic approach vs CC for detecting the presence of neoplastic cells in CSF, in patients with aggressive B-NHL who are at high risk of CNS relapse. *Methods*. A total of 63 CSF samples were analysed (total volume: 0.8 to 4ml; median: 2.3 mL) in newly diagnosed patients with aggressive B-NHL, from a total of 19 different hospitals (DLBCL: 40; BL: 18; follicular lymphoma transformed to DLBCL (tFL): 2; T-cell-rich B-NHL: 2 and plasmablastic lymphoma (PL): 1). Of the 63 patients studied, 37 were men (59%) and 26 women (41%) with a mean age of 57±18 years (range: 13-86 years). In all cases, CSF samples were analysed simultaneously using CC at the institution of origin and FCM, centrally at a single institution. For the FCM analysis of the CSF, stabilised samples (Transfix, Immunostep SL) were systematically stained with the following combination (FITC/PE/PERCPCY5.5/PECY7/APC/APCCY7) of monoclonal antibodies: CD8-lambda/CD56-kappa/CD4-CD19/CD3/CD20/CD45. If CSF infiltration was found in the FCM test, an additional 6-color antibody panel was used for full phenotypic characterisation of the disease. *Results*. Haematopoietic cells were detected in all cases (mean: 1.5±2 cells/ul; range: 0.1-10 cells/uL). These cells included T cells in 98% of cases (mean: 0.6±0.8/uL; range: 0.04-3.6/uL) and monocytes in 95% of cases (mean: 0.6±1.2/uL; range: 0.01-9/uL). Furthermore, in 30%, 1.6%, 17% and 11% of the cases polyclonal B-lymphocytes (mean: 0.05±0.06/uL; range: 0.01-0,2/uL), plasma cells (0.09/ul), natural killer cells (0.02±0.02/ul; range: 0.01-0.08/uL) and neutrophils (mean: 0.9±1.2/uL; range: 0.03-3/uL) were also detected, respectively. Of the 63 cases studied, 10 (16%) showed infiltration by neoplastic B-cells by FCM, while CC only showed infiltration in four of these patients (6%), with two other cases being suspicious (3%). Cases with CNS infiltration by both methods included 1/2 tFL -14 neoplastic cells/ul (82%) by FCM-, 2/18 BL -8 neoplastic cells/ul (68%) and 187 neoplastic cells/uL (99%) using FCM- and 1/40 DLBCL -0.96 neoplastic cells/ul (28%) by FCM-. Infiltrated cases by FCM and suspicious by CC included two cases of BL with 165 (98%) and 0.9 (75%) neoplastic cells/ul by FCM. Those four patients with FCM+/CC- CSF samples displayed lower percentages of pathological B-cells (2% and 16% in 2/40 patients with DLBCL and 1% and 0.1% in 2/18 BL, respectively). Two of these four patients had neurological symptoms (meningism and facial paralysis). *Conclusions*. Although preliminary, these results suggest that FCM is more sensitive that CC in detecting CSF infiltration by neoplastic B-cells in aggressive B-NHL, particularly in cases where these cells are present in a relatively low numbers.

0866

HIGH INCIDENCE OF AUTOIMMUNE COMPLICATIONS IN 82 PATIENTS WITH NON-MALT MARGINAL ZONE LYMPHOMAS

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Background. Non-MALT marginal zone lymphomas (MZL) include splenic (SMZL) and nodal (NMZL) types which are rare indolent non-Hodgkin lymphomas; the incidence of autoimmune complications is reported between 10 and 15%. *Aim*. We performed a retrospective analysis in our patients with non-MALT MZL to evaluate the incidence of autoimmune phenomena, their relationship with clinical and biological characteristics and their impact on survival. *Patients and Methods*. We retrospectively evaluated 82 patients with non-MALT MZL, diagnosed between 1988 and 2007 at our Institution. Seventy-four were affected by SMZL and 8 by NMZL. In 45/74 patients with SMZL the diagnosis was made by bone marrow biopsy and in 29 by spleen and bone marrow histologies. The patients with NMZL were diagnosed by bone marrow biopsy in 3 cases and by lymph-node and bone marrow biopsies in the remaining ones. Fifty-two patients were males and 30 females (ratio 1,73) with a median age at diagnosis of 64 years (range 34-86). In 82% of SMZL patients splenomegaly was present, while all the NMZL patients had peripheral lymphadenopathy. Serum M-component was detectable in 29/82 (35,3%) patients. Anti-HCV seropositivity (RIBA) was found in 14/62 (22,6%) tested patients. *Results*. In 20/82 patients (24,4%) clinically relevant autoimmune complications occurred; we found 8 cases of autoimmune haemolytic anaemia (AIHA), 2 of autoimmune thrombocytopenia, 2 of Evans' syndrome, 3 of anti-MAG-associated neuropathy, and one case of rheumatoid arthritis, Sjogren's syndrome and symptomatic crioglobulinemia respectively. Moreover one patient presented both AIHA and antiphospholipid syndrome (APS) and another autoimmune thrombocytopenia and APS. We also found 7 cases of clinically asymptomatic crioglobulinemia and 8 patients with seropositivity for Rheumatoid factor or ANA. Incidence of autoimmune complications was higher in females (p<0.05) while we found no statistically significant correlation between autoimmune disorders and: age, presence of splenomegaly, LDH or β2-microglobulin levels, anti-HCV seropositivity and leukemic presentation. In 14/20 patients autoimmune complications occurred at presentation or within 3 months from diagnosis; in 3 patients autoimmune phenomena preceded the diagnosis of lymphoma of 60, 18 and 3 months respectively. Treatment of symptomatic autoimmune complications consisted of: steroids (13 patients) associated with alkylating agents (7) or splenectomy (5), Rituximab + polichemotherapy (5), other drugs (2). Median overall survival of all patients was 108 months; the occurrence of autoimmune complications did not significantly influence survival. *Conclusions*. In our series of non-MALT marginal zone lymphomas the incidence of autoimmune complications was higher than previously reported. In the majority of cases they occurred at diagnosis or even before it. Survival was not influenced by the occurrence of autoimmune phenomena.

0867

COMPREHENSIVE GERIATRIC ASSESSMENT-ADAPTED CHEMOTHERAPY IN 100 ELDERLY PATIENTS (>70 YEARS) WITH DIFFUSE LARGE B-CELL NON-HODGKINS LYMPHOMA

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Background. Rituximab plus CHOP (R-CHOP) is the standard chemotherapy (CT) regimen for elderly patients (pts) with CD20 positive DLBCL. However, many pts aged 70 years (yrs) or more are often unable to received R-CHOP and the majority of them are excluded from clinical trials. Moreover, comprehensive geriatric assessment (CGA) has been demonstrated a useful instrument to predict the clinical outcome of elderly pts with cancer even if it has never been tested in a prospective way. *Aims*. Within the GOL (Gruppo Oncoematologico Linfomi) from June 2000 to March 2006 we started a phase II prospective study with the aim to evaluate feasibility and activity of a CGA-driven CT for elderly pts with DLBCL. *Methods*. Rituximab was used in all pts after its introduction in the marketing in Italy (February 2002). Pts with no comorbidity received CHOP or R-CHOP; in pts with mild cardiopathy epirubicin was used instead of doxorubicin (CEOP or R-CEOP); in pts with moderate or severe cardiopathy the use of antracyclines was omitted (CVP or R-CVP); pts with diabetes didn't receive prednisone (CHO, CEO or R-CHO, R-CEO); pts with neuropathy received CHP or R-CHP or CEP or R-CEP (vincristine was omitted). The dosage of CT was decided according to CGA: pts with a good score of CGA (i.e. ADL=6 and IADL>6) received full doses of CT; pts with an intermediate score (ADL=5 and IADL>4) received 75% of the planned dose; pts with a poor score (ADL<5 and IADL<5) received 50% of the planned dose. All pts received prophylactic filgrastim. *Results*. One hundred pts (41 males and 59 females) have been treated and no patient was excluded from this approach. The median age was 75 yrs. Stages III-IV were diagnosed in 51% of pts. Sixty-one per cent of pts received full doses of CT; 25% of pts received 75% of the planned dose and 14% of pts 50% reduced dose of CT. Overall, 86% of pts received an antracycline (doxorubicin in 56% and epirubicin in 30%) and 54% received rituximab plus CT. The used regimens were: R-CHOP 22%, CHOP 16%, 75%-R-CHOP 10%, 75%-CHOP 8%, CEOP 11%, R-CEOP 4%, 75%-R-CEOP 9%, 75%-CEOP 6%. The remaining pts received CVP in 5% of cases and reduced R-CVP in 9% of cases. Toxicity was quite acceptable. Grade 3-4 neutropenia was observed in 29% of pts, mucositis in 13%, peripheral neuropathy in 9%, febrile neutropenia in 13%, cardiac toxicity in 3% and skin toxicity in 1%. Four toxic deaths were observed (2 septic shock, 1 acute respiratory failure and 1 acute myocardial infarction). Overall, 76% of pts achieved a complete remission (CR) and with a median follow-up of 24 months, only 16% of them have relapsed. Seventy-three pts are alive and 63% are alive in CR. *Conclusions*. Our results demonstrated that a CGA-driven approach is feasible and highly active in elderly pts with DLBCL. Moreover this strategy allows a potentially curative approach to all pts with aggressive NHL avoiding both to under-treat elderly pts with a curable disease and to over-treat elderly pts with comorbidities.

Acute myeloid leukemia - Biology I

0868

LESTAURTINIB INDUCES INCREASED CYTOTOXICITY IN PRIMARY AML BLASTS WITH A FLT3 INTERNAL TANDEM DUPLICATION COMPARED TO THOSE WITH WILD TYPE OR TYROSINE KINASE DOMAIN MUTATED FLT3

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Background. The presence of an internal tandem duplication (ITD) in the fms like tyrosine kinase 3 (FLT3) gene predicts an adverse outcome in young adults with acute myeloid leukaemia (AML). We have recently demonstrated that mutations within the tyrosine kinase domain (TKD) of FLT3 are not associated with a similarly adverse prognosis (Mead *et al.* [2005] Blood, 106, 101a). The different prognosis associated with the two different classes of mutation may have implications for the use of FLT3 inhibitors in patients with FLT3 mutated AML. *Aims*. To compare the *in vitro* cytotoxic effects of lestaurtinib (CEP701) and PKC412 in primary AML blast cells from patients of different FLT3 mutational status and to assess cytotoxicity induced by FLT3 inhibition in combination with cytarabine. *Methods*. Primary blast cells from patients with AML (n=22) were incubated in the presence of lestaurtinib (5-100 nM), PKC412 (5-100 nM), cytarabine (0.01-100 mcg/mL) or a combination of cytarabine and lestaurtinib for 48 hours. The number of viable cells remaining was assessed by measuring the optical density following the addition of MTS tetrazolium compound (Promega). Evidence of synergy was calculated using the Chou-Talalay equation (Calcusyn software). *Results*. In primary AML blast cells, both lestaurtinib and PKC412 induced cytotoxicity, although higher concentrations of PKC412 were required to induce the same degree of kill. The cytotoxicity induced by lestaurtinib was greater in the 6 FLT3/ITD-positive cases (35±10% [SEM] kill at 5nM lestaurtinib) than in the 5 FLT3/TKD-positive cases (10±4%, p=0.06) which did not differ from the 11 FLT3/WT cases (5±4%, p=0.38). The difference between FLT3/ITD and FLT3/WT cases was significant (p=0.03). When cytarabine was used as a single agent, the effect was similar in FLT3/ITD and FLT3/WT cases across all concentrations used but in FLT3/TKD-positive cases there was a suggestion that a greater degree of kill was achieved at the highest concentration (100 mcg/mL) (p=0.05). When cytarabine and lestaurtinib were used in combination, there was strong evidence of synergy between their cytotoxic effects which was most marked in FLT3/ITD cells, with a mean combination index of 0.3, 0.6 and 0.4 in FLT3/ITD, TKD and WT cells respectively. *Conclusions*. These data suggest that there are important differences in the effect of FLT3 inhibitors on AML blasts depending on the type of FLT3 mutation present. FLT3/ITD mutated AML blasts were more sensitive to FLT3 inhibition than FLT3/TKD mutated or FLT3/WT blasts, suggesting that blasts with a FLT3/ITD are more likely to be dependent on FLT3 signalling for survival.

0869

HIGH RESOLUTION SNP ARRAYS AND MIRNA GENE EXPRESSION REVEALS PREVIOUSLY UNIDENTIFIED ABERRATIONS IN PATIENTS WITH AML AND NORMAL KARYOTYPE

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Acute myeloid leukemia (AML) is a clinically and molecularly heterogeneous disease. Effective risk stratification is especially difficult for patients with normal karyotype, which account for more than 40% of all cases. Our aim was to use a high-resolution Affymetrix 50K-SNParray (SNP-A) to study the whole genome of a series of 47 well genetically characterized patients with AML and normal karyotype, and its relationship with genetic aberrations known to have a prognostic impact in AML. We only took into consideration common aberrations detected by the CNAT and the dChipSNP softwares. Expression of 157 mature microRNAs was analyzed by real-time PCR. We found a subgroup of cases with no cryptic changes (14/47, 29%) that were heterogeneous at the molecular level (8 had ITD-FLT3, 6 no mutation), and 10 cases had cryptic deletions and/or duplications (21%). Twenty eight cases (60%) had LOH by uniparental disomy (UPD). Interestingly, some of these recurrent regions are frequently deleted or rearranged in AML: 4q21.21-

q35.2 (5 cases), 5q23.1-5q31.1 (2 cases), 7q22.1-q32.1 (5 cases), 11q23.3-11q24.2 (2 cases), and 13q12-q22.1 (7 cases). We analyzed if these regions contain homozygous mutations in significant genes in AML, and in 2 cases with 13q12 UPD we identified a homozygous mutation in FLT3. Although it would be necessary to confirm it in a larger series, LOH by UPD does not seem to have impact in the outcome of the patients; however, UPD could lead to alterations in expression levels of imprinted genes, and could be associated with specific gene expression patterns. Fisher's Exact test demonstrated no association ($p>0.05$) between the presence of LOH and variables with a prognostic meaning in AML: FLT3 mutation, age>60, and no complete remission. LOH by UPD usually occurs in fragile sites in the genome, a common location for miRNA. Recently, there has been major progress in the identification of miRNA expression profiles that could be associated with prognostic factors. Moreover, miRNAs seem to have a role in leukemia pathogenesis. To study the relationship between LOH regions and miRNAs expression we profiled expression of 157 miRNAs by using real-time PCR in 23 patients, and in MO from normal donors. Twenty two miRNAs showed differentiation associated expression changes. Consistent microRNA down-regulation was seen in miR-198, miR-211, miR-139, miR-302b, miR-127, miR-214, miR-182*, miR-205, miR-105, miR-138 and miR-204; whereas miRNA upregulation was seen in miR-374, miR-181a, miR-181b, miR-146, miR-210, miR-34a, miR-213, miR-219, miR-155, miR-17-5p and miR-30e. In conclusion, our results confirm other studies by showing that the prevalence of LOH by UPD in *de novo* AML is high, 60% in our series with normal karyotype. Although it would be necessary to confirm it in a larger series, this does not seem to have impact in the outcome of the patients. Interestingly, we identified a subgroup of 14 patients with no cryptic genomic aberration changes that was heterogeneous at the molecular level and had different outcome, confirming that the subclassification of AML patients with normal karyotype should carry on looking for molecular profiles, including miRNA differential expression.

0870

SEGMENTAL UNIPARENTAL DISOMY IS THE MOST COMMONLY ACQUIRED GENETIC ABNORMALITY IN RELAPSED ACUTE MYELOID LEUKEMIA

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Background. Relapse is the commonest cause of death in acute myeloid leukaemia (AML), but the mechanisms leading to relapse are unclear. Recently, acquisition of segmental uniparental disomy (UPD) by mitotic recombination (MR) has been reported in 15-20% of AML patients at diagnosis using whole genome single nucleotide polymorphism (SNP) arrays. These abnormalities are cytogenetically invisible and are associated with homozygous mutations in several types of malignancy. Clonal evolution from heterozygous to homozygous mutations by MR could provide a mechanism for relapse. *Aims*. To identify regions of UPD acquired at relapse of AML and associated gene mutations within the homozygous region. *Methods*. DNA from 27 pairs of diagnostic and relapsed AML samples were analysed using Affymetrix 10K SNP arrays. Copy number and loss of heterozygosity were analysed using in-house software. Regions of deletion, gains and segmental UPD were documented and compared between diagnosis and relapse. *Results*. Segmental UPDs were acquired at relapse in eleven AML patients (40%). Six of these were segmental UPDs of chromosome 13q. FLT3 exon 14-15 lay in the region of homozygosity. Sequencing of the six cases demonstrated a change from heterozygosity at diagnosis to homozygosity at relapse for an internal tandem duplication (ITD) mutation of FLT3, confirmed by PCR fragment analysis. The mutation was identical between each diagnosis and relapse pair. Another AML patient acquired segmental UPD of 19q, which lead to homozygosity of a CEBPA substitution mutation at position 957, changing from C to T. This is a stop codon that has previously been described to produce a truncated protein. One AML patient acquired segmental UPD of chromosome 4q. There is likely to be an associated homozygous mutation in the region of UPD, but in view of the large region involved, it was not possible to investigate further. Another three AML patients had evidence suggesting an acquired subclone, with UPD of chromosome 13, at relapse. The heterozygous calls across chromosome 13 at diagnosis became *no calls* at relapse because the calling algorithm was unable to interpret a change in the proportion of alleles. This was confirmed by showing a change in the relative allele signals between diagnosis and relapse. In one of these cases, there was also a rise in the FLT3 ITD level at relapse suggesting the subclone harboured a homozygous FLT3 mutation. *Summary/Conclusions*. This study suggests acquisition of segmental UPD by mitotic recombination is the most commonly acquired genetic abnormality at relapse of AML. It shows there can be possible clonal heterogeneity in the acquisition of UPD, which suggests acquired UPD may be under detected. Finally, it suggests AML cells with acquired UPD are resistant to chemotherapy.

0871

DERIVATION OF A GLOBAL MAP OF DNA HOMOZYGOSITY IN ACUTE MYELOID LEUKEMIA (AML)

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Background. Cytogenetic abnormalities are the most important prognostic factor in AML, but recent small studies have demonstrated frequent acquired regions of homozygosity invisible by conventional karyotyping. These regions have a normal copy number, so are called segmental uniparental disomy (UPD). They result from mitotic recombination or non-disjunction events and can lead to homozygosity for mutated genes within the region. *Aims*. To provide a high-resolution map of homozygosity and copy number changes (CNCs) in a large and unselected cohort of patients with acute myeloid leukemia. Materials and *Methods*. Diagnostic samples from 463 patients with AML from the MRC AML10 trial were studied using Affymetrix 10K 2.0 single nucleotide polymorphism (SNP) arrays. This array identifies 10,204 SNPs across all chromosomes excepting the Y-chromosome. DNA from ten unrelated non-leukemic individuals were used as a control. Data was analyzed using in-house developed software, Genome Oriented Laboratory File (GOLF). *Results*. 380 CNCs and regions with homozygosity were identified in 161 patients (35%). Copy number neutral homozygosity due to segmental or whole chromosome UPD was observed in 17%, deletions in 14.5% and gains in 12%. Of all the 380 aberrations, 46.5% were deletions, 31.2% were UPDs and 21.6% were gains. UPDs were most frequent on chromosomes 11, 13, 2, 1 and 6. The most recurrent regions were on 13q, 11p and 11q. The most frequent deletions were of 7/7q, 5q, 6p, 6q, 11p and most frequent gains were of 8, 11q, 21q. UPD was seen across all cytogenetic risk groups. The proportion of patients with deletions, gains and UPDs amongst the poor-risk cytogenetic group were 71%, 34% and 23%, respectively. The good risk cytogenetic group had 9% deletions, 9% gains and 11% UPDs, and the intermediate risk cytogenetic group had 9%, 7.5% and 16.5% respectively. UPDs of 13q (5.4% of patients) were observed exclusively in intermediate risk AML. Within the intermediate risk group, there were more UPDs amongst normal karyotype (NK) AML patients (19%) than those with cytogenetic abnormalities (10.5%). SNP array analysis was able to map in detail copy number changes in poor risk, complex karyotype AML patients. Monosomy or deletions of 18/18q (14.3%) and gains of 5p (5.7%) were observed exclusively in poor-risk AML. Deletions of 12p, 17/17p, 3/3q, 20/20q (11.4% patients for all four) were more frequent in poor risk patients than any other group, which is in accordance with other reports. Deletions of 8/8p, 15/15q and 16/16q were observed in 11.4%, 8.6% and 14.3% of poor-risk patients, respectively. The results of 369 patients by SNP array analysis largely matched those from cytogenetic karyotyping available. Of 302 samples that were normal on SNP arrays 12% showed CNCs by cytogenetic analysis. SNP arrays could also detect many aberrations missed by karyotyping. The two experimental approaches therefore complemented each other. *Summary/Conclusions*. We have identified recurrent regions of UPD in a large cohort of AMLs. UPD occurs in all cytogenetic risk groups, but is more frequent in NK AML. This map is an initial step towards identifying areas containing novel candidate genes important in AML.

0872

MN1 INDUCES LEUKEMIA IN MICE AND PREDICTS ATRA RESISTANCE AND SENSITIVITY IN AML PATIENTS

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Recently, we found that overexpression of wild-type MN1 is a negative prognostic factor in acute myeloid leukemia (AML) patients with normal cytogenetics. We evaluated whether MN1 plays a functional role in leukemogenesis. Strikingly, we now demonstrate using retroviral gene transfer and bone marrow transplantation that MN1 overexpression is sufficient to induce a rapidly lethal AML in mice (disease latency 35 days). Insertional mutagenesis and chromosomal instability were ruled out as secondary aberrations. MN1 dramatically increased resistance to all-trans retinoic acid (ATRA)-induced cell-cycle arrest and differentiation (by >3,000-fold) in an *in vitro* differentiation model. We show by chromatin-immunoprecipitation that MN1 directly interferes with RARα signaling through binding to regulatory sequences and transcriptional repression of the RARα-target genes Rarbeta, C/ebpepsilon, and PU.1. The morphologically and functionally evident block in differentiation of MN1-transduced cells thus appears to be mediated by repression of RARα signaling. This could be confirmed functionally by fusion of a transcriptional activator (VP16) to MN1 in order to mask the potential repressive activity of MN1. MN1VP16-transduced bone marrow cells gave rise to immortalized cell lines, however, these cells differentiated to mature granulocytes and eventually went into cell cycle arrest upon treatment with ATRA. We then evaluated whether MN1 expression levels in AML patients (excluding the M3 subtype) correlated with resistance to ATRA treatment in a cohort of patients older than 60 years uniformly treated within treatment protocol AML HD98-B. Of a total of 83 patients 40 were treated with standard chemotherapy (idarubicin, cytarabin, and etoposide), and 43 patients were treated with standard chemotherapy plus ATRA. MN1 expression levels at diagnosis were measured by quantitative RT-PCR. Patient characteristics were equally distributed between ATRA versus no-ATRA treated patients including event-free survival (EFS) and overall survival (OS). Patients with MN1 expression above the median expression level did not differ in EFS or OS whether they were treated with ATRA or not. Strikingly, patients with low MN1 expression who received ATRA had a significantly prolonged EFS (p=0.008) and OS (p=0.04) compared to the remaining patients. Our data show that MN1 is a unique oncogene in hematopoiesis that both promotes proliferation/ self-renewal and blocks differentiation. Such findings provide tools and impetus to unravel the mechanisms of MN1 function. Of immediate consequence, MN1 expression levels allow to identify a subset of AML patients that appear to benefit from treatment with ATRA and thus may become useful as a predictive marker to guide the treatment in AML patients.

Acute lymphoblastic leukemia - Biology

0873

DIFFERENTIAL CHROMOSOMAL BREAKPOINTS IMPACT LEVEL OF LMO2 EXPRESSION IN T-ALL

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Background. Human T-cell acute lymphoblastic leukemias (T-ALLs) are associated with chromosomal translocations involving TCR genes and proto-oncogenes. The t(11;14)(p14;q11), which is considered the paradigm for TCR-associated translocations, is presumed to arise from erroneous TCRD V(D)J recombination and to result in ectopic LMO2 activation. However, the molecular mechanisms underlying formation of this translocation and the resulting LMO2 activation are poorly defined. *Aims* .We aimed to investigate how t(11;14)(p13;q11) aberrations lead to LMO2 activation in T-ALL. *Methods*. Following genomic breakpoint analysis in 9 T-ALL cases, breakpoints were cloned and tested in an *ex vivo* recombination substrate assay and in silico using a RIC algorithm. LMO2 levels in T-ALL were determined by real-time quantitative PCR. Occurrence of TCRD-LMO2 translocations was evaluated in normal thymocytes by nested PCR. *Results.* In our T-ALL cases we identified two t(11;14)(p13;q11) translocation mechanisms: 1) RAG mis-targeting of LMO2 through cryptic recombination signal sequences (cRSSs), and 2) V(D)J post cleavage synaptic complex repair mistakes. Furthermore, contrary to the common concept of TCR-enhancer driven activation, we provide compelling evidence that removal of a T-cell specific negative regulatory element (NRE) upstream of LMO2 is the main determinant for LMO2 activation in the majority of t(11;14)(p13;q11) cases. Activation levels are determined by the exact LMO2 breakpoint position and the configuration of the derivative chromosome. *Summary/Conclusions*. Our combined *in vivo*, *ex vivo*, and in silico analyses on nine new t(11:14)(p13;q11) positive T-ALL cases as well as normal thymocytes illustrate the critical role of the exact LMO2 breakpoint location in influencing the level of LMO2 activation and the consequent pressure for pre-leukemic selection and T-ALL development.

0874

EVIDENCE FOR POSTNATAL ORIGIN OF CHILDHOOD T-LINEAGE ALL

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Background. T ALL is an aggressive hematological malignancy that comprises about 15% of childhood ALL. One hallmark of T ALL is the activation of oncogenic transcription factors that results from deregulation, deletion or, rarely though, chromosomal translocations. The latter are considered early events in leukemogenesis that require further complementary mutations for the clinical manifestation of the disease. Such genetic alterations as well as the clone-specific T cell receptor (TCR) rearrangements characterize the leukemic clone and thus can be used to scrutinize neonatal blood spots. If they can be detected at birth, this would indicate that the leukemia originates already in utero. A fetal origin has been suggested for the majority of genetic subgroups of childhood B cell precursor ALLs. *Aims*. The aim of this study was to assess whether childhood T ALL also originated in utero by backtracking leukemia-associated molecular markers back to birth. *Patients and Methods*. Neonatal blood spots from 18 children, 1,5 - 11,2 years old at the time of diagnosis of T ALL, were included into this study. Molecular markers were identified and used for highly sensitive and specific PCR (RQ-PCR or nested PCR) approaches. A sensitivity to detect one cell with the particular marker among 100.000 normal cells was required for inclusion into this study. The targets comprised TCRD-LMO2 breakpoint regions (n=2), TAL1 deletions (n=3), Notch1 mutations (n=1), and TCRD or TCRG rearrangements (n=15). Between one third and half of a neonatal blood spot was

used per patient for these analyses. *Results*. The molecular marker (TCRD-LMO2 breakpoint region, n=1, TCR rearrangement, n=2) was detected in only three of 11 patients 3,3 years old or younger but in none of the older ones. The positivity at birth did not correlate with other clinical or biological features as for example leukemia subtype, presence of a thymus tumor or extent of bone marrow and peripheral blood infiltration. *Summary and Conclusions*. This is the first comprehensive study performed in children with T ALL to assess the in utero origin of their disease. Our data provide first evidence for a predominant postnatal origin of T ALL in the majority of cases with a rare prenatal initiation only in young children. These data suggest, in line with mouse and zebra fish models for T ALL and the data derived from the T ALL-like disease in two children with retroviral gene transfer for SCID-X1, that T ALL is not only a rapidly proliferating disease when fully malignant, but that the latency, the time from initiation to the clinical manifestation, might also be short. The results are in sharp contrast to B cell precursor ALL in children for whom an in utero initiation was demonstrated not only in the majority of cases with genetically diverse subgroups but also for children, who were older at the clinical manifestation of the leukemia.

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EML1-ABL1 IS ACTIVATED BY COILED COIL MEDIATED OLIGOMERIZATION AND INDUCES T-CELL ACUTE LYMPHOBLASTIC OR CHRONIC MYELOID LEUKEMIA IN A MOUSE BONE MARROW TRANSPLANT MODEL

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Background. BCR-ABL1 (BCR-ABL) is a potent oncogenic tyrosine kinase that is activated by coiled coil mediated oligomerization and by coiled coil independent mechanisms. BCR-ABL1 is common in CML and B-ALL, but is rarely found in T-ALL. The EML1-ABL1 fusion is a variant ABL1 fusion gene that we identified in a T-ALL patient. *Aims*. We aimed at comparing the oncogenic properties of BCR-ABL1 and EML1-ABL1 using *in vitro* and *in vivo* models. Characterization of ABL1 fusions such as EML1-ABL1 could also provide valuable insights to better understand the oncogenic properties of BCR-ABL1. *Methods*. We generated BCR-ABL1 and EML1-ABL1 constructs as well as deletion constructs and transduced those to the murine IL-3 dependent Ba/F3 cell line. EML1-ABL1 and EML1-ABL1 deletion variants were assayed for autophosphorylation and for their ability to transform Ba/F3 cells to IL-3 independent growth. Using a mouse bone marrow transplant model, we determined the transforming properties of EML1-ABL1 *in vivo*. *Results*. Both BCR-ABL1 and EML1-ABL1 transformed Ba/F3 cells to IL-3 independent growth, although EML1-ABL1 showed weaker autophosphorylation compared to BCR-ABL1. EML1-ABL1 forms homodimers, and deletion of the coiled coil domain of EML1 abrogated its transformation in Ba/F3 cells. These observations suggest that EML1-ABL1, in contrast to BCR-ABL1, only depends on oligomerization for its activation, which may explain the observation of weaker kinase activity of EML1-ABL1. In addition, deletion of part of EML1, resulting in a direct fusion between the EML1 coiled coil domain and ABL1 resulted in increased autophosphorylation. In a murine retroviral bone marrow transplant model, 50% of the mice transplanted with EML1-ABL1 transduced bone marrow cells developed a transplantable fatal T-ALL, characterized by leukocytosis, splenomegaly, and massive infiltration of bone marrow and spleen by $CD4^+/CD8^+$ blast cells. The remaining 50% of recipients of EML1-ABL1 transduced bone marrow cells developed either a non-transplantable CML-like disease (30% of mice) with leukocytosis, splenomegaly, and infiltration of bone marrow and spleen by $Mac1^+/Gr1^+$ maturing granulocytes, or a mixed T-ALL/CML-like disease (20%). All EML1-ABL1 associated diseases had a latency of 96 -119 days, which is significantly longer than the 15-21 days latency associated with BCR-ABL1 induced CML-like disease. The longer latency for EML1-ABL1 associated disease indicates that the acquisition of additional mutations is required. Some of the mice displayed expression of transcription factors LYL1, TAL1 and LMO2, known to be associated with human T-ALL. Activating NOTCH1 mutations could not be detected. In contrast to EML1-ABL1 induced disease, mice transplanted with the more active deletion variant with the EML1 coiled coil domain fused to ABL1 developed a CML-like disease with much shorter latency (36 days). *Conclusions*. In contrast to BCR-ABL1 which is activated by coiled coil dependent and independent mechanisms, coiled coil mediated oligomerization is necessary and sufficient for EML1-ABL1 kinase activity and transforming properties. Our data suggest that kinase activity of ABL1 fusions may determine in part the disease phenotype. The mouse model we describe for EML1-ABL1 induced T-ALL can be further explored to identify co-operating events in the pathogenesis of T-ALL and for testing novel therapies.

0876

ANALYSIS OF PAIRED PRESENTATION AND RELAPSE SAMPLES IN T-ALL SUGGESTS THAT, AT LEAST IN SOME PATIENTS, NOTCH-1 MUTATIONS MAY BE ACQUIRED AS A SECONDARY EVENT

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Background. Activating mutations in Notch-1 are present in over 50% of patients with T-cell acute lymphoblastic leukaemia (T-ALL). It has been proposed that acquisition of these mutations may be an early event in leukaemogenesis, committing common lymphoid progenitors towards a T-cell fate before the acquisition of other secondary mutations. They may thus represent potential targets for minimal residual disease (MRD) analysis which can be limited, particularly in adults, by the inability to identify suitable and stable gene rearrangements in the T-cell receptor (TCR). *Aims*. To determine the stability of Notch-1 mutations in paired presentation-relapse samples. *Methods*. Presence of Notch-1 mutations in the HD-N, HD-C, TAD and PEST domains was investigated in 51 presentation T-ALL samples (34 adults, 17 children) and 14 paired relapse samples using PCR and denaturing HPLC (Transgenomic WAVE®). Abnormal chromatograms were sequenced to identify the mutation, with purification of low level mutations where necessary. Relative mutant level was quantified in products with a size difference (insertions or deletions) using fragment analysis (Beckman Coulter CEQ8000). T-cell receptor (TCR) clonality was performed at the TCRα locus using standard techniques. Same patient identity was confirmed in the paired presentation-relapse samples using analysis at 4 short tandem repeat loci. *Results.* At presentation a total of 49 mutations were detected in 36 patients (70%), 12 of whom had more than one mutation. Seven of the mutations appeared to be low level from the WAVE pattern. Quantification was possible in four of them and confirmed that the relative mutant level was 6-10% of total despite the presence of greater than 85% blasts. Furthermore, 3 of the patients with low level mutations also had a high level mutation in another domain, suggesting the former were acquired as a secondary event in a subclone. Of 14 matched pairs, 7 were wild type at both presentation and relapse. Four of the 7 mutant-positive patients at presentation relapsed with the same mutation(s), with no change in mutant level which was high. Three patients had evidence of a change in mutant at relapse. One patient lost a low-level HD-C mutation but gained a high level HD-N mutation which could not be detected at presentation using mutant-specific qPCR at a sensitivity of 1×10^{-4}. The patient had the same two TCR Vα1 clones at both presentation and relapse. Another patient lost a high level PEST mutation at relapse whilst a low level HD-C mutation progressively increased at both first and second relapse (6%, 21% and 33% respectively). The same TCR Vα11 clone was detected at each time point. A third patient lost both a high level HD-N and PEST mutation and gained a different PEST mutation; in this case, the TCR clone at relapse was different, suggestive of a secondary T-ALL. *Summary*. These data suggest, at least in some cases, Notch-1 mutations post-date the TCR rearrangement and can be acquired as a secondary event. The loss of mutations at relapse in 3 of 7 mutant-positive patients indicates that caution must be exercised in using them as single MRD targets.

0877

PAX5/TEL TRANSDUCED PRE-BI CELLS ARE RESISTANT TO TGF-β1 AND MIGRATE TOWARDS SDF-α

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Background. PAX5 is a transcription factor essential for B cell development. Recent data indicate that PAX5 aberrancies are present in approximately 30% of pediatric B-cell precursor ALL. We previously cloned the PAX5/TEL chimeric gene, originated from the translocation t(9;12)(q11;p13) in an ALL patient. PAX5/TEL is likely to be an aberrant transcription factor, resulting from joining the 5' region of PAX5 to the 3' region of the TEL/ETV6 (Ets-family DNA binding domain) gene. We have demonstrated a specific nuclear localization of the chimeric protein in NIH3T3 by immunofluorescence analysis. Moreover, in IL-3 dependent murine proB Ba/F3 cells, PAX5/TEL recruits mSin3A corepressor, suggesting its function as transcriptional suppressor. Aim of the study was to investigate the functions of the PAX5/TEL chimeric protein in preBI cells purified from mice fetal liver. *Methods.* murine PAX5$^{-/-}$ (B220^{+}/C-KIT^{+}/CD19^{-}) and wild type (B220^{+}/C-KIT^{+}/CD19^{+}) preBI cells were transduced with pMIGR-PAX5/TEL-IRES-GFP retroviral construct. Both PAX5-/- preBI cells and wild type preBI cells were cultured on OP9 stromal cells in IMDM +2%FCS and IL-7. *Results.* PAX5/TEL transduced wild type preBI cells showed down modulation of CD19 and slight increase in c-KIT level expression, while B220 antigen progressively became negative. PAX5$^{-/-}$ preBI cells transduced with PAX5/TEL did not show any difference with the parental cells. Transwell assay showed increased migration index of PAX5/TEL wt preBI cells towards SDF-1α chemokine, indicating a tendency to migrate into bone marrow. RQ-PCR data indicates that this could be mediated by modulation of CXCR4 receptor levels. Without IL-7, after 24-48 of withdrawal, PAX5/TEL wt preBI cells were still proliferating, while control cells died; although they did not show a complete cytokine independence, after 72-96 hours of starvation, PAX5/TEL cells were still alive, showing an advantage in cell survival. PAX5/TEL cells were resistant to TGF-β1 anti-proliferative and apoptotic effects, continuing to actively proliferate in presence of the cytokine, with a three-fold increased growth rate than control cells. *Conclusions.* PAX5/TEL showed a role of transcriptional suppressor in wt preBI cells, down regulating CD19 expression; this, in addition to B220 decrease, indicates that PAX5/TEL can drive the regression of preBI cell into a previous B cell differentiation stage, e.g. proB cells. PAX5/TEL do not replace PAX5 functions in PAX5$^{-/-}$ cells; indeed it cannot activate PAX5 target genes as CD19, important for restoring B cell differentiation potential. Both during the culture without the IL-7 and during the culture in presence of TGF-β1, PAX5/TEL gives proliferation and survival advantage to cells, by offering them a chance to resist to apoptotic stimuli, thus potentially predisposing for further events leading cell transformation.

Thrombosis and bleeding disorders

0878

SOLUBLE P-SELECTIN AND THROMBOSIS RISK OF PATIENTS WITH A PERSISTENT LUPUS ANTICOAGULANT IN A PROSPECTIVE COHORT STUDY

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Background. Increased soluble P-selectin (sP-selectin) was associated with an about 10-fold increased risk of recurrent venous thromboembolism in a case/control study. No prospective data on the thrombosis risk of LA-patients with elevated sP-selectin levels are currently available. *Aims*. Therefore, it was the aim of our study to assess the value of elevated sP-selectin levels as a predictive parameter for thromboembolic events in LA-patients. *Methods*. Diagnosis of LA was made according to established criteria. sP-selectin was determined by ELISA (Human soluble P-Selectin Immunoassay, R&D Systems, USA). Elevated sP-selectin was defined as a value exceeding the 95th percentile of control group of 129 individuals (cutoff=55.1 ng/mL). LA-patients were asked to participate in the study at the time of diagnosis of LA or at the time of their first presentation at the outpatient ward if the diagnosis of LA had already been established. After patients had given their informed consent their medical history was recorded and they were followed prospectively. Study endpoints were objectively confirmed arterial or venous thromboembolic events, death or loss of follow-up. The study design was approved by the Ethics Comittee of the Medical University Vienna. *Results*. Ninety-seven LA-patients were included (80 women, 17 men; median age 42, range 17-86 years). Sixty-nine LA-patients had a history of thrombotic events (59 women, 10 men) and 52 of these (75%) were under oral anticoagulation at the time of study inclusion. Twenty-eight LA-patients (21 women, 7 men) did not have a history of thrombosis and nobody in this group was under oral anticoagulation. sP-selectin levels were significantly higher in LA patients with a history of thrombosis (median 44.1 ng/mL, range 9.8-130.1) than in those without (median 35.5 ng/mL, range 15.6-74.1, p=0.008). An odds ratio for past thrombosis of 6.5 [95% confidence interval: 1.4-29.8] was calculated for elevated sP-selectin. During follow-up (median observation time: 911 days, range: 80-1973) ten thrombotic events were observed (four deep vein thromboses, one pulmonary embolism, three strokes and two myocardial infarctions), of these were seven recurrent events in patients with a history of thrombosis and three primary events. Elevated sP-selectin was present in 25 patients (26%). The cummulative probability of thrombosis was 12.2% after one year and 16.8% after five years in patients with elevated sP-selectin, whereas it was 2.8% after one year and 14.5% after five years for LA-patients with sP-selectin below the cutoff (Figure 1).

Figure 1. Venous and arterial thrombotic events in LA-patients with (continuous line) and without (discontinued line) elevated sP-selecti levels.

Considering the whole observation time elevated sP-selectin was associated with a hazard ratio for thrombosis of 2.0 [95% confidence interval: 0.5-8.2, p=0.34] adjusted for age, sex, oral anticoagulation and positive thrombosis history in multivariate cox regression analysis. *Conclusions*. Elevated sP-selectin levels were associated with past thrombosis and thrombosis within the first year after sP-selectin determination in LA-patients, whereas it was not associated with thrombosis after five years of follow-up. Whether high p-selectin levels qualify as a predictive parameter for thrombosis in LA-patients needs confirmation.

0879

P-SELECTIN GENE HAPLOTYPES MODULATE SOLUBLE P-SELECTIN LEVELS AND CONTRIBUTE TO THE RISK OF VENOUS THROMBOEMBOLISM

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Background. The cell adhesion molecule P-selectin, which mediates the interaction of activated platelets or endothelial cells with leukocytes, is central to the pathogenesis of thrombosis. In arterial and venous thromboembolism (VTE) increased soluble P-selectin (sP-selectin) levels have been found, and associations of P-selectin genotypes with thrombotic disease have been discussed. *Aims*. The aim of our study was to assess the effect of certain haplotypes in the P-selectin gene comprising single nucleotide polymorphisms (SNPs) in the promotor region and SNPs in the coding region altering amino acids (SNPs C-2123G, S290N, N562D, T715P). *Methods*. We conducted a case-control study of 116 high risk patients with a history of objectively confirmed recurrent VTE and at least one event of an unprovoked deep venous thrombosis or pulmonary embolism. One-hundred-twenty-nine age- and sex-matched healthy individuals served as controls. The SNPs -2123 (C>G), S290N (G>A), N562D (A>G) and T715P (A>C) were analyzed by allele-specific PCR. The genotype results were used for computer-assisted haplotype construction. sP-selectin (ng/mL) was measured by ELISA. The difference in the haplotype distribution between patients and controls was described by logistic regression models. Differences in P-sel levels were analysed using linear regression models. *Results*. In patients (53 women; mean age ±SD:56±12 yrs) nine haplotypes and in controls (66 women; mean age ±SD:53±11 yrs) ten haplotypes were identified. Failure for haplotyping occurred in three controls. Six haplotypes in patients and seven in controls had a frequency of above 5% and together they covered 93% and 96% of the genetic variation, respectively. Compared to haplotype-6 (CGAA, major allele at all positions) odds ratios for VTE were 2.1 (95% CI: 1.1-4.3) for haplotype-2 (GGAA) and 4.8 (2.1-12.0) for haplotype-8 (CAGA). Statistical analysis showed that haplotypes were significantly associated with sP-selectin levels (p<0.001). Compared to haplotype-6 (mean ±SD: 39.1±12.8) sP-selectin levels differed in mean by +11.4 (p<0.001) for haplotype-8, by -8.3 (p=0.009) for haplotype-3 and by -8.9 (p=0.008) for haplotype-7. *Conclusions*. According to our data P-selectin haplotypes are associated with the risk of VTE and affect plasma levels of sP-selectin.

0880

A HIGH PLATELET COUNT INDEPENDENTLY PREDICTS VENOUS THROMBOEMBOLISM IN CANCER PATIENTS RESULTS FROM THE VIENNA CANCER AND THROMBOSIS STUDY

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Background. Cancer patients are at increased risk of venous thromboembolism (VTE), laboratory parameters with predictive value could help to assign a patient to a high or low risk group. *Aims*. We aimed to assess high platelet count as thrombosis risk predictor for VTE. *Methods*. This is a report from the ongoing prospective observational CATS. Patients with newly diagnosed cancer or progression of disease who had no chemotherapy within the last three months were enrolled from 10/2003 to 07/06 and followed prospectively via phone and mail. Occurrence of VTE and information on the patients´ anti-cancer-treatment within the follow up period have been recorded. VTE has always been confirmed by imaging. Observation ends with occurrence of VTE, death or at least after 2 years. Kaplan Meier and Cox regression analysis were applied for statistical calculation. *Results*. We included 489 patients (243 female/246 male, median age 63 yrs, 341 newly diagnosed) with solid tumours. Main tumour entities were malignancies of the breast (n=124), lung (n=82) , upper (n=29) and lower gastrointestinal tract (n=96), pancreas (n=36), kidney (n=19) and prostate (n=64). Thirty-nine patients had other tumour types than aforementioned. Distant metastases were found in 256 patients at time of recruitment. During the observation period VTE occurred in 27 patients (13 female/14 male, median age 62 yrs.). The cumulative probability of VTE was 6.2% after one year. High platelet count (above 459 G/L = 95th percentile, 27 patients) [HR:4.9, 95% CI 1.9-12.4], surgery [HR: 6.8, 95% CI 2.6-17.9] and radiotherapy [HR: 3.2, 95% CI 1.2-8.4] were statistically significant risk factors for VTE in multivariate analysis including platelet count, age, sex, distant metastases, surgery, chemotherapy and radiotherapy. *Summary / Conclusion.* High platelet count is a clinically important independent predictive parameter for VTE in cancer patients. Measurement of platelet count at diagnosis of cancer would help to individualize the decision for thrombosis prophylaxis during their course of disease.

0881

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-SC on VWF with bleeding severity score (BSS) calculated at enrollment. Diagnoses of VWD were confirmed by the coordinating center using multimeric 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. *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 hemarthrosis (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 [5.5 (3.1-9.8)], VWF:RCo <10 U/dL [3.2 (1.7-5.9)] and FVIII:C <20 U/dL [4.1 (2.4-7.0)] were significantly associated with high risk of bleeding. By multivariate model including all the variables, BSS [5.5 (2.8-10.8)] was 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%) VWD. On the other hands, patients with VWF:RCo >30 U/dL and FVIII: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 and surgeries in types 1 (65%) and 2 (35%) VWD and 452 injections of VWF concentrates were used to treat bleeding and surgeries in type 3 (75%), type 2 (34%) and type 1 (15%) VWD. *Conclusions*. 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 VWF:RCo >30 U/dL and FVIII:C >40 U/dL bleeding occurs rarely in agreement with their relatively low BSS.

0882

CISPLATIN INDUCES THROMBIN GENERATION VIA FORMATION OF PROCOAGULANT ENDOTHELIAL MICROPARTICLES IN VITRO

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Background. Cisplatin-based chemotherapy is a risk factor of venous thromboembolism in cancer patients. We hypothesized that cisplatin causes an increased release of procoagulant microparticles from endothelial cells (EC). *Aims*. (1) To quantify numbers and procoagulant activity of endothelial microparticles (EMP) released following cisplatin treatment and (2) to investigate EMP-associated procoagulant mechanisms. *Method*s. Two EC lines (HUVEC, HMVEC-L) were exposed to cisplatin (1, 2.5, 5, 10, and 20 M) for up to 120 h. Cell viability was assessed by quantification of mitochondrial dehydrogenase activity. To check for involvement of apoptosis in cisplatin-related EC death, cellular annexin V-binding and activities of caspases-3 and -7 were determined. Counts and procoagulant activity of EMP were measured by flow cytometry and a thrombin generation assay, respectively. Analysis of tissue factor (TF) expression by EC included RT-PCR and flow cytometry. EMP-associated TF was analyzed by ELISA and an assay to measure TF-dependent procoagulant activity, respectively. *Results*. Cisplatin, via induction of apoptosis, decreased EC viability in a dose- and time-dependent manner. This was accompanied by an increasing release of EMP into culture media (maximum: HUVEC + 544%; HMVEC-L + 1738%) and an increase of generated thrombin (maximum: HUVEC + 150%; HMVEC-L: + 493%), respectively. Cisplatin did not induce TF expression on EC. EMP-associated TF antigen and TF-dependent procoagulant activity were measurable at marginal levels only at very high cisplatin concentrations. EMP-associated thrombin generation was almost abolished by annexin V but could not be reduced by omission of factor VII. *Conclusions*. Cisplatin induced a marked release of procoagulant EMP. Negatively charged phospholipids but not TF on EMP were decisive for total thrombin generation. Further studies are warranted to investigate the cisplatin-induced release of EC-derived MP *in vivo*.

Myeloma / MGUS / WM - Biology

0883

IMPACT OF CONSOLIDATION WITH BORTEZOMIB, THALIDOMIDE AND DEXAMETHASONE ON RESIDUAL MULTIPLE MYELOMA CELLS AFTER AUTOLOGOUS TRANSPLANTATION ASSESSED BY QUALITATIVE AND QUANTITATIVE PCR

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Background. Molecular remissions (MR), detected by PCR analysis, are rare after autologous transplantation (ASCT) in multiple myeloma (MM). However several studies indicate that the achievement of MR is an important requisite to obtain long-term disease control. *Aims*. This study evaluates, by qualitative and quantitative PCR, if a post-transplant consolidation treatment with Bortezomib/Thalidomide/Dexamethasone (VTD) might impact the kinetic of minimal residual disease (MRD) and increase the rate of MR. Inclusion criteria were: 1) a documented complete remission (CR) or very good partial remission (VGPR) following ASCT; 2) a molecular marker based on the IgH rearrangment. *Methods*. The VDT had to be started within 6 months from ASCT. Each cycle consisted of: a) Bortezomib at the dose of 1.6 mg/m^2 as an IV injection once weekly (on days 1, 8, 15, 22) followed by a 13-day rest period (days 23-35); b) Thalidomide at the initial dose of 50 mg/day PO once daily, with increments of 50 mg every 7 days to acceptable tolerance (maximum 200 mg); c) Dexamethasone 20 mg/day PO once daily, on days 1 to 4, 8 to 11 and 15 to 18 followed by a 17-day rest period (days 19-35). A total of 4 cycles were delivered. Molecular analysis were performed on bone marrow (BM) samples at study entry, after two courses, at the end of treatment and then at six months intervals. The tumor marker was based on the IgH rearrangment and MRD was evaluated by qualitative nested PCR and quantitative real time PCR (Voena *et al.*, Leukemia 1997; Ladetto *et al.*, Biol Bone Marrow Transpl 2000). *Results*. 31 patients have entered the study and are evaluable at study entry. MR rate at study entry after ASCT is very low as expected (6%). Two enrolled patients are not evaluable due to toxicity. 25 patients are currently evaluable after two VTD courses. Notably four of them converted from PCR-positivity to PCR negativity. At the end of treatment 18 patients are evaluable. One more patient converted to PCR negativity at this phase. MR was maintained at six months follow-up evaluation in two of three PCR negative evaluable patients. Real-time PCR has been so far performed in five persistently PCR-positive patients. In four of them a clear reduction of tumor burden (median reduction 0.75 log) was observed during VTD consolidation. *Conclusions*. Consolidation with VTD can convert at least a minority of CR/VGPR MM patients from PCR-positivity to PCR-negativity. In addition it has a measurable anti-tumor effect also in persistently PCR-postive patients. The study is still ongoing. A longer follow-up and a larger patient sample will allow to verify if conversions to MR following VTD persist over time and positively impact patient outcome.

0884

THE PROTEASOME INHIBITOR BORTEZOMIB STIMULATES THE OSTEOGENIC DIFFERENTIATION OF HUMAN MYSENCHIMAL CELLS IN VITRO AND MAY RESTORE THE NUMBER OF OSTEOBLASTIC CELLS IN MULTIPLE MYELOMA PATIENTS

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Bone formation process is profoundly impaired in multiple myeloma (MM) patients. Recent data suggest that ubiquitin-proteasome pathway, the major cellular degradative system and therapeutic target in myeloma cells, also regulates osteoblast differentiation. Preliminary observations obtained in MM patients treated with the proteasome inhibitor Bortezomib show an increase of bone specific alkaline phosphatase in responder patients as compared to non-responder ones. Currently it is not know whether Bortezomib have a direct effect on osteoblast and bone formation *in vitro* human cultures and *in vivo* in MM patients. To clarify this issue first we checked the effect of Bortezomib either on osteoblast 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 progeni-

tors Colony Forming Unit-Fibroblast (CFU-F) and late ones Colony Forming Bone nodules (CFU-OB). 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 osteoblast markers was observed after 24 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 β-catenin levels. Consistently we found that Bortezomib at low dose induces bone nodule formation in human mesenchymal cells after 21 days of exposition. Similar results were obtained using specific proteasome inhibitors as MG-132 and MG-262 suggesting that the pro-osteogenic effect of Bortezomib was due to its capacity to block proteasome activity. To extent our *in vitro* observation we have evaluated the potential effect of Bortezomib *in vivo* in MM patients. Bone histomorphometry as well as immunostainig for Runx2/Cbfa1 was performed on BM biopsies obtained from 21 MM patients before and after 6-8 cycles of Bortezomib administrated in monotherapy. A significant increase in the number of osteoblastic cells X mm^2 of bone tissue and in the number of Runx2/Cbfa1 positive osteoblastic cells was observed only in responder patients showing an early increase of the serum alkaline phosphatase. In conclusion our data indicate that Bortezomib may increase osteoblast differentiation in human mesenchymal cells without affecting the proliferation, survival and function of mature osteoblasts. *in vivo* and *in vitro* observations support the hypothesis that both direct and indirect effects on bone formation process could occur during Bortezomib treatment.

0885

ANGIOPOIETIN1/ANGIOPOIETIN2 RATIO IS REDUCED AND CORRELATES WITH DISEASE SEVERITY IN RELAPSED/REFRACTORY MYELOMA PATIENTS. NORMALIZATION OF THE RATIO POST BORTEZOMIB THERAPY

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Background. Angiogenin, angiopoietin-1 (ang1), angiopoietin-2 (ang2), vascular endothelial growth factor (VEGF) and VEGF-A (the main angiogenic VEGF fraction) are cytokines involved in the process of neoangiogenesis and possibly in multiple myeloma (MM) pathophysiology. Ang1 and ang2 are competitive ligands for the same receptor, Tie-2. There is very limited information concerning the effect of bortezomib on angiogenic cytokines. *Aim*. to evaluate pre- and post-bortezomib serum levels of angiogenin, ang1, ang2, VEGF and VEGF-A in refractory/relapsed MM. *Patients and Methods*. Thirty-five patients (24M/11F, median age:68 years) were studied. Twenty-four patients had IgG, 8 IgA, and 3 light-chain MM. According to ISS, 10 patients had stage 1, 7 stage 2 and 18 stage 3 disease, while according to Durie-Salmon (DS) staging, 3 patients had stage I, 20 stage II and 12 stage III MM. Seventeen patients received bortezomib as 2nd line treatment, while 18 patients were in ≥2nd relapse. Median time from diagnosis to bortezomib was 30 months. Bortezomib was given at the standard dose, while dexamethasone was added to 15 patients who did not respond after 2 cycles of therapy. Serum cytokines' levels were measured pre-bortezomib (baseline) and on day 21 of the 4th cycle, using ELISA methodology (R&D Systems, Minneapolis, USA, for all, except VEGF-A: Diaclone, Bensancon, France). *Results*. The objective response (OR) rate was 71%. Baseline levels of all studied angiogenic cytokines, except of ang1, were elevated in patients, while the ratio of ang1/ang2 was reduced compared to healthy individuals (HI) (Table 1). Baseline ang2 levels were higher in patients with ISS 3 or DS III compared to lower stages (p=0.04), and correlated with β2M (0.008). Baseline ang1 levels correlated negatively with DS stage and time from diagnosis to bortezomib (p=0.05 and 0.01, respectively). More importantly, the ratio of ang1/ang2 at baseline correlated negatively with β2M, DS stage and PS (p<0.05). Baseline angiogenin presented positive correlation with β2M, ISS, PS, bone marrow infiltration and number of previous therapies (p<0.03). Finally, baseline VEGF-A levels correlated with PS, bone marrow infiltration and CRP (p<0.04). Following bortezomib administration, serum levels of ang1 increased, while ang2 decreased; consequently, the ratio of ang1/ang2 increased and normalized. No other alterations were observed post-bortezomib therapy. Patients who achieved an OR showed a significant elevation of ang1 (p=0.01) and reduction of ang2 (p=0.01) compared with those who did not (p=NS); therefore, ang1/ang2 ratio increased and normalized in OR patients (p=0.025). The median follow-up of the patients was 8.7 months and the median survival has not been reached yet. However, patients with no or slight increase of ang1/ang2 ratio had inferior survival (median: 11.8 months) compared to patients with a significant increase (median survival has not been reached yet, p=0.02). *Conclusions*. The angiogenic cytokines studied, with the exception of ang1, were elevated in relapsed/refractory MM. The ratio of ang1/ang2 and angiogenin correlated with disease severity. Bortezomib administration was accompanied by normalization of this ratio in responders. In our cohort of patients the ang1/ang2 normalization post-bortezomib predicted for better survival.

Table 1.

0886

STABLE ASYMPTOMATIC MYELOMA AND MGUS IN THE PRESENCE OF T(4;14)

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Background. The t(4;14)(p16;q32) is associated with a poor outcome in myeloma and is reported to be underrepresented in MGUS. Patients with the t(4;14) translocation are less likely to present with bone disease, possibly due to an association with a more rapidly progressing form of myeloma. We present data to show that this is not always the case. *Methods*. FISH for t(4;14) was performed centrally on CD138-selected plasma cells from bone marrow samples received from hospitals throughout the UK. Cases were also tested for deletion of chromosome13 and p53, t(11;14), t(14;16), t(14;20), t(6;14) and ploidy. The majority of patients received had a diagnosis of myeloma although some had MGUS or asymptomatic myeloma (AMM) based on the IMF diagnostic criteria. *Results*. Results were available for the 4;14 probe on 1724 patients (158 MGUS, 109 AMM, 1457 MM). Four MGUS (3%), 15 AMM (14%) and 162 myeloma (11%) were positive for the translocation. The pre-myeloma t(4;14) cases had a similar incidence of deletion 13 (90% vs 93%), but lower incidences of deletion of p53 (5% vs 11%), loss of the derivative 14 (16 vs 27%) and hypodiploidy (68% vs 83%), although none reached statistical significance. There was a higher proportion of IgA paraprotein type in t(4;14) myeloma patients (58/162 cf 276/1191 p=0.001) and fewer patients with IgG (63/162 cf 711/1191 p<10-6) but this was not found in AMM, with a higher proportion of IgG (68% cf 62%, not significant) and similar proportion of IgA (23% in both) to the overall figures. In MGUS 2 patients were IgG and 2 IgA. One MGUS was lost to follow-up, the two IgG patients have remained as stable MGUS for 33 and 73 months, while the fourth showed a rise in paraprotein after 36 months, resulting in reclassification to AMM. Among the patients defined at diagnosis as AMM, 3 have progressed to myeloma within 1 year, one with a rising paraprotein at 24 months does not yet qualify as myeloma, 4 showed slow progression (myeloma diagnosed at 33, 34, 54 and 78 months) and 7 remain stable at 9, 14, 23, 32, 33, 38 and 39 months. *Conclusions*. This study has shown that, although rare in MGUS, there is at least as high an incidence of t(4;14) in AMM as in myeloma. Although some patients presenting with apparent MGUS or AMM plus t(4;14)

have an early manifestation of typical myeloma, the majority show only slow progression for up to 6 years. The detected FISH abnormalities in these stable patients are not significantly different from those in the more aggressive cases suggesting that other as yet unidentified factors play a significant role in the phenotype of t(4;14) disease. These results confirm that treatment decisions for this group of patients should be based on clinical behaviour rather than the presence of a *poor prognosis* genetic abnormality.

0887

DELETIONS OF CHROMOSOMES 6Q AND 13Q, AND TRISOMY 4 ARE THE MOST COMMON CYTOGENETIC ABNORMALITIES IN WALDENSTROM MACROGLOBULINEMIA. PRELIMINARY RESULTS OF A MULTICENTRIC STUDY

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The genetic bases of Waldenström Macroglobulinemia (WM) are poorly understood. We studied by conventional cytogenetic (CC) and Fluorescence *in situ* hybridization (FISH) analysis a cohort of 83 untreated WM patients, enrolled in a prospective randomized trial from the French Cooperative Group on Chronic Lymphocytic Leukemia and Waldenström Macroglobulinemia (FCG-CLL/WM). The sex ratio was 57M/26F, the mean age at diagnosis was 67 years [40-85]. The mean percentage of lymphoplasmacytic cells was 50% [8-90]. CC was systematically performed on bone marrow or peripheral blood, and FISH analysis carried out using 7 probes CEP4, CEP12, 13q14, 11q22 (ATM), 17p13 (TP53), IGH Abbott, 6q21 Q-Biogene, on metaphases and interphase nuclei. Out of 67/83 successful karyotypes, 45% showed abnormalities. There were 7 (11%) 6q deletions, 5 (8%) trisomy 4/partial trisomy 4, 3 (5%) trisomy 18, 2 (3%) trisomy 12. Using FISH deletions of 6q21 were observed in 14/50 cases (28%), 13q14 in 8/54 cases (15%), TP53 5/54 cases (9%), ATM 3/53 cases (6%). Trisomy 4 was present in 7/53 cases (13%), and trisomy 12 in 3/55 cases (5%). No rearrangement of IGH was observed in the first 17 analyzed cases. The 6q deletion is the most frequent reported cytogenetic abnormality in WM. We found 28% of 6q deletion, a low percentage compared to the literature [39-54%]. This could be explained either by the difference in the probe used or we did not select for lymphoplasmacytic cells before cytogenetic analyses. Interestingly we confirmed our recent observation that trisomy 4 was frequent in WM (15% if partial trisomy 4 was included). Furthermore we observed in this large series a frequent 13q14 deletion (15%). In conclusion, cytogenetic abnormalities in WM differ from those commonly reported in other B-cell neoplasms and confirm the originality of this disease. 6q deletion is frequent compared to CLL or marginal zone lymphoma (MZL) and 13q14 deletion is rare compared to CLL. In our series trisomy 12 is rare compared to atypical-CLL and MZL. We didn't observed cytogenetic involvement of the IGH locus, which is frequent in multiple myeloma or lymphoplasmocytic lymphoma. Finally trisomy 4 is present in WM but not reported in other B-cell malignancies. Searches for correlations with clinical and other biological parameters are ongoing.

Genomics and proteomics

0888

PHOSPHOPROTEOMIC PROFILING OF PEDIATRIC B-ALL PATIENTS USING REVERSE PHASE PROTEIN ARRAYS

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Background. The outcome for children with Acute Lymphoblastic Leukemia (ALL) is improved in the last few decades with current therapies, but 30% of patients still resist to conventional therapies. Leukemia patients with similar molecular aberrations react differently to drug treatment, proving that there must be altered signalling pathways other than those already targeted in therapy. Although some genetic defects had been associated to different therapeutic responses, for many malignancies the signal transduction pathways (STPs) involved in the neoplastic process are not yet identified. *Aims*. Our study aims to provide such information in pediatric B-ALL patients using phosphoproteomic measurement of STPs in order to identify patient subgroups characterized by aberrantly activated phosphoproteins. The identification of differently altered STPs could offer possibilities for targeted therapy and a means of patient stratification. *Methods*. Reverse Phase Protein Arrays (RPPA), which can quantitatively measure hundreds of phosphorylated proteins, were employed to profile the working state of cellular STPs in 120 pediatric B-ALL specimens collected prior to treatment at the Pediatric Oncohematology Laboratory (University of Padova, Italy). Bone marrow white blood cells lysates were immobilized in dilution curves on nitrocellulose coated slides, and antibody staining (1 slide =1 antibody) was revealed using DAB. Images of antibody-stained arrays were analyzed using the Microvigene Software. The data processing generates a single value for each sample relative to each phosphorylated protein. Molecular network analysis and phosphoprotein profiling were performed using commercially available software. The phosphorylation status of 92 key signalling proteins was analyzed. Clinical data, such as immunophenotype, response to therapy and chromosomal translocations, were collected for all the patients. Different patients subgroups were compared in order to find differentially activated phosphoproteins to better map the biology of the disease and to identify new drug targets relative to the deranged pathways. *Results*. In a first study, prednisone good responder (PGR) patients were compared with prednisone poor responder (PPR) patients. Profiling of phosphoproteins shows that an aberrantly activated pathway in PPR patients results in the hyperactivation of the transcription factor NFkappaB, a known target of steroid therapy. NFkappaB hyperactivation could explain why these patients are not able to respond to prednisone, thus aberrantly activated proteins upstream to NFkappaB could represent new targets for kinase inhibitors. In the same patient cohort, non-translocated patients were compared to MLL rearranged patients. Analysis of MLL rearranged patients samples reveal an activated pathway that leads to the hyperactivation of Bcl-2. This activation could provide a survival advantage to the blast cells, blocking the normally functioning apoptotic pathway. *Conclusions*. Proteins in the deranged NFkappaB and Bcl-2 signalling pathways observed within these B-ALL patients may represent new targets for targeted kinase inhibitors already used in other contexts or yet to be developed. The discovery of the molecular mechanisms related to drug resistance could play an important role for individualizing therapy and may reveal new strategies to improve therapy stratification and treatment outcome.

0889

MIRNA EXPRESSION PROFILING IN ADULT ACUTE MYELOID LEUKEMIA IDENTIFIES DISTINCT SUBCLASSES

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Background. Acute myeloid leukemia (AML) encompasses a large number of morphologically similar but at the molecular level quite distinct variants. Recurrent cytogenetic aberrations have been shown to constitute markers of diagnostic and prognostic value. However, despite recent successes in detecting novel molecular markers like FLT3, CEBPA, and NPM1 mutations, and despite recent advances in gene expression profiling treatment stratification is still difficult and the biology underlying dis-

tinct AML subclasses is still not fully understood. Recently, deregulated-expression of microRNAs (miRNAs) has been correlated with various cancers including leukemias, and evidence was provided that miRNAs can function both as oncogenes and tumor suppressors. *Aims*. In order to determine a potential role of differential miRNA expression in AML we profiled miRNA expression in a large series of adult AML patients to better characterize AML on the molecular level. *Methods*. We analyzed 91 samples which encompass the spectrum of cytogenetic and molecular genetic aberrations in AML using DNA microarray technology. We used a miRNA microarray platform validated by quantitative RT-PCR and Northern Blot analyses that contained approximately 250 human miRNAs. *Results*. By unsupervised hierarchical cluster analysis of our 91 AML samples based on the miRNA expression of 202 filtered miRNAs we were able to identify two large AML subgroups. While these two groups were in part characterized by the differential expression of the miR-17-92 cluster, which recently has been identified to be deregulated in many cancer types, especially Myc-regulated tumors, we also identified several miRNAs not yet known to be differentially expressed in leukemia. Interestingly, correlation of the unsupervised cluster defined groups revealed an association with leukemia morphology as determined by FAB (French-American-British) classification, but there was no significant correlation with cytogenetically or molecular-genetically defined leukemia AML subgroups. On the other hand, by supervised analysis using the significance analysis of microarrays (SAM) methodology we were able to identify miRNA signatures characterizing acute promyelocytic leukemias with a t(15;17) and normal karyotype AML carrying mutations of NPM1. *Summary/Conclusions*. While these findings already support a potential role of differential miRNA expression in AML pathogenesis, future analyses correlating miRNA expression with global gene expression, which are currently underway, are likely to provide additional insights into AML biology, thereby helping to unravel the role of miRNAs in leukemogenesis.

0890

IDENTIFICATION OF CANDIDATE GENES IN HIGH-LEVEL DNA AMPLIFICATIONS IN AML WITH COMPLEX KARYOTYPE USING AN ARRAY-BASED APPROACH

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Background. Complex karyotype acute myeloid leukemia (AML), commonly defined as the presence of three or more chromosome abnormalities without specific fusion transcripts, is seen in approximately 10-15% of all AML cases. In this subset of cases, genomic losses and gains are much more frequent than balanced translocations, indicating other mechanisms of leukemogenesis. One possible mechanism is the activation of (proto-) oncogenes through high-level DNA amplifications. *Aims*. To detect high-level DNA amplifications and to identify corresponding candidate genes, we applied comparative genomic hybridization to microarrays (array-CGH) in 150 cases of complex karyotype AML and correlated the findings with gene expression profiling (GEP) data. *Methods*. For array-CGH a custom-made 2.8k-microarray was used consisting of 2799 different BAC- or PAC-vectors with an average resolution of approximately 2 Mb. Hybridization experiments were performed in a dye-swap setting; significant aberrations were defined as mean plus/minus three times the standard deviation of a set of balanced clones for each individual experiment. In selected cases correlation with global gene expression studies was performed to further delineate regions harboring candidate genes. *Results*. We identified 70 high-level DNA amplifications in 25 different genomic regions. Amplifications occurring in at least two cases mapped to (candidate genes in the amplicon) 11q23.3-q24.1 (n=16; ETS, FLI1, APLP2); 11q23.3 (n=15; MLL, DDX6, LARG, SC5DL); 21q22 (n=9; ERG, ETS2); 9p24 (n=4; JAK2); 13q12 (n=4; CDX2, FLT3, PAN3); 8q24 (n=4; C8FW, MYC); 12p13 (n=2; FGF6, CCND2); 11q13 (n=3; STARD10, GARP, RAD30, DLG2), 20q11 (n=2; ID1, BCL2L1); and. 22q11 (n=2; CHEK2, NF2 To better characterize the genomic architecture of the amplicons, we additionally applied array-CGH using an 8.0k-microarray with an average resolution of approximately 1 Mb. Using this approach highly complex amplicon structures with several distinct amplicon peaks were identified e.g. in the amplified regions 8q24, 11q23, and 13q12. Parallel analysis of array-CGH and GEP in a subset of 43 cases displayed overexpressed candidate genes in the critical amplified regions; for some of these genes an oncogenic role has been implicated e.g. C8FW and MYC in 8q24, ETS1, FLI1 and APLP2 in 11q24.1, as well as FLT3 and CDX2 in 13q12. *Summary/Conclusions*. Using high-resolution genome-wide screening tools such as array-CGH, a large number of high-level DNA amplifications was identified in AML with complex karyotype suggesting a more general role for protooncogene activation in this AML subset. This high-resolution technique allows the detection of complex amplicon structures with several distinct amplicon peaks pinpointing to selective candidate genes. In addition, correlation with GEP studies facilitates the delineation of overexpressed candidate genes within the amplified regions.

0891

AN INTERNATIONAL MULTI-CENTER RESEARCH STUDY TO DEFINE THE APPLICATION OF MICROARRAYS IN THE DIAGNOSIS AND SUBCLASSIFICATION OF LEUKEMIA (MILE STUDY): A REPORT ON 2926 CASES

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Background. Microarray analysis can identify differentially expressed genes associated with distinct clinical and therapeutically relevant classes of both pediatric and adult leukemias. *Aims*. Recently, 11 MILE (Microarray Innovations in Leukemia) study centers from the European Leukemia Net (seven laboratories), USA (three laboratories), and Singapore (one laboratory) completed their analysis phase of archived patient samples using whole genome microarrays. *Methods*. Using a standardized laboratory protocol for Affymetrix HG-U133 Plus 2.0 microarray analysis, the classification accuracy of gene expression profiles for 16 acute and chronic leukemia subclasses (mature B-ALL with t(8;14), Pro-B-ALL with t(11q23)/MLL, c-ALL/Pre-B-ALL with t(9;22), T-ALL, ALL with t(12;21), ALL with t(1;19), ALL with hyperdiploid karyotype, c-ALL/Pre-B-ALL without t(9;22), AML with t(8;21), AML with t(15;17), AML with inv(16)/t(16;16), AML with t(11q23)/MLL, AML with normal karyotype or other abnormalities, AML complex aberrant karyotype, CML, CLL), MDS, as well as non-leukemia/healthy volunteers as a control group is compared to the current routine diagnostic workup of each center. *Results*. All participating laboratories demonstrated high proficiency of microarray analysis in a so-called pre-phase where cell lines had been tested during laboratory standardization and proficiency analysis. The subsequent MILE study Stage I included in total 2156 samples. Strict quality acceptance criteria of the gene expression results were met in >98% of the samples. Gene expression profiles from this study were further combined with previous microarray data obtained by the research groups from Munich (Haferlach) and Memphis (Downing). The emerging data set of 2926 patient samples and subsets thereof then was used to train linear discriminant classification algorithms to assess the prediction accuracy for 18 distinct sample classes. The average accuracy of three 30-fold cross-validations resulted in 94.44% concordant calls comparing goldstandard diagnosis and microarray result. Miscalls between the classes were predominantly observed in the distinction between MDS samples, characterized by an underlying AML-like gene expression signature, and AML with normal karyotype. For an independent test data set with 92 specimens covering 12 of the 18 classes, the accuracy was 87/92 (94.57%). Moreover, based on distinct gene expression profiles, CLL can be further classified into IgVH mutated or unmutated subgroups (98.41% accuracy of cross-validation with fixed probe sets, n=289 samples), or ZAP70 positive or negative cases (95.51%, n=256 samples), respectively. As a next step, a customized microarray for leukemia classification was designed using 1,449 probe sets. *Conclusions*. This international multi-center study demonstrated a very high accuracy of leukemia classification using whole-genome gene expression profiling and indicated the feasibility of a routine diagnostic application of microarrays. As started in December 2006 additional 2,000 samples will be prospectively analyzed in the MILE study Stage II with a custom research AmpliChip Leukemia microarray to test these accuracy estimates in a blinded study.

0892

INTEGRATION OF GENOME WIDE SNP ARRAYS AND GENE EXPRESSION DATA OF CHILDHOOD ALL WITHOUT KNOWN GENETIC ABERRATIONS IDENTIFY SUBGROUPS WITH SPECIFIC GENETIC HALLMARKS

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Background. Approximately 25% of childhood ALL patients cannot be classified according to cytogenetic detectable hallmarks. Gene-expression (GE) and SNP-arrays represents powerful tools for the identification of hidden genetic abnormalities in cancer. Genotype and gene expression data can be integrated to directly infer the variation of transcript levels in deleted or amplified regions, and to identify variation in gene expression of genes indirectly affected by genomic alterations. Aim of the study was to identify cryptic abnormalities in unclassified childhood ALL patients by performing an integrative analysis of GE and SNP arrays data. *Methods*. 29 patients followed the inclusion criteria: a) B-cell precursor childhood ALL b) DNA index 1; c) negativity at t(4;11), t(12;21), t(9;22), t(1;19) RT-PCR screening; d) normal karyotype when cytogenetics available. All analyzed patients were part of a 125 B-ALL patients cohort analyzed by GE profiling in the context of international *Microarray Innovation in Leukemia* (MILE) study. Affymetrix GeneChip Mapping 100K SNP arrays and HG-U133Plus2 Probe Arrays were used. *Results*. The presence of del(9)(p21) was found in 10/29 (30%) patients, with an homozygous commonly deleted region involving CDKN2A in 7/29. Hemizygous losses of 9p13 including the PAX5 gene were found in 7/29 (24%) patients. Three patients (10%) showed specific deletions involving the TEL gene on chromosome 12p13.2. Three patients (10%), including two with TEL deletion, showed the presence of the intrachromosomal amplification of chromosome 21 (iAMP21). Five patients did not show copy number change, and random microdeletions were found in single cases. We found a statistically significant reduction of CDKN2A probe sets expression in 9p21 deleted cases. Due to the retention of the non deleted allele, no significant down regulation of TEL and PAX5 specific probes was found in 12p13 and 9p13 deleted cases, respectively. More interestingly, a list of differentially expressed genes was generated by supervised clustering analyses comparing patients with and without the TEL, CDKN2A, and PAX5 gene deletions, respectively. Although a list of down regulated genes was generated for the CDKN2A and PAX5 deleted cases, likely due to heterogeneity and large extension of both deletions, a specific signature was not confirmed. Conversely, the JAG1 gene encoding for Jagged 1 ligand of the Notch receptor was among a list of differentially expressed (upregulated) genes in TEL deleted cases. The expression level of the JAG1 specific probes was evaluated in the GE dataset of 76/125 B-ALL patients of the MILE study, including t(4;11), t(9;22), t(12;21) and 24 unclassified patients analyzed by SNP arrays, indicating statistical significant JAG1 upregulation in TEL deleted and TEL/AML1 positive subgroups relative to BP-ALL with other genetic lesions, also confirmed by RQ-PCR. *Conclusions*. The integration of genomic variation and gene expression profiling allowed us not only to identify hidden genetic lesions but also to discover genes differentially expressed in new genotype-specific subgroups. Larger studies are mandatory to extend and further confirm the observation of JAG1 over expression in TEL-related leukemia; moreover, functional studies are required to understand the role of Notch pathway in this context.

Myeloproliferative disorders - Clinical

0893

HIGH TROUGHPUT SEQUENOM BASED ASSAY FOR MONITORING JAK2 V617F POST ALLOGENEIC STEM CELL TRANSPLANTATION FOR CLASSIC MYELOPROLIFERATIVE DISORDERS

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Background. AlloSCT is currently the only curative treatment in classic myeloproliferative disorders (MPD) and is often performed in advanced disease stages. JAK2 V617F mutation is thought to play a causative role in the pathogenesis of MPD. There is limited data on patients (pts) with JAK2 V617F undergoing alloSCT and on the course of this mutation in the post transplantation period. *Methods*. To investigate the course of JAK2 V617F mutation in pts undergoing alloSCT we developed a sensitive and quantitative high throughput assay. JAK2 mutation detection was carried out using a chip-based matrix-assisted laser desorption-time-of-flight mass spectrometer (Sequenom, San Diego, CA) with a specific primer extension assay designed to detect the JAK2 V617F mutation. The level of mutation was calculated by the following formula: % T allele = (AUC T allele) x 100 / (AUC T allele +AUC G allele). T allele proportions were compared with routine methods for chimerism detection. *Results*. Fifteen pts underwent alloSCT in the study period (1.2001-6.2006), mean age 59 (34-72) years, 9 males and 7 females. Their diagnoses included idiopathic myelofibrosis -5, post polycythemia myelofibrosis -2, post essential thrombocythemia myelofibrosis -2 and sAML -6. Nine pts underwent SCT with reduced intensity conditioning regimen and 6 with myeloablative conditioning, donors were matched siblings-11 and MUD-4. JAK2 V617F was found in 9/15 (56%) pts. Compared to pts without JAK2 V617F, more pts with JAK2 V617F underwent alloSCT due to sAML (4/9 vs. 1/6) and more have undergone splenectomy (4/9 vs. 1/6) (ns). Disease duration was 180 and 131 months in pts with and without JAK2 V617F, respectively (ns). Before the SCT, all but one pt had more than 50% of the mutated allele (mean T allele 74%, range 16-98%), suggesting the existence of a homozygous clone for this mutation in most pts at the time of alloSCT. After alloSCT, level of the mutant T allele decreased in a timely manner in 5 pts, there was a limited decrease in 1 pt, it remained unchanged in 1 pt and it was not evaluable in 2. There was a very high correlation between the decrease in the mutant T allele and the decrease in host cells as detected by I-FISH analysis for XY chromosomes in sex mismatched SCT (n=4) and PCR for STR (n=3) ($r=0.97$, $p<0.001$). After a median follow up of 16 months (range 2-58 months) 9 pts are alive and in CR. Two years survival is 59% (95%CI 33-84) with no difference between JAK2 V617F positive and wild type pts. The level of JAK2 V617F mutation has reached 0% in all surviving pts. *Conclusions*. In our cohort, pts with JAK V617F MPD and clinical indication for alloSCT were mostly homozygous for the mutation and they seemed to have a more aggressive clinical behavior than pts without the mutation. The good correlation between JAK2 V617F and other methods for chimerism detection in the post alloSCT period suggests that assay may be used for follow up in pts with MPD undergoing alloSCT.

0894

JAK2 EXON 12 MUTATIONS OCCUR FREQUENTLY IN IDIOPATHIC ERYTHROCYTOSIS PATIENTS WITH LOW SERUM ERYTHROPOIETIN LEVELS

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Background. Polycythaemia vera (PV) is a clonal haematological malignancy, characterised by hyperplasia of the erythroid, granulocytic and megakaryocytic lineages, that is commonly associated with the gain-of-function mutation, V617F JAK2. In contrast to PV, idiopathic erythrocytosis (IE) is associated with variable serum erythropoietin (Epo) levels and characterised by an elevated haematocrit without hyperplasia of the megakaryocytic or granulocytic lineages. IE can arise from genetically diverse origins, with defects being identified in the Epo receptor in those patients with low serum Epo levels, and in the oxygen-sensing pathway in individuals with raised or inappropriately normal Epo levels. Howev-

er, the molecular pathogenesis of most IE cases remains unclear, with the vast majority of cases being negative for the V617F JAK2 mutation. Recently, four novel acquired mutations located in JAK2 exon 12 have been described in V617F-negative individuals presenting with a myeloproliferative syndrome characterised in part by an isolated erythrocytosis. These mutations are located around 80 amino acids upstream of V617 and confer Epo-independent growth to erythroid progenitors *in vivo*. *Aims*. To determine the frequency of JAK2 exon 12 gain-of-function mutations in IE patients with low to normal serum Epo levels, and to identify any consequences of these mutations. *Methods*. DNA was isolated from peripheral blood and screened by allele-specific PCR (AS-PCR) for the F537-K539delinsL, H538QK539L, K539L and N542-E543del JAK2 mutations. Potential JAK2 mutations were confirmed by direct sequencing granulocyte DNA. Erythroid progenitor cells from patient peripheral blood mononuclear cells were cultured in the presence and absence of Epo. Bone marrow morphology was assessed on haematoxylin- and eosin-stained sections prepared from trephines taken at diagnosis. *Results*. Eight of the 58 IE patients screened were positive for a JAK2 exon 12 mutation, five of whom had the N542-E543del allele. All affected individuals presented with a low serum Epo concentration, and fulfilled the diagnostic criteria for IE. The haematological features of mutation-positive and mutation-negative patients were similar, although Epo-independent erythroid cell growth occurred exclusively in the mutation-positive patients ($p<0.0005$). Immunohistochemical analysis of bone marrow trephines from seven of the eight mutation-positive patients confirmed the marked isolated erythroid hyperplasia previously associated with JAK2 exon 12 mutations, with myeloid to erythroid ratios ranging from 1:1 to 1:6. Granulopoiesis and megakaryopoiesis, in contrast, appeared normal. *Summary*. Eight of 29 IE patients with low serum Epo levels were positive for a JAK2 exon 12 mutation. To date, these gain-of-function mutations are the most common molecular defect identified in the low serum Epo subgroup of IE patients. Consequently, IE patients presenting with low serum Epo-associated erythrocytosis and/or Epo-independent erythroid colony formation should assessed for the presence of a JAK2 exon 12 mutation.

0895

THE IMPACT OF V617F JAK-2 GENE MUTATION ON PLATELET, GRANULOCYTE AND PLASMA HEMOSTATIC AND INFLAMMATORY MOLECULES IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background. Thrombosis is the principal cause of morbidity and mortality in patients with Essential Thrombocythemia (ET). The pathogenesis of these complications remains to be clarified. An acquired gain-of-function mutation (V617F) in the tyrosine kinase JAK2 gene has been recently demonstrated in neutrophils and platelets from about 50% of patients with ET. Clinical data indicate an association between the presence of this mutation and the severity of the disease. *Aims*. It is unknown whether the V617F JAK2 mutation may affect the hemostatic system. Aim of this study was to evaluate whether the presence of the V617F JAK2 mutation identifies ET patients with specific hemostatic abnormalities. *Methods*. Seventy five consecutive ET patients, 37 V617F JAK2 carriers and 38 JAK2 wild-type, and 50 control subjects, were enrolled into the study. Platelet hemostatic and adhesive molecules, platelet-polymorphonuclear leukocyte (PMN) aggregates, and PMN surface activation molecules, were analysed by flow cytometry. In the same subjects, the plasma levels of hypercoagulation markers [prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), D-Dimer, Elastase and Thrombomodulin (TM)] were measured by ELISA. *Results*. Platelets from the overall ET patient group expressed significantly higher membrane tissue factor (TF) and P-selectin ($p<0.01$), and lower CD41 and CD42b ($p<0.01$), compared to healthy controls. Platelet surface TF was significantly higher in the V617F JAK2 carriers compared to wild-type subjects: this was confirmed by the measurement of total platelet TF antigen levels. The expression of circulating platelet/PMN aggregates was significantly ($p<0.05$) greater in the JAK2 mutation carriers versus both wild-type ET and control subjects. All PMN surface activation and inflammatory markers (i.e. CD14, TF, CD11b and LAP) were significantly higher in ET versus controls, with CD14 and LAP being highest in the JAK2 mutation carriers ($p<0.05$). Furthermore, the plasma levels of hypercoagulation markers were significantly greater in the plasma of ET patients compared to healthy controls. However, no statistically significant differences were observed between the V617F JAK2 carriers and wild-type ET except for the TM level, which was significantly higher ($p<0.01$) in the V617F JAK2 carrier subgroup. All the differences found 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. *Conclusions*. These data show that the V617F JAK2 mutation in ET influences specific patterns of hemostasis and inflammation, being associated to increase in platelet expression of TF, platelet/PMN aggregates, PMN CD14 and LAP expression, and plasma TM levels. The alterations of cellular (i.e. leukocytes and platelets), as well as plasma (i.e. TM), compartments of blood coagulation associated to the presence of the V617F JAK2 mutation support the hypothesis of an increased hypercoagulable condition in V617F JAK2 ET carriers. Prospective studies are required to define the role of these hemostatic abnormalities in predicting thrombotic events in ET patients.

0896

HIGHER LEUKOCYTE COUNT IS A RISK FACTOR FOR THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA

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Background. Thrombotic complications represent one main features 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 online) and PV patients (Landolfi R, Blood Nov2006 online) is associated with thrombosis during follow-up. *Aims*. 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, 430-3125) and median leukocyte count was 9.1×10^9/L (range, 3.9-35). Median follow up was 71 months (range, 1-317). Major cardiovascular (CV) events were recorded in 159 patients (22.5%). 94 patients (59%) had thrombosis at diagnosis whereas 65 (41%) had a CV events 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 increased from 15% with leukocyte count <5 to 17% with leukocyte 5-7.5, to 22% with 7.5-10, to 28% with >10-12.5×10^9/L leukocytes ($p=0.01$, X2 test for trend). Considering as the reference population patients with leukocyte count $<7.5\times10^9$/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 Htc levels, and lower platelet count, confirming previous observations. The percentage of patients with CV events was 23% and 19% in the presence or absence of mutation, respectively ($p=0.3$). Due to the role of JAK2V617F on 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 were associated with thrombosis irrespectively of the presence of JAK2V5617F allele (Chi-square test for trend, $p=0.05$ for both). The role of leukocytes was mantained 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 thrombo-embolism ($p=0.03$) while no overt effect was seen as concerned arterial events. *Conclusions*. We conclude that higher leukocyte count constitues 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 result in the modification of current criteria for patients risk stratification and prompt prospective trials aimed at evaluating the impact of cytotoxic therapy on thrombosis prevention.

0897

THE PHENOTYPE OF JAK2V617F MUTANT PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA IS NOT RELATED TO THE BURDEN OF MUTANT ALLELE

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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. *Aims*. 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*. 223 patients with ET, diagnosed according to PVSG or WHO criteria, were studied. Median age was 51 years (range, 16-93). JAK2V617F allele ratio was evaluated at diagnosis by a Taqman one-step assay on granulocytes DNA. *Results*. 142 patients (64%) were JAK2V617F mutant, among which 92% presented levels of mutated allele between 1% to 50% (corresponding to an heterozygous status) and 8% higher than 50% (homozygous status), accounting for 58% and 5% of patient population, respectively. Median level of mutant JAK2 allele was 20% (range 1-87%), that is significantly lower than the level found in a population of PV patients (n=135; 54%, range 1-100; $p<0.001$). By dividing patients into discontinuous classes, 81 (36%) were wild-type, 88 (40%) presented a JAK2V617F ratio within the 1-25% ratio class, 43 (19%) in the 26-50% and 11 (5%) in the 51-100%. JAK2V617F mutant patients had significantly higher leukocyte count ($p=0.03$), hemoglobin ($p<0.0001$) and Htc levels ($p<0.0001$), LAP level ($p=0.05$) and lower platelet count ($p=0.09$), confirming previous observations. Thrombotic complications were referred by 14% and 25% of wild-type and JAK2V617F mutant patient, respectively ($p=0.5$). Among JAK2V617F mutant patients, age at diagnosis was significantly correlated with the load of JAK2V617F allele ($p=0.0007$, CI 95% 0.1-0.4). Haematological parameters such as hematocrit, haemoglobin, leukocyte count, MCV, platelet count, LDH and LAP, examinated at diagnosis, were not significantly correlated with the load of mutant allele. On the contrary, the frequency of patients presenting splenomegaly progressively increased according to JAK2V617F allele burden ($p=0.07$ chi-square test); furthermore, there was a significant correlation between JAK2V617F ratio and patients with spleen size larger than 15 cm ($p<0.006$, chi-square test). Unlike in a population of PV patients (Vannucchi AM, ASH 2006), we found no correlation with pruritus, systemic symptoms, thrombotic events and need of chemotherapy. On the other hand we observed a significant correlation between EEC and the percentage load of JAK2 mutant allele while, nothwithstanding PRV-1 expression levels were significantly higher in mutant than in wild-type patients ($p=0.05$), there was no overt correlation with the JAK2V617F allele burden. There was no difference in $CD34^+$ cell count in the peripheral blood in patients having, or not, the mutation. *Conclusions*. Unlike in PV patients, where most of clinical and laboratory characteristics were shown to have a continuous, allele burden-dependent correlation with JAK2 mutation, patients with ET are highly heterogenous under this respect, suggesting that, in addition to the lower allelic burden, other genetic and/or host factors have a major role in modulating disease presentation.

Acute myeloid leukemia - Biology II

0898

UNRAVELING LEUKAEMOGENIC MECHANISMS AND TARGETS OF NUP98/HOMEO-DOMAIN RECOMBINATIONS IN AML THROUGH A NEW NUP98/HHEX FUSION GENE

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An increasing number of chromosomal translocations leading to fusion genes involving the nucleoporin 98 (NUP98) gene are being identified in patients with acute leukaemia and adverse prognosis. All of the resulting fusion proteins involve the NUP98 N-terminus fused to various C-terminal partners most frequently involving homeodomain (HD) transcription factors. We have identified a patient with acute myeloid leukaemia (AML) and a t(10;11)(q23;p15) as sole cytogenetic abnormality. Fluorescent *in situ* hybridization (FISH) and PCR analysis revealed a translocation involving the N-terminus of NUP98 fused to the DNA binding domain of the haematopoietically expressed homeobox gene (HHEX), an essential transcriptional regulator of early blood formation. Whereas HHEX acts as a transcriptional repressor, the NUP98/HHEX fusion activates transcription in transcriptional repression assays. Significantly when co-expressed the NUP98/HHEX fusion is transdominant over repression by HHEX. Expression of NUP98/HHEX in primary murine bone marrow cells leads to aberrant self-renewal capacity and a block in normal differentiation as shown in serial replating assays and liquid cultures. *in vitro* transforming activity of the NUP98/HHEX fusion is dependent on the presence of the NUP98 GFLG repeat and the HHEX homeodomain. Transplantation of bone marrow cells retrovirally expressing NUP98/HHEX leads to acute leukaemia characterized by extensive infiltration of leukaemic blasts expressing myeloid ($Gr1^+$, $Mac1^+$) as well as markers of the B-cell lineage ($B220^+$). A latency period of 8 months suggests that NUP98/HHEX is necessary but not sufficient for disease induction. Expression of GFP-NUP98/HHEX fusions showed a highly similar nuclear localization pattern as for other NUP98/HD fusions such as NUP98/HOXA9 or NUP98/PMX1. Like NUP98/HHEX, these fusion genes exert their transforming activity by aberrant transcriptional activation depending on the NUP98 GLFG repeats and the HD suggesting the possibility of a common gene expression program. Currently ongoing comparative gene expression profiling experiments in primary murine bone marrow cells will provide evidence for the presence of common targets. Validation of critical targets may open new avenues for targeted therapeutic approaches for acute leukaemias harboring NUP98- or other transcription factor fusion genes.

0899

THE ALTERNATIVELY SPLICED ISOFORM AML1-ETO9A IS DETECTABLE IN ALL T(8;21)-POSITIVE ACUTE MYELOID LEUKEMIAS: CORRELATION OF THE EXPRESSION LEVEL WITH CLINICAL OUTCOME

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Acute myeloid leukemia (AML) with translocation t(8;21)(q22;q22) creating the AML1-ETO fusion gene is a distinct type of AML generally associated with a favorable prognosis. However, a significant proportion of these patients relapse and survival after 5 years is approximately 50%. These findings together with studies from murine models suggest that additional genetic lesions are underlying the clinical heterogeneity of t(8;21)-positive AML. Recently, an alternatively spliced isoform of the AML-ETO transcript, AML-ETO9a, was identified in a large proportion of t(8;21)-positive leukemias by RT-PCR. AML-ETO9a includes an extra exon, exon 9a, of the ETO gene encoding a C-terminal truncated AML1-ETO protein. As expression of AML1-ETO9a leads to rapid development of leukemia in mice, the coexpression of AML1-ETO9a might contribute to induce leukemia development. The aim of our study was to analyze a large series of t(8;21)-positive AML pts. from the AMLSG study trials for the expression of AML1-ETO9a and to correlate the expression levels with clinical outcome. Total RNA of peripheral blood (PB) and/or bone marrow (BM) cells from diagnosis was analyzed from 42 t(8;21)-positive AML using a Taqman real-time quantitative (RQ) PCR assay allowing the sensitive and specific detection of the AML1-ETO9a splice variant with primers located in exon 8 and exon 9a; expression of AML1-ETO was determined according to Europe Against Cancer (EAC) standard protocols.

The fusion transcript copy number in each sample is reported as the normalized value of AML1-ETO9a or AML1-ETO per transcript copy number of a housekeeping gene, β2-microglobulin, as a control, respectively. All patients were enrolled in one of our AMLSG trials [AML HD93, AML HD98A, AMLSG 07-04] for younger patients (16 to 60 years). Postremission therapy was high-dose cytarabine-based in all trials. Using a sensitive RQ-PCR assay AML1-ETO9a expression was detected in all diagnostic t(8;21)-positive AML samples. The fusion transcript copy number ranged from 0,12% to 54%. There was no correlation between pre-treatment variables such as WBC, LDH, amount of blasts in BM or PB and the level of AML1-ETO9a expression. In addition, there was no correlation between these variables and the AML1-ETO9a/AML1-ETO ratio. Using maximally selected log-rank statistics a cut point for the dependent variable overall survival for AML1-ETO9a fusion transcript copy number of 12% in BM was identified (p=0.03). For patients with AML1-ETO9a fusion transcript copy numbers higher than 12% relapse free (p=0.001) and overall survival (p=0.0001) were significantly worse compared to patients with expression levels below that cut point. *Summary/ Conclusions*. In contrast to recent studies the alternatively spliced isoform AML1-ETO9a can be detected in all t(8;21)-positive AML samples using a sensitive and specific RQ-PCR assay. Our preliminary results indicate that not the presence of AML1-ETO9a splice variant *per se* but the level of expression might have an impact on clinical outcome. However, additional studies in larger patient series will be performed to further evaluate these findings.

0900

CRYPTIC 3Q26 ABERRATIONS PARTLY EXPLAIN ABNORMAL EXPRESSION OF EVI1 IN AML CASES WITHOUT CYTOGENETICALLY RECOGNIZABLE TRANSLOCATIONS IN THIS LOCUS

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Background. Ecotropic viral integration site 1 (EVI1) is a proto-oncogene located on chromosome 3q26. Activation of EVI1 expression often occurs through chromosome 3 translocations and inversions, which leads to development of leukemia. We previously (Barjesteh van Waalwijk *et al*. 2003) showed that EVI1 is expressed in 8% of AML patients in the absence of 3q26 aberrations and is a poor prognostic marker in this intermediate risk group. The mechanisms of EVI1 deregulation remain unclear. *Aims*. In this study we wish to confirm the prognostic value of EVI1 in an independent AML cohort. We aim to determine the mechanisms of the aberrant expression of EVI1 in AML patients without 3q26 abnormalities. We hypothesize that EVI1 expression occurs through cryptic aberrations, which causes EVI1 to come under the regulatory control of the RPN1 gene, located on chromosome 3q21. *Methods*. We determined the relative expression of EVI1 by RT-Q-PCR using specific primers and probes. FISH analysis was performed using fluorescent-labeled probes located on EVI1 (3q26), MDS1 (3q26), RPN1 (3q21) and 3qter. *Results*. In an independent cohort of 272 AML patients we determined the EVI1 expression and confirmed that high EVI1 expression predicts a poor 5-years OS (6% v 33%; p=0.0001) and EFS (6% v 24%; p=0.0001). Within the intermediate risk AML cases the poor prognostic value of EVI1 remained (5-year OS 11% v 28%; p=0.009, EFS; 11% v 25%; p=0.008). EVI1 gene structure analysis revealed five splice variants; 1A, B, C, D and 3L. Previously, we had determined the expression of splice variant EVI-1D only. Here, we determined the expression of each splice variant in a cohort of 557 AML patients. Analyzing EVI1-D variant separately, 35 patients were positive, whereas 44 patients were EVI1 positive (EVI1$^+$) for one or more splice variants. Importantly, EVI1 remained a significant strong independent marker (EFS; HR=1,95 and OS; HR=2,11). Nine of the EVI1$^+$ patients carried a known 3q26 abnormality. An EVI1-MDS1 FISH analysis was performed in the remaining available EVI1$^+$ patients to investigate whether EVI1 over-expression was caused by cryptic translocations. FISH showed cryptic inv3(q21;q26) in six cases. In the remaining 18 EVI1$^+$ patients FISH revealed no abnormalities, suggesting other mechanisms of EVI1 expression in those cases. *Conclusion*. We showed that EVI1 is a strong prognostic marker in AML patients with and without 3q26 aberrations. By analyzing the different EVI1 splice variants we identified more patients with EVI1 expression. Cryptic inversions involving 3q26 were found in 6 patients, resulting in aberrant expression of EVI1 controlled by RPN1 regulatory sequences. The remaining patients revealed no translocations by FISH analysis. We hypothesize that EVI1 regulation in this group is different as compared to patients with a (cryptic) 3q26 aberration. To gain more insights into the molecular basis of high EVI1 levels in the other cases, research is needed to determine other possible mechanisms of EVI1 over-expression such as copy number changes or mutations in regulatory regions.

0901

THE T(6;9) ASSOCIATED DEK/CAN FUSION PROTEIN DOES NOT BLOCK DIFFERENTIATION OF EARLY HEMATOPOIETIC STEM CELLS BUT INCREASES THEIR SELF RENEWAL POTENTIAL

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Background. More than 60% of acute myeloid leukemia (AML) harbor specific chromosomal aberrations mainly translocations. Translocations such as t(15;17), t(11;17), or t(8;21) lead to the formation of chimeric genes encoding for aberrant fusion proteins such as PML/RAR, PLZF/RAR and AML-1/ETO. These fusion proteins are able to induce and to maintain the leukemic phenotype by blocking terminal differentiation of early hematopoietic progenitors and by interfering with the biology of the leukemic counterpart of the hematopoietic stem cells. The t(6;9) with its DEK/CAN fusion protein is of particular interest because i.) it is more frequent in young patients; ii.) it is associated with a poor prognosis. The t(6;9)-DEK/CAN fusion occurs with an incidence of 1-5% in adult patients with AML and in most of the cases t(6;9)-positive AML is classified as FAB-M2 or M4 (90%) and rarely as M1 (10%). Nearly nothing is known about the biology of DEK/CAN and whether it shares common features with other fusion proteins which could account for its leukemogenic potential. In contrast to PML/RAR, PLZF/RAR or AML-1/ETO the overexpression of DEK/CAN does not block VitD3-induced monocytic differentiation in U937 cells. These findings called in question the leukemogenic potential of DEK/CAN. *Aims*. To further disclose the role of DEK/CAN in the leukemogenesis we investigated its effect on the biology of early hematopoietic stem cells (HSC) in comparison to PML/RAR. Therefore we retrovirally transduced Sca1+/lin- murine HSC (SL cells) with DEK/CAN and PML/RAR and performed both differentiation and stem cells assays (morphology, surface marker expression, replating efficiency, colony forming unit - spleen-CFU-S). *Results*. Here we show that i.) DEK/CAN in contrast to PML/RAR was apparently unable to block terminal differentiation of SL cells as revealed by morphology and the expression of the myeloid differentiation marker Gr-1 and Mac-1 as well as of the stem cell marker Sca-1 and c-Kit; ii.) DEK/CAN increased the replating efficiency of SL cells, but did not reach the level of PML/RAR with a reduced number of either colony forming units with respect to PML/RAR; iii.) the increased replating efficiency was related to an stem cell capacity as revealed by the fact that in contrast to mock infected control cells DEK/CAN-positive cells gave origin to a positive CFU-S assay even after one or two plating rounds in semi solid medium similar to PML/RAR. *Conclusions*. Apparently DEK/CAN seems to share with other LAFP only the capacity to increase the self renewal of HSC, but not to block terminal differentiation. These findings strongly suggest that the effect of DEK/CAN is limited to a very small subset of cells within the stem cell compartment which might represent the origin of a DEK/CAN-positive leukemia. Actually the capacity of DEK/CAN to give origin to leukemia *in vivo* is under investigation.

0902

BORN TO BE EXPORTED: C-TERMINUS NUCLEAR EXPORT SIGNALS OF DIFFERENT STRENGTH ENSURE CYTOPLASMIC ACCUMULATION OF NUCLEOPHOSMIN LEUKEMIC MUTANTS

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Nucleophosmin (NPM1) gene mutations are the most common genetic lesion in Acute Myeloid Leukemia (AML) (Falini, NEJM, 352:254, 2005) and specifically identify a distinct molecular and clinico-pathological entity (Falini, Blood, 109:874, 2007). NPM1 mutations result in aberrant NPM cytoplasmic dislocation due to two indispensable alterations at C-terminus of NPM protein: 1) disruption of both tryptophans (W) 288 and 290 (or 290 only) that constitute the Nucleolar Localization Signal; 2) creation of a new carboxy-terminal Nuclear Export Signal (NES), with 6 molecular variations observed to date. We previously showed that there is correlation between the new NES sequence and the number of mutated tryptophan(s). In fact, the most common NES motif (LxxxVxxVxL) always associates with loss of both W288 and W290 (e.g. NPM mutant A), while leukemic mutants retaining W288 always carry rare NES variant sequences (e.g. LxxxLxxVxL in NPM mutant E). These findings sug-

gest diverse sequences of NPM mutant NES motifs function differently. Hereby, we provide evidence of how C-terminus alterations functionally cooperate to delocalize NPM mutants to cytoplasm. We first investigated whether different NPM leukemic mutants differ in their ability to be exported in the cytoplasm. NIH 3T3 cells were transfected with eGFP-tagged NPM mutants A and E. After incubation with low doses of Leptomycin B (a specific inhibitor of Crm1, the protein responsible for NES-mediated nuclear export), NPM mutant A was almost completely nuclear whilst NPM mutant E was still markedly cytoplasmic. This demonstrate that NPM mutant E is less sensitive to Crm1 inhibition than NPM mutant A. To directly measure the export efficiency of each of the six different NPM C-terminal NESs so far identified, we isolated and cloned them into a pREV(1.4)-eGFP plasmid expressing a mutagenized REV protein lacking its NES but retaining its Nuclear Localization Signal, an assay that allows to measure the export efficiency of various NES sequences (Henderson, Exp Cell Res 256:213, 2000). The REV(1.4) fusion protein containing the most common NPM mutant NES LxxxVxxVxL (never found with W288) was nuclear in the majority of transfected cells (indicating a functional NES with weak activity), whilst all variant NESs were mostly cytoplasmic (indicating a stronger activity). These findings prove that NPM mutants carry NES motifs with different nuclear export efficiency. NPM subcellular localization is dictated by opposing balance of forces (tryptophans and NES), and no NPM mutant from AML leukemic patients has ever been found to contain the weak LxxxVxxVxL NES in the presence of W288. We therefore investigated the consequence of artificially combining these two features on NPM subcellular traffic in NIH 3T3 cells. Notably, these artificial NPM mutants were not exported efficiently into cytoplasm, since the force (W288) driving mutants towards the nucleolus overwhelmed the force (NES motif) exporting them into cytoplasm. These findings show that NPM leukemic mutants must carry a strong NES motif if W288 is retained in order to ensure efficient cytoplasmic accumulation. This reveals a mutational selective pressure toward efficient NPM nuclear export and points to this event as critical for leukemogenesis and therefore as a potential therapeutic target.

Table 1. NESs from NPM leukemic mutants: types, incidence upon NPM mutants, and correlation with Tryptophan loss.

Chronic myeloid leukemia - Biology

0903

BMS-214662 TARGETS AN EARLY PROGENITOR POPULATION IN PRIMARY CML AND INDUCES APOPTOSIS IN THE QUIESCENT FRACTION AFTER SENSITIZATION BY THE MEK 1/2 INHIBITOR U0126

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Background. Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder that cannot be eradicated by the targeted Abl tyrosine kinase inhibitor (TKI), imatinib mesylate (IM; Gleevec, Gilvec) or dasatinib (Sprycel; a more potent multi-targeted TKI) as these drugs reversibly arrest proliferation of CML stem/progenitor cells but do not induce apoptosis or kill the most primitive quiescent cells. Farnesyltransferase inhibitors (FTI) inhibit farnesylation of oncogenic RAS as well as of other intracellular proteins involved in hematological malignancies. BMS-214662, a cytotoxic FTI has been shown to preferentially kill non-proliferating cells, to induce potent tumour regression, and has anti-leukemic activity in acute myeloid leukemia. *Aims.* We tested BMS-214662 for ability to target CML stem/progenitor cells. *Methods.* Primitive $CD34^+38^-$ cells, derived from CML patients in chronic phase and normal donors, were treated with BMS-214662 alone or in combination with a pharmacologic inhibitor, U0126, and analysed for caspase-3 activation by flow cytometry. Western blotting analysis was used to investigate BMS-214662 mechanism of action. *Results*. BMS-214662 significantly and selectively increased caspase-3 activity in CML versus normal cells (27.7 and 6.4%, respectively, after 48 hours of treatment). Remarkably, since $CD34^+38^-$ cells are almost exclusively quiescent, this highlights for the first time the effectiveness of BMS-214662 against the more quiescent primary CML stem cell population. Moreover, neither IM, nor dasatinib, nor lonafarnib (Sarasar), nor nilotinib (Tasigna) showed similar apoptotic activity to BMS-214662. In CML $CD34^+$ progenitor cells, BMS-214662 potently blocked the pro-survival MAPK pathway by inhibiting phosphorylation of RAF-1 and ERK. In addition, the inhibition of MEK with U0126, together with BMS-214662 resulted in a dramatic synergistic enhancement of apoptosis. In an attempt to understand how BMS-214662 induces apoptosis, we analysed expression level of the Bcl-2 family proteins. For both CML cell lines and primary $CD34^+$ cells Mcl-1, Bcl-2 and BimXL levels were unchanged after treatment with BMS-214662. However, BMS-214662 decreased the level of IAP-1, a known suppressor of apoptosis. *Conclusions*. Our group is the first to report that BMS-214662 selectively kills quiescent cancer stem cells and therefore offers potential for eradication of CML in chronic phase.

0904

THE P-LOOP MUTATIONS Y253H AND E255K/V MAY DEVELOP MORE FREQUENTLY THAN T315I DURING NILOTINIB THERAPY AFTER IMATINIB FAILURE AND ARE ASSOCIATED WITH PROGRESSION IN PATIENTS WITH PH-POSITIVE LEUKEMIA

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Background. In vitro data suggest a central role for BCR-ABL mutations in clinical resistance to nilotinib for patients with Philadelphia-positive (Ph^+) leukemia. Fifteen nilotinib resistant mutations were identified in resistance screens (Blood, 2006;108:1328-1333, Blood, 2006;108:2332-2338). With the exception of T315I, all mutations were effectively suppressed with nilotinib concentrations of 2000 nM, which falls within the peak-trough plasma levels (3600-1700 nM) measured in patients treated with 400mg BID. *Aims*. We aimed to determine the effect of mutations *in vivo* on nilotinib resistance and molecular response of patients treated with 400mg nilotinib BID after imatinib failure. *Methods*. Sixty-eight patients were treated in a Phase II trial (Ph^+ALL, n=4; CML-blast crisis [BC], n=15; CML-accelerated phase [AP], n=6; CML-chronic phase [CP], n=43). Patients were followed by RQ-PCR and mutation analysis while receiving nilotinib therapy for a median of 6 (range 2-15) months. The results were correlated with disease status. Molecular response was defined as the reduction of BCR-ABL to ≤ 1% (minor) and ≤ 0.10% (major) on the international scale. Loss of molecular response was defined as >2-fold rise of BCR-ABL and loss of a minor molecular response. *Results*. Prior to nilotinib (baseline), 22 different mutations were detected in 33/68 patients (49%). Molecular response occurred in 25/68 patients (37%). A major molecular response occurred in 16/68

(24%). The frequency of molecular response was significantly higher in patients without baseline mutations (54% versus 18%), p=0.005. During therapy, 18/68 patients (26%) developed 7 different mutations that were not detectable at baseline (Table 1) (5/18 did not have any baseline mutation). These seven mutations were predicted from the *in vitro* screening studies to be resistant to nilotinib. Progression was documented in 21 patients (4/4 Ph^+ALL, 8/15 BC [53%]; 2/6 AP [33%]; 7/43 CP [16%]). There was no significant difference in the frequency of progression for those with baseline mutations (36%) and those without (26%). Of the 21 patients with progression, 12 developed mutations during therapy; 5 had mutations at baseline that were identified in the resistance screens (3, F359V; 1, F359I; 1, Q252H) but had no further mutation at progression; and 4 did not have a mutation at any time. Of the 20 patients with molecular response who did not have any of the mutations that were identified in the *in vitro* resistance screens, none has lost molecular response whereas 3/5 who developed mutations during therapy lost response, p=0.004. At 90% of assessment timepoints the patients with mutations identified in the resistance screens received at least 400mg BID (9% 600mg BID). *Summary/Conclusions*. Progression was associated with the detection of mutations that were previously identified in nilotinib resistance screens. Rather than the predominant emergence of T315I with resistance, as predicted by the *in vitro* studies, the mutations that developed most frequently were Y253H, E255K and E255V. Among the 7 mutations that developed, only T315I has an IC50 value >1000 nM and is the only one predicted to be insensitive to nilotinib concentrations of 400 mg BID. This might be due to intracellular concentrations of nilotinib being lower than the plasma concentrations predicted from pharmacokinetic studies.

Table 1. Mutations that developed during nilotinib therapy.

Mutations that developed during nilotinib therapy*	No. of patients who developed the mutations	No. of patients with disease progression
Y253H	6	6
E255K	1	1
E255V	1	1
E255V / E255K / G250E	1	1
E255K / G250E	1	1
T315I	1	1
F359V	1	1
G250E	2	0
G250E / F359I	1	0
F359I	1	0
*disease status unavailable for 2 additional patients (1 E255V, 1 T315I)		

0905

NOVEL PATHWAY IN BCR-ABL SIGNAL TRANSDUCTION INVOLVES AKT-INDEPENDENT ACTIVATION OF PLC-GAMMA/MTOR/P70-S6 KINASE

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Background. In CML, defining new, additional therapeutic targets in the pathways, activated by BCR-ABL is critical for the development of new treatment strategies, especially for patients resistant or refractory to imatinib or other tyrosine kinase inhibitors. While studying the involvement of PI3K/Akt/mTOR signaling pathway in the development of such resistance we have uncovered the existence of additional, Akt-independent mechanism of activation of mTOR/p70-S6 Kinase pathway. *Aims*. In this work, we characterize a new, PhosphoLipaseC-gamma (PLCγ) dependent pathway for activation of mTOR/p70-S6Kinase and its importance for the control of proliferation and apoptosis of BCR-ABL-positive cells. *Methods*. BCR^-ABL^+ cell lines LAMA84, AR320, KCL22, K562, Ba/F3-BCR-ABL were treated with various inhibitors and their effect on cell signaling was analyzed by Western blotting using phosphorylation-specific antibodies. In addition to the chemical inhibition, siRNA-mediated knock down of PLCγ was utilized to better understand the necessity/sufficiency of PLCγ for full activation of mTOR/p70-S6K pathway in the BCR^-ABL^+ cells. The function of PLCγ/mTOR/p70-S6K pathway for the BCR-ABL driven cells was also analyzed in proliferation (MTS) and apoptosis assays where cells were treated with imatinib alone or in combination with U73122, BAPTA-AM, RAD001 or PKC412 inhibitors. *Results*. Short term treatment with imatinib (1 μM, 4h) of BCR^-ABL^+ cell lines caused downregulation of phosphorylation of p70-S6K and of S6 ribosomal protein without decreasing phosphorylation levels of Akt, as detected by Western blotting using the respective phosphorylation-specific antibodies p-p70-S6K (Thr389), p-S6 (Ser240/244) and p-Akt (Ser473). Inhibition of Akt by the specific inhibitor SH-6 (10 μM, 4h) did not affect the phosphorylation of p70-S6K and S6. These results were consistent in all analyzed cell lines, and led us to consider alternative mechanism for mTOR/p70-S6K pathway activation. One such mechanism, recently described in FGF9 signaling is a PLCγ-controlled Calcium signaling pathway involving Ca/Calmodulin (CaM) and Ca/Calmodulin-dependent Kinase (CaM-K). In all BCR^-ABL^+ cell lines analyzed, we detected strong PLC-γ activation (examined by p-PLCγ-Tyr783 antibody), which was effectively suppressed by imatinib (1 μM, 4h). Incubation of the cells for 30 min with 10 μM U73122, a specific PLC inhibitor, in contrast to the inactive analog U73343, significantly blocked p70-S6K and S6 phosphorylation. siRNA mediated suppression of PLCγ also reduced S6 phosphorylation. In general, activation of PLCγ leads to activation of various PKC isoform and increased Ca-dependent signaling. By employing inhibitors of the Ca-signaling (BAPTA-AM, EDTA), CaM-K (KN-93) and the PKC inhibitor PKC412 we studied the participation of these molecules in the pathway. Inhibition of PLCγ led to suppression of cell proliferation and enhanced apoptosis. Combined treatment with imatinib and U73122 drastically increased the apoptosis rate of the cells. In addition, this combination was able to suppress proliferation and induce apoptosis in imatinib resistant cells. Combination of these two inhibitors was more efficient in Ba/F3 p185-F317V cells exhibiting moderate imatinib resistance as compared to Ba/F3 p185 E255K and T315I cells exhibiting strong resistance to imatinib. *Conclusions*. In summary, we demonstrate the existence of additional, Akt-independent, PLCγ -dependent mode of activation of mTOR/p70-S6K which operates in Bcr-Abl-positive cells. This alternative pathway may prove novel therapeutic targets for CML treatment.

0906

DELETIONS OF THE DERIVATIVE CHROMOSOME 9 DO NOT INFLUENCE RESPONSE TO IMATINIB IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS (A GIMEMA WORKING PARTY ANALYSIS)

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Background. BACKGROUND Extensive submicroscopic deletions adjacent to the breakpoint on derivative chromosome 9 [der(9)] have been reported in a subset of Chronic Myeloyd Leukemia (CML) patients and have been associated with an adverse outcome with conventional drugs and α-interferon (α-IFN). Huntly 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. *Aims.* To establish the relationship of der(9) deletions with the response to imatinib in early CP patients,we performed a sub-analysis within 3 simultaneously running trials of the GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) CML WP (n.CML/021, phase II - ima 800 in intermediate Sokal risk; CML/022, phase III- ima 400 vs 800 mg in high Sokal risk, n . CML/023, observational - ima 400 mg). *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 (3-33 months). Fluorescence in situ hybridization (FISH) analysis of bone marrow cells was performed at diagnosis using BCR/ABL extra-signal, D-FISH or dual-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%/43%, 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: the cytogenetic and molecular response rates 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 MMolR 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, AIL.

0907

THE INTRACELLULAR CONCENTRATIONS OF IMATINIB AND NILOTINIB, CAN BE SUBSTANTIALLY ALTERED BY INTERACTION WITH OTHER DRUGS: STUDIES WITH PROTON PUMP INHIBITORS

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Background. There is accumulating evidence that dose intensity is a key determinant of molecular response in CML patients treated with imatinib. Furthermore it has been demonstrated that trough imatinib blood levels are predictive of cytogenetic and molecular response. However, it is the concentration of imatinib within haemopoietic cells, not plasma that is the major determinant of ABL kinase inhibition. Factors that increase or reduce imatinib intracellular uptake and retention (IUR) may therefore impact on response. *Aims.* To assess the impact of drugs known to inhibit efflux pumps on the IUR of imatinib and nilotinib. Proton Pump Inhibitors (PPIs) pantoprazole and esomeprazole, were selected because they interact with efflux pumps, and are commonly used in CML patients treated with either imatinib or nilotinib. *Methods*. Using the IUR assay and [14C]-labelled nilotinib or imatinib the effect of co administration of PPI's were assessed after 2 hours exposure at 37oC. Further using the same approach the effect of unlabelled imatinib on [14C]-nilotinib uptake, and unlabelled nilotinib on [14C]-imatinib uptake were examined. Using cell lines, the IC50 for imatinib and nilotinib were also assessed in the presence and absence of PPI. *Results*. (Table 1). These results demonstrate a significant decrease in the IUR for imatinib in the presence of either PPI. A similar though not significant decrease was observed with the addition of nilotinib. In contrast a statistically significant increase in the IUR for nilotinib was observed when either PPIs or imatinib were added. Furthermore serial IC50 experiments in K562 cells demonstrated a dose dependant decrease in the IC50nilotinib when either PPI was incorporated into the assay (IC50nilotinib:0 µM pantoprazole-350 nM, 200 µM pantoprazole-145 nM and 400 µM pantoprazole -83nM). In contrast the IC50 for imatinib in the presence of PPI was increased (IC50 0 µM pantoprazole-4.2 µM, 200 µM pantoprazole-10 µM and 400 µM pantoprazole -7 µM). *Conclusions*. Imatinib and nilotinib are transported differently by haemopoietic cells. We have shown this clearly with regard to influx mechanisms.[1] We now show that imatinib increases nilotinib intracellular concentration significantly, but the converse does not apply. The effects of PPIs are also contrasting with both pantoprazole and esomeprazole significantly increasing nilotinib, but not imatinib intracellular concentration. We speculate that these interactions are due to competition and inhibition of efflux pathways, most likely ABCB1 but possibly ABCG2. Monitoring plasma concentrations of imatinib and nilotinib may not be sufficient to ensure optimal intracellular concentrations of these kinase inhibitors. Drug interactions are well recognised to alter plasma levels by affecting hepatic metabolism. Our findings suggest such drug interactions may also lead to excessive toxicity (in off-target cells) or reduced efficacy, by significantly altering intracellular concentrations of these kinase inhibitors. These effects will not be reflected in changes to plasma drug levels.

Table 1.

Median % change in IUR from no PPI control	1uM [^{14}C]-labelled nilotinib		1uM [^{14}C]-labelled Imatinib	
	+200 µM PPI	**+400 µM PPI**	**+200 µM PPI**	**+400 µM PPI**
+ Pantoprazole *(n=9)*	29.6%	29.9%	-24.8%	-28.6%
p value	***0.02***	***0.033***	***0.016***	***0.004***
+ Esomeprazole *(n=6)*	37.8%	32.7%	-11.6%	-20.7%
p value	***0.001***	***0.015***	*0.089*	***0.033***
% change in IUR from control	**+1uM imatinib**	**+2uM imatinib**	**+1uM nilotinib**	**+2uM nilotinib**
Median (n=5)	31.2%	47.8%	-26.8%	-22.2%
p value	***0.008***	***0.008***	***0.015***	*0.74*

Reference

1. White, D.L., et al. Blood 2006:108:697-704.

Chronic lymphocytic leukemia and related disorders - Biology

0908

EXPRESSION LEVELS OF CLLU1 AND LPL : NEW DISEASE-ASSESSMENT TOOLS IN CLL PATIENTS IN BINET STAGE A ?

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Background. IGVH mutational status is a strong predictor for outcome in Chronic Lymphocytic Leukemia (CLL) but its determination remains unadapted to routine practice. Several reports have shown that the expression levels of other genes (such as LPL) could be used as surrogate markers. Recently Buhl *et al.* identified a novel gene (CLLU1), exclusively upregulated in CLL cells, and whose high expression mRNA levels could predict poor clinical outcome in patients younger than 70 years. *Methods*. Non-purified peripheral blood mononuclear cells from a cohort of 177 untreated Binet stage A-CLL patients from 4 centers were examined for CLLU1 transcripts (cDNA1) and LPL expression levels by real time quantitative PCR and compared to the same pool of purified B cells from healthy individual donors (n=3). We correlated the results with the previously determined IGVH mutational status (MT: mutated; UNM: unmutated), expression of ZAP70, chromosomal aberrations and clinical characteristics. *Results*. The selected cohort included 61% of male and median age at diagnosis was 67 (38-90) years. With a cut-off value arbitrary defined as 1 for the CLLU1/β2M and for the LPL/β2M ratios from the control pool, the median level of up-regulation of CLLU1 and LPL expressions were 12,2 and 54 fold above the level found in normal B cells, respectively. This level was not surprisingly lower than previously described for CLLU1 (27 fold) since our cohort included only stage A patients. Expression levels of CLLU1 and LPL were significantly associated with the IGVH mutational status [median values: 9.4 and 21 in MT (n=129) versus (vs) 173 and 886 in UM (n=43) (p=0.0000 for the two genes) , with ZAP70 expression [median: 86 and 631 in ZAP^+ cases (n=47) vs 6,5 and 22,6 in ZAP^- cases (n=96) (p=0.0012 and p=0.0000), respectively] and with poor prognosis cytogenetic markers such as del17p (p=0.012 and p=0.0018) and del11q (p=0.00019 and$p$=0.00003). CLLU1 and LPL expression level was in an unexpected way (and more significantly for LPL) associated with the presence of trisomy 12 (p=0.016 and p=0.00096). A slight correlation was found between the young patient age at diagnosis and CLLU1 (p=0.02), between LPL expression and male gender (p=0.001) and between LPL and CLLU1 (p=0.0041). *Conclusions*. Our study demonstrates in a large cohort of recently diagnosed stage A CLL patients that CLLU1 and LPL mRNA quantifications 1/ are good surrogate markers of usual prognosis leukaemia-cell parameters, 2/ represent a reliable alternative to IGVH genes sequencing. Moreover, these quantifications do not require purification of B lymphocytes, a significant advantage for routine practice. CLLU1 and LPL mRNAs levels could then be considered as new disease-assessment tools in CLL. Follow up of our patients will determine if these new parameters add independent prognostic information to the definitions of smoldering and nonsmoldering CLL in Binet stage A.

0909

QUANTITATIVE GENE EXPRESSION ANALYSIS OF SURROGATE MARKERS FOR GENETIC RISK GROUPS AND SURVIVAL IN CLL

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Background. The genetic factors VH mutation status, V3-21 gene usage, and genomic deletions at 11q22-q23 and 17p13 have been shown to be important prognostic markers in CLL. Given the high complexity of these analyses in the recent years several molecular surrogate markers were developed aiming at the facilitation of routine prognosis assessment. *Aims*: To assess the value of potential surrogate markers for the prediction of genetic risk groups and survival. *Methods*: Real-time RT-PCR (RQ-PCR) of candidate genes was performed in a CD19-purified and a non-purified CLL cohort each comprising the relevant genetic subgroups (VH mutated, VH unmutated, V3-21 usage, 11q-, 17p-). 17 markers (ADAM29, ATM, CLLU1, DMD, GLO1, HS1, KIAA0977, LPL, MGC9913, PCDH9, PEG10, SEPT10, TCF7, TP53, Vimentin, ZAP-70, ZNF2) identified in previous studies were investigated in the non-purified cohort of 102 CLL patients. Of these, 10 markers, either with an overexpression in non-CLL cells or an impact on survival or risk group prediction, were analyzed in the purified cohort of 112 cases. VH sequencing and FISH screening for genomic aberrations were carried out for all cases. Survival information was available for 80 (purified) and 88 cases (non-purified). Logistic regression was performed to test the predictive value of gene expression for genetic risk groups, Cox proportional hazards statistics for survival analysis. *Results*. The genetic risk groups in both cohorts showed the expected correlation with survival with significantly shorter survival of VH unmutated, 17p-, and 11q- cases indicating a representative composition of the cohorts under study. In non-purified cases, the best predictive marker for VH status was LPL (p=0.001). While no reliable predictive markers were identified for V3-21 usage or 17p-, lower ATM expression was predictive for 11q-. In survival analysis including all candidate genes TCF7 (p=0.001) and KIA0977 (p=0.016) were of prognostic value. In multivariate survival analysis including candidate gene expression and the genetic risk factors as variables, only 17p- remained as a significant parameter. In the purified cohort, significant markers (p<0.05) for genetic risk groups were: ZAP70, LPL, and TCF7 for VH mutation status (TCF7 expression associated with mutated VH); SEPT10, ZAP70, and ADAM29 for V3-21 usage; ATM and TCF7 for 11q- (both with a negative association); ZNF2 for 17p- (negative association). In multivariate analysis, the parameters 17p-, 11q-, V3-21 usage, TCF7, and ZAP70 expression were identified as independent prognostic factors. In contrast to ZAP70, TCF7 expression was positively correlated with survival times. *Conclusions*. Several results obtained in CD19-purified cases could not be reproduced in unpurified cases strongly arguing for a tumor cell selection prior to expression analysis. In purified cases, ZAP70, LPL, and TCF7 were the best predictors for VH mutation status. Additional markers such as ATM and ZNF2 may help to identify genomic risk groups such as 11q- and 17p-. Multivariate survival analysis suggests TCF7 as a strong survival predictor and points to a pathogenic role for this gene in CLL. The patient series have been extended to 115 non-purified and 150 purified cases, statistical evaluation is currently ongoing.

0910

FUNCTIONAL IMPAIRMENT OF THE P53 PATHWAY DETECTED BY FLOW CYTOMETRY IS A NOVEL INDEPENDENT ADVERSE PROGNOSTIC FACTOR IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. The p53 pathway plays a central role in cancer biology by limiting clonal expansion, maintaining genomic integrity and contributing to the action of chemotherapy. In chronic lymphocytic leukaemia (CLL), loss of wild-type p53 or ATM (activates p53 in response to DNA damage) is strongly predictive of an adverse clinical outcome. However, p53 dysfunction may arise through alternative mechanisms. Furthermore, the degree of functional impairment might be clinically important. We have previously developed a simple test that measures the functional integrity of the ATM-p53 pathway in CLL cells. In the present study, we have evaluated this test for its potential value as an independent prognostic factor. *Aim*. To examine the prognostic value of p53 functional analysis in CLL in relation to other biological variables of prognostic importance. *Methodology*. A representative cohort of 121 unselected patients with CLL was tested for p53 dysfunction using a simple flow cytometric assay in which CLL cells were analysed for up-regulation of p53 and one of its transcriptional targets, p21CIP1, following exposure to ionizing radiation (IR). Results were expressed as p53 and p21CIP1 index values (= MFI in irradiated cells ÷ MFI in non-irradiated cells). The receiver operating curve (ROC) method was used to identify appropriate cut-off values. Univariate analysis of clinical outcome was performed using the Kaplan-Meier method and log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. A likelihood-ratio test was used to estimate P values, with sequential backward removal of variables

with a p value of ≥ 0.10. Sets of measurement data were compared for statistical significance using the Student's t test or Mann-Whitney U test. Sets of enumeration data were compared by χ^2 and Fisher's exact tests. Two-sided tests with an a level of 0.05 were used in all analyses. *Results*. 74% of informative cases had stage A disease, 43% had unmutated IgVH genes, 30% had high CD38 expression and 20% had adverse cytogenetic abnormalities (loss of p53 or ATM). The median time from diagnosis to blood sampling was 6.5 months and the median follow-up time from diagnosis 35.6 months. Low p53 or p21CIP1 index values were present in one third of patients and were associated with a significantly shorter overall survival and treatment-free interval (Figure 1). Although p53 dysfunction was strongly associated with loss of p53 or ATM, the majority of patients with p53 dysfunction did not have either of these adverse chromosomal abnormalities. Sub-group analysis revealed p53 dysfunction to be a powerful predictor of adverse outcome among patients classified as good risk by clinical staging, chromosomal analysis, IgVH mutation analysis or CD38 expression. In multivariate analysis, p53 dysfunction was the only independent predictor of adverse outcome in patients with early-stage disease, those without adverse chromosomal abnormalities, and those with mutated IgVH genes (hazard ratio 7.8, 3.1 and 14.6 respectively). *Summary/Conclusions*. These findings identify p53 dysfunction as a novel independent prognostic factor in CLL. In addition, they provide the first demonstration in human cancer that clinical outcome can be predicted by functional probing of a DNA-damage response pathway.

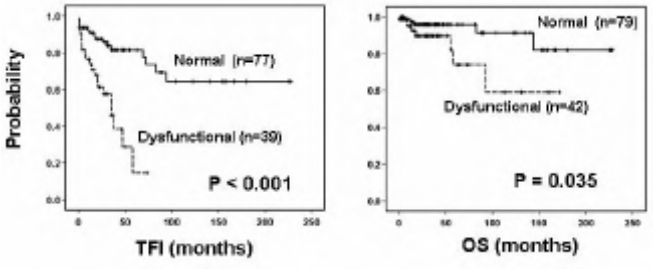

Figure 1. TFI and OS of the two groups of CLL patients.

0911

THE ANTITUMOR ACTIVITY OF LUMILIXIMAB IS MEDIATED THROUGH THE INDUCTION OF CASPASE-9DEPENDENT APOPTOSIS AND IS SYNERGISTIC WITH RITUXIMAB AND FLUDARABINE IN CD23+ CLL AND LYMPHOMA CELLS

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Background. The effectiveness of monoclonal antibody therapy in treating patients with chronic lymphocytic leukemia (CLL) has prompted interest in other antibodies specific for alternate B-cell antigens. The CD23 antigen, a transmembrane glycoprotein that functions as a low-affinity receptor for immunoglobulin E, is highly expressed on most CLL cells and represents a potential therapeutic target. *Aims*. We examined the ability of lumiliximab, an IgG1 chimeric monoclonal antibody against CD23, to mediate cytotoxicity via direct apoptosis, antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) in CD23+ malignant B-cell lines and CLL cells. We also evaluated the antitumor activity of lumiliximab in a disseminated human lymphoma model. *Methods*. Cytotoxicity was determined using flow cytometry to assess the induction of apoptosis via caspase-3, 51CR-release to determine ADCC, and flow cytometry analysis of propidium iodide staining to quantify CDC. The activation of caspase-8 and -9 and expression of pro- and anti-apoptotic proteins were assessed before and after treatment with lumiliximab using Western blot analysis. The anti-tumor activity of lumiliximab was evaluated *in vivo* using the CD23+ SKW6.4 human lymphoma/SCID mouse model. *Results*. In CD23+ B-cell lines, lumiliximab induced apoptosis, ADCC, and CDC. Lumiliximab induced apoptosis and ADCC in CLL cells; however, the degree of ADCC was low to moderate compared with that seen in CD23+ B-cell lines. There was no evidence of CDC induction in primary CLL cells after exposure to lumiliximab. Treatment with lumiliximab in the presence of an anti-human IgG Fc crosslinking antibody resulted in increased staining of activated caspase-3 in CLL cells'the median percentage of apoptosis was 46% compared with 16% with the isotype control (p<0.001). Western blot analysis also showed cleavage of caspase-9 and PARP but not caspase-8 in CLL cells incubated in the presence of lumiliximab. Lumiliximab treatment decreased the expression levels of the anti-apoptotic proteins Bcl-2 and XIAP and inhibited Akt activation. Lumiliximab in combination with rituximab or fludarabine mediated synergistic cytotoxicity against both CD23+ B-cell lines and primary CLL cells. In addition, lumiliximab as single-agent treatment produced significant inhibition of disease progression compared with a control antibody in a SCID mouse model of human B-cell lymphoma (p<0.01). The antitumor response achieved with lumiliximab was comparable to that observed with rituximab at the same dose and with the same treatment schedule. Notably, the use of lumiliximab in combination with either rituximab or fludarabine prolonged survival compared with each single-agent treatment alone. *Conclusions*. These data indicate that lumiliximab induces apoptosis primarily through the mitochondrial pathway via activation of caspase-9, resulting in the cleavage and activation of caspase-3 and PARP. Furthermore, lumiliximab synergistically increases the cytotoxicity of rituximab or fludarabine in a CD23+ malignant B-cell line and leukemia cells from CLL patients and enhances the antitumor activity of these agents *in vivo*. These observations provide a rationale for clinical trials in patients with CLL to assess the efficacy of lumiliximab as part of a novel chemoimmunotherapy regimen containing rituximab and fludarabine.

0912

IN VITRO MODEL FOR LYMPHOID MICROENVIRONMENT AND INHIBITION OF APOPTOSIS IN B-CLL CELLS: INVOLVMENT OF PI3-K/AKT PATHWAY AND PTEN

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Background. B-CLL is considered as a disease of accumulation due to the long survival and resistance of apoptosis of the leukemic B-cells. Recent data suggest the presence of proliferation pool of B-CLL cells in the lymphoid tissues and that the activation of anti-apoptotic mechanisms in the leukemic B cells in chronic lymphocytic leukemia (B-CLL) through the interaction with their microenvironment may lead to prolonged survival and the accumulation of the malignant clone. However, the signaling molecules, which are responsible for these processes are not completely defined. *Aims*. The aim of this study is to provide an *in vitro* model, which mimics the *in vivo* situation in the lymphoid tissue, and to elucidate the role of the lymphoid microenvironment in the activation of the potent anti-apoptotic PI3-K/Akt pathway and prolonging survival and maintenance of proliferation of B-CLL cells. *Methods*. Stromal fibroblasts in the lymphoid tissues are known to produce several growth factors, cytokines and matrix proteins, which are essential for supporting hematopoiesis. Therefore, bone marrow (BMF), spleen (SF) and lymph gland (LGF) fibroblasts were used as an *in vitro* model for lymphoid microenvironment under serum free condition. Pharmacological inhibitors and siRNAs against PI3-K and Akt were applied to explore the anti-apoptotic effect of the PI3-K/Akt pathway in B-CLL. *Results*. co-cultivation of B-CLL cells with human BMF, LGF, and SF significantly inhibited apoptosis and prolonged survival of the leukemic cells in comparison to suspension cultures. The data also demonstrated the presence of proliferation islands of B-CLL cells, which were in close contact with the stromal cells. To explore the involvement of PI3-K/Akt pathway in the anti-apoptotic effect of stromal fibroblasts, co-cultures were performed in presence of PI3-K inhibitors (wortmannin or LY294002) or siRNAs against PI3-K and Akt1. These inhibitors significantly reduced the supportive effect of stromal fibroblasts and induced apoptosis in the resting and proliferating B-CLL cells. The leukemic cells were more sensitive to PI3-K inhibition than T cells, monocytes and stromal fibroblasts. Induction of apoptosis was associated with a significant decrease in the intracellular PIP3, PI3-K, PDK1 and Akt1 and dephosphorylation (activation) of PTEN. Since PTEN activity, as a negative regulator for PI3-K signalling, is controlled by its phosphorylation at the tail domain, we studied the pattern of PTEN protein expression in B-CLL. Western blotting demonstrated that the total PTEN in PBMC of B-CLL patients (n=40) is comparable to healthy individuals (n=8). However, using phosphospecific anti-PTEN antibody demonstrated that samples of B-CLL patients highly express phosphorylated (inactive) forms of PTEN in comparison to healthy persons. *In conclusion*. The lymphoid microenvironment model allows demonstrating that the interaction between the leukemic cells and the lymphoid microenvironment may lead to the activation of PI3-K pathway and inhibition of apoptosis of B-CLL cells. The data also demonstrate that the *in vitro* model may allow large-scale drug screening and indicate that PI3-K is a feasible therapeutic target in B-CLL.

Red cells and iron

0913

PLASMA LIPOCALIN-2 LEVELS IN PATIENTS WITH THALASSEMIA INTERMEDIA: CORRELATION WITH IRON METABOLISM AND ERYTHROPOIESIS

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Background and Aims. Members of the lipocalin protein family are small, secreted proteins with a variety of functions. Although the physiological role of lipocalin-2 has not been fully elucidated, a few pivotal functions have recently been reported, namely the regulation of the apoptosis of leukocytes. Unexpectedly, lipocalin-2 is abundantly expressed in erythroid progenitor cells. *in vitro* culture experiments demonstrated that lipocalin-2 induces apoptosis and inhibits differentiation of erythroid progenitor cells. During acute anemia, the expression of lipocalin-2 was reduced in erythroid cells by a feedback system. Furthermore, injection of recombinant lipocalin-2 in mice suffering from acute anemia retarded the recovery of red blood cell (RBC), suggesting the importance of reduced expression of lipocalin-2 for accelerated erythropoiesis. Lipocalin-2 is, also, an iron trafficking protein, a member of the non-transferrin-bound iron (NTBI) pool and an alternative to the transferrin-mediated iron-delivery pathway. Of note is, NTBI, which is elevated in thalassemic patients, induces cellular toxicity. In this study we investigated the possible association of lipocalin-2 with parameters of iron overload and erythropoiesis in patients with Thalassemia Intermedia (TI). *Methods*. Forty patients with TI were included in the study. In terms of clinical severity, 16 of them were never transfused, while 24 patients were of severe phenotype and only 8 of them were rarely transfused. Twenty healthy individuals served as controls. Patients and controls were evaluated for a possible renal impairment as lipocalin-2 has been implicated in renal dysfunction. Lipocalin-2 levels were determined in plasma using an immunoassay technique. NTBI levels were determined using graphite furnace atomic absorption spectrometry. Erythroid marrow activity was estimated by measuring soluble transferrin receptors (sTfR) levels with a turbidimetric technique. *Results*. The main results of the study showed: a) lipocalin-2 levels were significantly higher in patients with TI compared to controls (139.1±86.1 vs 51.2±11.8 mg/L, $p<0.0001$). Only 4 patients had lipocalin-2 levels within the control group range, b) no correlation was found between NGAL levels and neither the parameters of erythropoiesis Hb, Hb F and sTfR, ($p>0.66$, $p>0.67$ and $p>0.81$ respectively), nor with those of iron metabolism ferritin and NTBI ($p>0.90$ and $p>0.95$ respectively). *Conclusions*. The increased lipocalin-2 levels observed in TI patients in this study are in agreement with the elevated expression of lipocalin-2 observed in thalassemia mouse models. We postulate that the induction of lipocalin-2 in these patients may represent either a survival response, facilitating the survival of the less damaged thalassemic erythroid precursors or a consequence of the abnormal iron regulation in TI.

0914

ZOLEDRONIC ACID INCREASES BONE MINERAL DENSITY IN PATIENTS WITH THALASSEMIA INTERMEDIA-INDUCED OSTEOPOROSIS DESPITE THE CONTINUOUS BONE MARROW EXPANSION

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Background. Osteoporosis represents an important cause of morbidity in adult patients with thalassemia. The pathogenesis of osteoporosis in thalassemia is multifactorial, and includes mainly bone marrow expansion, endocrine dysfunction and iron overload. Patients with thalassaemia intermedia (TI) seem to have a more expanded bone marrow with pressure on cortical bone, which causes pain and bone loss in several cases. Soluble transferrin receptor (sTfR) and erythropoietin (Epo) serum levels are considered as accurate markers of erythropoietic activity in thalassemia. Bisphosphonates are potent inhibitors of osteoclast activity and have been used for the management of thalassemia-induced osteoporosis. The aim of this study was to evaluate the effect of zoledronic acid, the most potent aminobisphosphonate, on bone mineral density (BMD) of patients with TI and osteopenia/osteoporosis and explore possible correlations with bone marrow expansion and erythropoietic activity. *Patients and Methods*. Thirty-five patients with TI and osteopenia/osteoporosis (13M/22F, median age 45 years) were evaluated. Twenty-three were randomized to receive zoledronic acid, 4 mg, IV, every 3 months (n=12) or every 6 months (n=11), while 12 patients received placebo every 3 months. There was no difference in terms of the presence of gonadal dysfunction between the three studied groups. BMD of the lumbar spine (L), femoral neck and forearm was determined in all patients, using DEXA, before and 12 months after treatment. Bone marrow expansion was assessed by the measurement of sTfR and Epo serum levels, using an ELISA methodology (R&D Systems, Minneapolis, MN, USA), before and 12 months post zoledronic acid or placebo administration. In all patients markers of bone remodelling, such as C-telopeptide of collagen type-I (CTX) and bone specific alkaline phosphatase (ALP) were also measured by ELISA (serum CrossLaps®, Nordic Bioscience Diagnostics A/S, Herlev, Denmark, & Metra® BAP, Quidel Corporation, San Diego, CA, USA, respectively). Patients were asked to quantify their degree of bone pain on Huskisson's visual analogue scale (VAS) and the McGill'Melzack scoring system (MGM) before and 12 months post-therapy. *Results*. All patients had increased values of sTfR, Epo, CTX, & bALP compared with 40 controls of similar age and gender ($p<0.001$). Patients who received zoledronic acid showed a significant increase in their L-BMD ($p=0.01$), which was accompanied by a dramatic reduction in CTX & bALP (($p<0.001$). Zoledronic acid-groups showed also an increase of forearm BMD, which was of borderline significance ($p=0.07$). Placebo-group showed an aggravation of L-BMD ($p=0.041$) and markers of bone remodelling at 12 months, with no alteration in BMD of other sites. Zoledronic acid reduced bone pain, which remained stable in placebo group. There was only weak correlation between baseline sTfR levels and L-BMD. Serum sTfR and Epo increased significantly ($p<0.01$ and $p<0.02$, respectively) after 12 months of therapy in all studied groups. This increase was comparable between patients who showed an increase in their L-BMD and patients who did not. *Conclusions*. The improvement of BMD by zoledronic acid is irrespective of the continuous increase of bone marrow expansion in patients with TI-induced osteoporosis.

0915

PROTEOMIC AND BIOCHEMICAL PROFILING OF 4.1R DEFICIENT RED BLOOD CELLS

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Eryptosis is a term used to describe the highly regulated homeostatic process of erythrocyte programmed cell death. It mimics apoptosis in nucleated cells in that cell shrinkage and Phosphatidylserine (PS) exposure at the outer membrane leaflet are observed. Recently our group reported that the ligation of a specific synthetic peptide (4N1K), a natural ligand (thrombospondin-1) and a monoclonal antibody (BRIC-126) which all bind to red cell CD47 glycoprotein (also known as Integrin associated protein) can trigger PS exposure in erythrocytes. We have also demonstrated that several key red cell membrane proteins 4.1R/p55/Glycophorin C (GPC) are involved in the CD47 pathway. This is primarily because we have demonstrated a direct protein-protein interaction between CD47 and p4.1 and p55. In order to explore these interactions in more depth, we have studied red cells obtained from 4.1R deficient individuals using a combination of differential proteomic profiling and biochemical analysis of the red cell PS-exposure pathways. Interestingly GPC has a interaction with p55 and p4.1R which has been well established. We have used proteomic profiling, use of CD47 ligands to explore PS exposure pathways, protein immunoblotting and flow cytometry on 4.1R deficient red cells in these studies. Our findings have produced significant differences compared with that of normal control age-matched erythrocytes. We show that 4.1R deficient RBCs lack, or are significantly deficient in several membrane and membrane skeletal components, and give novel insight into the complexes that 4.1R maintains in the mature RBC. This may suggest it plays a key role in eryptosis, especially pertinent as it is known to have binding sites for PS. Protein immunoblotting showed absence of protein 4.1R and protein p55 confirming earlier studies. Glycophorin C was also diminished and Glycophorin A appeared equal as previously reported. However, previously unreported results included a deficiency of CD44, alterations in CD47 expression, lack of GPC dimerisation, and deficiency of aldolase A. Further we showed that PS exposure is increased in the CD47 pathway (when challenged with CD47 ligands

BRIC 126 and 4N1K) in cells lacking protein 4.1R compared with control cells, but decreased in the GPC pathway (when challenged with BRIC 10) in cells lacking protein 4.1R compared with control cells. 4.1R (Madrid) cells were also shown to be resistant to TSP-1 mediated eryptosis. This difference in PS exposure suggests an important regulatory role for 4.1R in both of these apoptotic pathways, and may suggest that CD47-induced PS exposure occurs independently of binding to 4.1R/p55. Studies are ongoing with red cells obtained from a further 4.1R deficient individual ($4.1R^{-/-}$ Lille) and initial analysis is confirms the alterations seen in the $4.R^{-/-}$ Madrid sample.

Zoe Plummer contributed equally to this work

0916

INHIBITION OF INCREASED SICKLE NEUTROPHIL ADHESION TO ICAM-1 BY NITRIC OXIDE DONORS AND ACTIVATION OF CGMP SIGNALING

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Background. Leukocytes play an important role in sickle cell disease (SCD); increased numbers of leukocytes are associated with increased morbidity and mortality in SCD and leukocytes participate significantly in the vaso-occlusive process. Nitric oxide (NO) has recently been implicated as important in SCD pathophysiology. NO plasma bioavailability is thought to be decreased in SCD, and NO therapy has been proposed for the treatment of SCD and vaso-occlusive crises. *Aims*. Since NO is an important inflammatory mediator and may be important for the inhibition of leukocyte adhesion and migration mechanisms, we compared the adhesive properties of neutrophils from control subjects and from SCD patients (SCD neutrophils)and looked at the effect of NO donating agents on this adhesion. Nitric oxide metabolites and cGMP levels (second messenger for NO) were also measured in these neutrophils. *Methods*. Neutrophils were isolated from whole blood by separating on a Ficoll gradient. Cell adhesion to recombinant ICAM-1 was compared utilizing static adhesion assays. NO metabolites/cGMP levels were measured in neutrophil extracts using specific assays. *Results.* Neutrophils from SCD patients demonstrated a significantly greater adhesion to ICAM-1-coated plates (10 μg/mL) than control neutrophils (19.51±9.02%, n=13; 10.98±3.95%, respectively n=9; p=0.025). Co-incubation of SCD neutrophils with the nitric oxide donors, sodium nitroprusside (SNP, 10 μM) and DEANO (1 μM) reduced their increased adhesion to ICAM-1 to levels similar to those of control neutrophils (19.12±3.00% reduced to 16.49±2.60, n=8; p=0.009 for SNP) and (19.62±2.50%, reduced to 10.17±0.986%, n=4; p= 0.002 for DEANO). In contrast, SNP and DEANO did not significantly affect normal neutrophil adhesion to ICAM-1 (12.33±0.25% to 11.95±0.62%, n= 5; $p< 0.05$) and (13.21±1.26% to 10.89±1.91%, n=3; $p<0.05$), respectively. Interestingly, co-incubation of SCD neutrophils with a guanylate cyclase activator, BAY41-2272 (150 nm), also decreased their adhesion to ICAM-1 (19.62±2.50% reduced to 14.99±1.88%, p=0.01; n=4) but not control neutrophil adhesion (13.21±1.26% reduced to 11.59±1.74%, $p> 0.05$; n=3). Levels of nitric oxide metabolites in SCD neutrophils did not differ significantly from those in normal neutrophils (17.34±7.02%, n=10; 16.30±7.00%, n=8; respectively). Furthermore, levels of cGMP (0.11±0.02 pMol/1x10^7 neutrophils, n=9) in SCD neutrophils were not significantly different to those observed in normal neutrophils (0.142±0.036 pMol/1x10^7 neutrophils, n=7; $p<0.05$). *Conclusions*. Thus, although NO bioavailability may be decreased in the plasma of SCD patients due to NO sequestration by cell-free hemoglobin, our data indicate that NO dynamics are not significantly altered in the leukocytes of SCD patients, as indicated by the fact that levels of NO metabolites and the major second messenger for NO, cGMP, are not significantly altered in SCD neutrophils. We speculate that increased neutrophil adhesive properties in SCD may not be the consequence of decreased NO bioavailability, rather, other factors such as altered cytokine levels may mediate increased cell adhesion. However, whilst increased adhesion appears not to be mediated by NO-dependent mechanisms, NO donors were able to reduce increased SCD neutrophil adhesion to ICAM-1, possibly via a cGMP-dependent pathway, indicating that NO and NO donating drugs may also benefit SCD patients by reducing increased leukocyte adhesion, a mechanism important for the vaso-occlusive process.

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0917

USEFULNESS OF THE EOSIN-5-MALEIMIDE CYTOMETRIC METHOD AS A FIRST-LINE SCREENING TEST FOR THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS: COMPARISON WITH EKTACYTOMETRY AND PROTEIN ELECTROPHORESIS

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Aims. To compare the flow cytometry (FC) based on the fluorescence of red blood cells after incubation with eosin-5-maleimide, with ektacytometry (EC) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in the diagnosis of hereditary spherocytosis (HS). *Methods*. Eighty patients with a suspicion of HS were studied by FC and EC, and 61 by SDS-PAGE. Other haemolytic conditions were also studied using FC [autoimmune haemolytic anaemia (n=20); Haemoglobin disorders (n=34)]. In addition, hospitalized newborns with (n=20) or without anaemia (n=9), healthy adults donors (n=20) were analysed. FC analyses were repeatedly performed on red cells stored at 4° C before labelling in 30 subjects. The results of the test were expressed as percentage of the fluorescence reduction compared to the mean fluorescence of the six normal controls. *Results*. According to the EC results, 52 patients were diagnosed as having HS and 13 as non HS. Fifteen results were considered as normal. SDS-PAGE was performed in 61 patients: there were 16 deficiencies of band 3, 6 of ankyrin, 2 of α spectrin, 1 of α spectrin, 1 of protein 4.1. The diagnosis of CDA II was highly suspected in 6 patients and confirmed secondary. In 30 patients no protein defect was noted, whereas the EC concluded HS for 23 of them. Agreement between the EC and FC methods was observed in 88% (46/52). In the 23 HS patients with no protein defect, a fluorescence reduction was noticed in 19 (83%). Surprisingly, the reduction of fluorescence intensity was higher in the ankyrin deficiency group than in the band 3 deficiency group. No false positive test was noted in other haemolytic anaemia, either in haemoglobin disorders or in autoimmune haemolytic anaemia. Analysis of the storage conditions revealed that performing the FC on red blood cells stored at 4° C in whole blood for up to 6 days was associated with a slight decrease in the fluorescence either in HS patients, or in other haemolytic anaemia, or in healthy donors: however, it did not affect the final results and, particularly in non HS haemolytic anaemia, no false positives were observed. *Conclusions*. Very good agreement was noted in our study between the FC and EC methods in the diagnosis of HS, and to a lesser degree, with SDS-PAGE since 88% of HS patients, based on abnormal EC results, had a decrease in fluorescence. The FC is a reliable and useful method in the diagnosis of HS as a first-line screening, particularly in patients with normal SDS-PAGE. It can be performed on very small quantities of blood (10 μL), on red cells stored for up to 6 days at 4°C, is a rapid method (<2 hours). In a few cases, it must be completed by other more specialized methods, such as EC or SDS-PAGE.

Non-Hodgkin's lymphoma - Biology

0918

JUNCTIONAL ADHESION MOLECULE C DIFFERENTIATES CD27 POSITIVE B LYMPHOCYTES INTO GERMINAL CENTER AND NON-GERMINAL CENTER CELLS AND CONSTITUTES A NEW DIAGNOSTIC MARKER FOR B CELL MALIGNANCIES

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Differentiation of naïve B cells into plasma cells or memory cells occurs in the germinal centers (GC) of lymph follicles or alternatively via a GC- and T cell independent pathway. It is currently assumed that B cell lymphomas correlate to normal B cell differentiation stages, but the precise correlation of several B cell lymphomas to these two pathways remains controversial. We describe the junctional adhesion molecule C (JAM-C), currently identified at the cell-cell border of endothelial cells, as a new B cell marker with a tightly regulated expression during B cell differentiation. Using RT-PCR as well as western blot analysis on sorted B cells, we first showed that the JAM-C molecule is specifically expressed on B cells. Subsequently, we produced a polyclonal antibody directed against JAM-C and we showed that JAM-C expression is regulated during B cell maturation: immature $CD10^+$ B cell in the bone marrow do not express JAM-C; naïve, mature, peripheral blood B cells express it weakly; $CD27^+$ memory B cells strongly; and bone marrow plasma cells are again JAM-C negative. Of particular interest, JAM-C expression divides $CD27^+$ tonsillar B cells into two subpopulations: JAM-Cneg cells, with a phenotype of germinal center B cells and a high expression of BCL-6, a nuclear proto-oncogene with a pivotal role in GC-formation, and JAM-Cpos cells, corresponding to non-germinal B cells, derived partly from the marginal zone. *In vitro* cultures of the different tonsillar B cell subpopulations ($CD27^+$Jam-C^+, $CD27^+$JAM-Cneg, CD27negJAM-C^+) confirmed these results, since Ig-secretion measured after 7 days of culture, was minimal in naïve CD27neg cells, $CD27^+$Jam-C GC cells produced mainly IgG, and non-GC JAM-C^+CD27^+ B cells mainly IgM. Simultaneous analysis of JAM-C and CD27 expression in peripheral blood lymphocytes (PBLs) from a series of 50 patients with various lymphoproliferative syndromes allowed a clear classification into two types of B cell malignancies: JAM-Cneg lymphomas (CLL, 20/20 patients tested; follicular lymphoma 5/5 patients tested; mantle cell lymphoma, 5/7 patients tested), and JAM-C expressing lymphomas (marginal zone B cell lymphoma (MZBL), 9/12 patients tested; hairy cell leukemia, 6/6 patients tested). Whether the three cases of JAM-Cneg marginal zone lymphomas correspond to *bona fide* MZBL, to a new subgroup of MZBL, or rather to misdiagnosed atypical CLL cases, will be discussed. In conclusion, we therefore suggest that JAM-C constitutes a new diagnostic marker for the characterisation of lymphoproliferative B cell syndromes, and in particular for the positive diagnosis of lymphomas derived from the marginal zone, e.g. marginal zone B cell lymphoma and hairy cell leukemia.

0919

ROLE OF JUNB IN ALCL LYMPHOMA DEVELOPMENT

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Background. Anaplastic large-cell lymphoma (ALCL), a highly malignant form of Non-Hodgkin's lymphoma, is often associated with the generation of the fusion protein NPM-ALK, involving the genes ALK (Anaplastic Lymphoma Kinase) and NPM (Nucleophosmin). Phenotypically, NPM-ALK positive ALCL is characterized by high expression of CD 30 and of the AP-1 transcription factor family member JunB. Mostly, JunB is viewed as an antioncogene, while the AP-1 family member cJun is a protooncogene. In ALCL, though, JunB seems to act as protooncogene. NPM-ALK is autophosphorylated, functioning as docking station for major signalling pathways (MAPK, JAK/Stat, PI3-K). *Aims*. In this study we investigate patho-physiological mechanisms underlying tumor formation by the NPM-ALK fusion protein and to improve diagnosis and treatment of ALCL patients. JunB could serve as diagnostic parameter for diagnosis of ALCL lymphomas. JunB or one of its target genes could also be established as a possible therapeutic target. *Methods*. We used both *in vivo* and *in vitro* models to define the role of JunB in lymphomagenesis. As *in vivo* model we crossed transgenic mice harboring the human NPM-ALK fusionprotein expressed under the CD-4 promoter with CD-4 Cre JunBf/f mice, thereby inducing a conditional T-cell JunB knockout (cre/lox system). As *in vitro* models we used different NPM-ALK positive or negative human ALCL cell lines. *Results*. AP-1 activity and composition of NPM-ALK positive and negative cell lines was compared by EMSA and supershift analysis. Our results show that AP-1 DNA-Binding activity is NPM-ALK dependent and that the Ap-1 complex in NPM-ALK positive cells mainly consists of JunB. Loss of JunB in T-cells of NPM-ALK transgenic mice does not result in a survival advantage, however, significantly reduced proliferation and apoptosis rates were found *in vivo*. The mRNA expression of JunB target genes p53 and BCL-X were markedly reduced, the expression of c-Kit and FosB increased. Stat3 protein expression, which was shown to be crucial in ALCL tumor formation, was increased. *Summary*. NPM-ALK positive ALCL is a highly malignant form of Non-Hodgkin's lymphoma. We showed in NPM-ALK positive ALCL cell lines that AP-1 complex, consisting largely of JunB is increased. Conditional T-cell specific loss of JunB results in reduced proliferation and apoptosis rates and in a shift in oncogene expression patterns. However, deletion of JunB in the late T-cell development stage does not result in an altered survival rate.

0920

THE ONCOPROTEIN NPM-ALK INDUCES TRANSLATION OF AP-1 FACTORS VIA MTOR SIGNALLING

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Background. The nucleophosmin- anaplastic lymphoma kinase fusion protein (NPM-ALK) is the product of the balanced chromosomal rearrangement t(2;5)(p23;q35) and occurs in about half of nodal anaplastic large cell lymphoma (ALCL). ALCLs are highly proliferating tumors and usually express AP-1 transcription factors which are implicated to play a crucial role in oncogenesis. *Results*. 1) Aberrant NPM-ALK expression induces activation of PI3Kinase/mTOR pathway and AP-1 activity in Ba/F3 cells. 2) mTOR activity is observed in 9/10 NPM-ALK positive compared to 1/15 NPM-ALK negative ALCL patient samples. 3) Inhibition of PI3K by LY294002 or of mTOR by rapamycin in NPM-ALK positive cells decreases proliferation and down-regulates protein levels of the AP-1 factors c-Jun, JunB, and Fra-2. 4) mRNA levels of c-Jun, JunB, and Fra-2 are not affected by PI3K/mTOR inhibition. 5) mRNAs of AP-1 factors are translated from large polysomes but are shifted to monosomes and ribo-nucleic particles (RNPs) upon PI3K/mTOR inhibition and serum starvation. 6) We found a highly conserved region within the untranslated region of AP-1 mRNA which is involved in translational regulation. *Conclusions*. Our findings reveal that AP-1 factors are a critical target of mTOR and are translationally deregulated in NPM-ALK positive lymphomas. This is the first study to demonstrate translational control of AP-1 transcription factors in human neoplasia.

0921

ANALYSIS OF MICRORNAS EXPRESSION PATTERN IN CLASSICAL HODGKIN LYMPHOMA

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Background. Mature microRNAs (miRNAs) are small non-coding RNA molecules of 21-25 nucleotides that act as negative regulators of translation of mRNA to protein. There is evidence that miRNAs play an important role in carcinogenesis and they can act as oncogene and tumour supressor gene. Is Known that miRNAs expression can define a tumour better than the mRNA expression pattern. *Aims*. The objective of this study was to analyze the miRNAs expression patterns in lymph nodes from patients with classical Hodgkin lymphoma (cHL) in comparison to reactive lymph nodes. Moreover, we investigated the miRNAs pattern of cHL depending on Epstein-Barr Virus (EBV) presence. *Methods*. We analyzed 157 mature miRNAs by Real time PCR in ABI PRISM 7500 in 49 lymph nodes from patients diagnosed with cHL (37 Nodular

sclerosis[NS] and 12 Mixed cellularity[MC]) and in 10 reactive lymph nodes (RLN). Patients median age was 32 years (range, 15-80), clinical stage was I-II (n=26); III-IV (n=23) and 25 were EBV+. RNA was obtained from formalin fixed paraffin embedded tissues. Data were analyzed by using BRB Array Tools and TIGR Multiexperiment Viewer. *Results*. Hierarchical clustering analysis categorized three well defined groups corresponding to NS, MC and RLN. We detect a distinctive miRNAs signature of cHL samples that was compound by a set of 25 miRNAs including miR-21, mir-134 and mir-138, which permits to differentiate between cHL samples and RLN. We applied PAM algorithm to an independent set of 24 samples and using the microRNAs signature of 25 miRNAs the samples were classificated in cHL or RLN correctly in all cases. We also found a specific set of 36 miRNAs differentially expressed between nodular sclerosis and mixed cellularity subtype. With respect to EBV presence, we found that miR-96, 128a, 128b, 129 and 205 were underexpressed in EBV+ cases, whereas miR-28, 130b, 132, 140 and 330 were overexpressed. We also found that miR-138 was overexpressed in clinical stages I-II vs. III-IV (p=0.004), and that miR-328 was overexpressed in stages III-IV (p=0.003). We analyze mir-21 and mir-138 by *in situ* hybridization and found that it were expressed by the tumoral cells. *Summary/Conclusions*. Classical HL have a specific miRNAs signature that can play a role in its pathogenesis and there are differences between NS or MC subtypes. The presence of EBV affect the miRNAs pattern.

0922

FREQUENT CRYPTIC ALTERATIONS DETECTED BY SNP-CHIPS IN BURKITT LYMPHOMAS

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Background. Burkitt lymphomas/leukemias (BL), highly aggressive B-cell lymphomas, are characterized by MYC locus rearrangement. Additional chromosomal abnormalities have been described; the most frequent are partial gain of 1q (+1q), 7q (+7q) and partial 13q deletion. We recently demonstrated in a conventional cytogenetic study of childhood mature B-cell lymphomas the independent negative prognostic impact of del(13q) and +7q. *Aims*. To characterize these prognostic additional chromosomal abnormalities and to look for cryptic genomic alterations in order to reveal candidate genes and/or cellular pathways involved in Burkitt lymphomagenesis. *Methods*. DNA from 36 BL (20 adults/16 children) was analyzed by 50K Xba SNP arrays (Affymetrix), a genome-wide study of single nucleotide polymorphisms (SNPs). This technique allows the detection of allelic imbalances and losses of heterozygosity (LOH). *Results*. The most frequent additional chromosomal abnormalities were refined: partial or total gain of 1q and 7q. Recurrent 13q amplification (minimal amplified region of 3.1Mb) was detected in 7 patients followed by a terminal deletion in 4 patients. Different candidate genes are under study. Many cryptic imbalances have been detected by this technique like 2p16.1 amplification containing 2 oncogenes (REL and BCL11a) in 7 patients or 9p21.3 deletion including the tumor suppressor gene (TSG) CDKN2A in 4 patients. 144 acquired partial uniparental disomies (pUPD) (defined as region of at least 50 SNPs) were found by SNP-arrays (4/patient). pUPD are characterized by loss of heterozygosity without chromosomal deletion and probably result from mitotic recombination(s). The physiopathogenic role of pUPD remains unclear. Firstly, it can be polymorphisms. Secondly, random pUPD can reflect genomic instability especially in complex karyotypes. We show a similar incidence of pUPD in BL with or without complex karyotype. Thirdly, non random pUPD may have a pathogenic effect, rendering the cell homozygous for a preexisting mutation leading to activation of an oncogene or inactivation of a TSG. Some regions seem to be non-random in the most probable pUPD of our series: 17p and 17q (n=4), 1p, 9p and 10q (n=3). We are now sequencing candidate genes (CDKN2A, TP53…) in these non random pUPD to unveil mutations of oncogenes or TSG. *Conclusions*. The SNP arrays allowed us to characterize additional prognostic chromosomal abnormalities in BL and to detect many cryptic non random alterations. Acquired pUPD seem to be localized in non random regions and can be involved in BL pathogenesis in cooperation with MYC deregulation.

Cytogenetics, molecular diagnostics and targeted therapies

0923

HIGH RESPONSE RATE AFTER SEQUENTIAL ADMINISTRATION OF AZACITIDINE, VALPROIC ACID, AND ATRA IN PREVIOUSLY UNTREATED OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH-RISK MYELODYSPLASTIC SYNDROME

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Background. Intensive chemotherapy is associated with very poor results in older patients with AML or high-risk MDS. Epigenetic modulation combining demethylating agent and HDAC inhibitor seems to be a promising alternative (Garcia-Manero Blood 2006, Soriano ASH 2006). We report here preliminary results of a pilot study using azacitine and valproic acid (VPA) followed by ATRA in this patient population. *Patients and Methods*. Patients with AML or high-risk MDS aged 60 years or more and considered as unfit for conventional chemotherapy were enrolled. Each treatment cycle comprised azacitidine (75 mg/m^2/d) and VPA (50 mg/kg/d) for seven days (D1-D7), followed by ATRA (45 mg/m^2/d) from D8 to D28, every 28 days. A total of 6 cycles was planned. Peripheral blood and marrow evaluation was performed after cycle 1, 3, and 6. AML responses were classified as CR, CRi, PR, NEL (no evidence of leukemia), stable disease with or without haematological improvement (HI), or progressive disease while MDS responses were classified as CR, PR, HI, or progressive disease, according to AML and MDS IWG criteria. Disease progression was assessed only after at least 3 cycles. *Results*. Between March 2006 and January 2007, 20 patients were treated (median age, 74 years; 12 AML and 8 MDS). AML patients had either severe comorbidities (n=6) or -7/complex karyotype (n=6). All MDS patients had high-risk IPSS Score including 3 patients with -7/3q abn/complex karyotype At the time of analysis, 17 patients were evaluable with a median cycle number of 5. Overall haematological response including HI was 65%. In AML patients, response rate was 66% (8/12) with 6 CR, 1 PR and 1 NEL. In MDS patients, response rate was 60% (3/5) including 1 CR, 1 PR, and 1 HI (HI-E + HI-P). Overall CR + PR rate was thus 53%. Only one death was observed during therapy (1 sepsis after the first cycle). Neurological Grade 3/4 toxicities were observed in 2 patients (transient encephalopathy attributed to VPA by both investigators and independent safety committee). Others side effects were pain at injection site and mild GI events, as described with azacitidine alone. Despite these toxicities, most patients could be treated as out-patients. *Conclusions*. Combined therapy with azacitidine and VPA followed by ATRA seems to be a relatively safe and very promising approach in older patients with high-risk myeloid disorders. Response rate (53% CR + PR) compares favorably enough with intensive chemotherapy to envision future prospective up-front comparison.

0924

TARGETING ICMT AMELIORATES K-RAS-INDUCED MYELOPROLIFERATIVE DISEASE AND LUNG CANCER

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Background. Hyperactive signaling through the RAS proteins is involved in the pathogenesis of many forms of cancer. The RAS proteins and many other intracellular signaling proteins are methylated at a farnesylated or geranylgeranylated cysteine residue at the carboxyl terminus by isoprenylcysteine carboxyl methyltransferase (ICMT). We previously showed that inactivation of Icmt results in mislocalization of the RAS proteins and blocks oncogenic RAS transformation of mouse fibroblasts suggesting that ICMT may be an attractive therapeutic target. However, nothing is yet known about the impact of inhibiting ICMT on the development of malignancies *in vivo*. *Aim*. To test the hypothesis that inactivation of Icmt would inhibit the development or progression of a K-RAS-induced myeloproliferative disease in mice. *Methods*. We used Cre-loxP techniques to simultaneously activate the expression of endogenous oncogenic K-RAS and inactivate the expression of Icmt in bone marrow cells. In this way, we could determine if the absence of Icmt would have an impact on the development of K-RAS-induced

malignancies *in vivo*. *Results*. Inactivation of Icmt reduced splenomegaly (Figure 1 C), the amount of immature myeloid cells in peripheral blood (Figure 1 B), and tissue infiltration. Moreover, in the absence of Icmt there was a dramatic reduction in the ability of K-RAS-expressing hematopoietic cells to form colonies in methylcellulose in the absence of exogenous growth factors (Figure 1D). Interestingly, the absence of Icmt had no impact on the proliferation and differentiation of normal, non-malignant hematopoietic cells. Finally, inactivation of Icmt reduced lung tumor development and myeloproliferation phenotypes in a second mouse model of K-RAS-induced cancer (Figure 1E-G). *Conclusion*. We conclude that inhibition of Icmt significantly ameliorates phenotypes of K-RAS-induced malignancies *in vivo* and that ICMT may be an attractive therapeutic target.

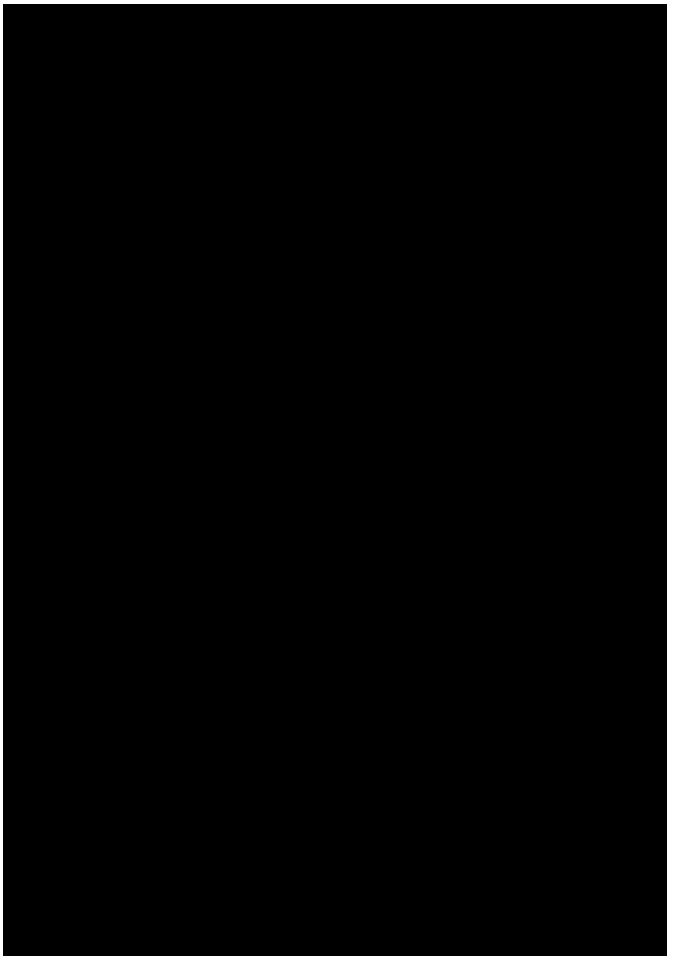

Figure 1. Knockout of Icmt and K-RAS-induced malignancies. Bergo et al.

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PROGNOSTIC IMPACT OF CHROMOSOMAL ABNORMALITIES IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA TREATED WITH HIGH-DOSE MELPHALAN (MEL140) AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Although chromosomal aberrations (CA) have emerged as important outcome predictors in multiple myeloma (MM), the prognostic significance of many recurring genomic changes is still unknown. Moreover, there is only scarce data on the implication of chromosomal abnormalities in elderly patients (pts.) receiving high-dose chemotherapy (HD-CTX) followed by autologous stem cell transplantation (ASCT). *Aims*. To evaluate the prognostic relevance of recurring chromosomal aberrations as detected by cIg-FISH for elderly pts. with MM receiving HD-CTX and ASCT. *Materials*. Between 05/2001 and 08/2006, 549 pts. 60-70 yrs. of age with newly diagnosed symptomatic MM entered the DSMM II trial of the Deutsche Studiengruppe Multiples Myelom to receive two cycles of HD-CTX (melphalan 140 mg/sqm) followed by ASCT after 3-4 cycles of dexamethason-based induction chemotherapy (IC; arm A1) or no IC (arm A2). cIg-FISH and DNA probes mapping to chromosome bands 1q21.2, 9q34, 11q25, 13q14, 14q32, 17p13, and 22q11 were applied to all pts. from whom sufficient bone marrow specimen were obtained. 110 pts. with a median age of 64 yrs. for that information on all genomic loci was available were included in the present analysis. The clinical database was last updated in 01/2006. *Results*. The median follow-up of time in the present series was 68 (0-238) weeks. Univariate analysis showed significantly shorter EFS in 11 out of 110 pts. (10%) with chromosome 17p13 deletion (17p'; 58 vs. 93 weeks, p=0.011) and 16 out of 110 pts. (14.5%) with translocation t(4;14) (78 vs. 93 weeks, p=0.027). Clinical and laboratory variables as well as all other CA had no significant impact on EFS. Most likely due to the short follow-up time, overall survival was not influenced by any parameter. *Conclusions*. 17p' and t(4;14), but not 13q' or +1q were associated with a significantly shorter EFS in elderly pts. receiving HD-CTX and ASCT. The next interim analysis of the DSMM II trial will take place in 04/2007 and molecular cytogenetic analysis of further cases is ongoing. This data (including multivariable analysis) will be presented at the meeting.

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0926

A NEW APPROACH TO MRD MONITORING IN AML, REAL-TIME QUANTIFICATION OF MULTIPLE AML-UPREGULATED GENES

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MRD monitoring in AML plays an important role in assessing the effectiveness of treatment and identifying patients at high risk of relapse, which enable intervention to prevent its onset. Several chromosomal translocations have been identified in AML, but are only detected in approximately 30% of patients. It is therefore vital to identify other type of MRD markers, such as those that are either have a restricted expression to or significantly upregulated in leukaemic cells, an example is WT1 gene, which is overexpressed at levels exceeding 104 copies in approximately 60% of AML patients. The main criterion of such markers is that they should be upregulatde by at least 3 logs, to enable accurate and sensitive protocols for MRD monitoring capable of detecting early increases in MRD level and therefore enable clinical intervention. We have examined a number of genes, using the above criterion (WT1, PRAME and CA9, XAGE1, CCL23, CLL1, ST18, RHAMM, and SPAG6). Using real-time RT-PCR (RQ-PCR) protocols for the quantification of these transcripts, we examined their levels in 214 samples from 103 AML patients at different phases of the disease (160 BM and 54 PB) and 15 normal BM and 7 normal PB samples. Presentation samples were (60 BM, 21 PB), post induction chemotherapy (27 BM, 6 PB), stable remission (42 BM, 12 PB), poor remission/before the onset of relapse (18 BM, 10 PB), and at haematological relapse (13 BM, 5 PB). Of the above genes, only WT1, PRAME, CA9, XAGE1, ST18 and SPAG6 conformed to the above criterion, and as such could be suitable as MRD markers in AML. These markers were able to offer accurate MRD monitoring for all 103 patients. Of the presentation samples from 60 patients WT1, PRAME, CA9, XAGE1, ST18, SPAG6 were shown as suitable for monitoring MRD in 43, 34, 12, 18, 21, and 18 patients respectively. The combination of WT1, PRAME with any third marker is sufficient to provide MRD monitoring for all 60 patients examined at presentation. However, the combination of all these 6 markers provided multiple markers for each of 52/60 patients. The levels of all transcripts decreased significantly in remission samples. Relapse samples showed levels corresponding to those detected at diagnosis, while patients examined 2-3 months before the onset of relapse showed a significant increase in the levels of the chosen markers, compared to those detected at remission. In 5 patients a single marker (WT1, PRAME, or XAGE1) did not show a significant increase before the onset of haematological relapse. However, in these patients, the second and third marker showed the significant increase in their levels to indicate imminent relapse. This finding shows the shortcoming of having a single AML-upregulated transcript, such as WT1, for MRD monitoring, as compared to having multiple AML-upregulated transcripts as MRD markers. These data show the value of this alternative approach to MRD monitoring in AML. These genes, taken together, were able to offer accurate MRD monitoring for all patients examined and were able to distinguish patients at high risk of relapse up to 3 months before its onset. This approach could simplify MRD monitoring with a choice of few markers only.

0927

MK-0457, A NOVEL MULTIKINASE INHIBITOR, IS ACTIVE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AND ACUTE LYMPHOCYTIC LEUKEMIA (ALL) WITH THE T315I BCR-ABL RESISTANCE MUTATION AND PATIENTS WITH REFRACTORY JAK-2 POSITIVE MYELOPROLIFERATIVE DISEASES (MPD)

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Background. MK-0457 (VX-680) is a small molecule inhibitor of aurora kinases A, B, and C, FLT3, and JAK-2 with nanomolar level broad spectrum pre-clinical anti-tumor activity. The T315I BCR-ABL mutation mediates high level resistance to imatinib, dasatinib and nilotinib. MK-0457 has *in vitro* activity against cells expressing wild-type or mutated BCR-ABL, including the T315I BCR-ABL mutation. *Aims*. The aim of this study was to determine the tolerability of MK-0457 in patients with refractory hematological malignancies. *Methods*. After IRB approval, fifty-one consenting patients with either refractory AML, CML, ph+ ALL or MPD were enrolled onto the study. The study regimen is a 5-day continuous IV regimen given every 2 to 3 weeks. To date, dose levels of 8 (N=4), 12 (N=5), 16 (N=3), 20 (N=6), 24 (N=15), 28 (N=4), 32 (N=3) and 40 (N=11) mg/m^2/hr have been investigated. *Results*. MK-0457 is a well tolerated agent in the treatment of hematological malignancies. At the 40 mg/m^2/hr dose level, grades 2 and 3 asymptomatic lipase elevations were observed and in one instance, it was determined to be a DLT. Additional toxicities possibly related to MK-0457 include grade 1 amylase elevation, nausea, alopecia, myalgias, althralgias, and cough, and grades 1 and 2 mucositis. Dose-proportional PK has been observed across dose levels. Myelosuppression is consistently seen with MK-0457 at all dose levels studied to date and appears to be dose-related. Of the 51 patients on part 1 of the study to date, 16 had CML, 5 had ALL, 21 had AML and 9 had rapidly progressive or transforming MPD. A nested PCR strategy followed by direct DNA sequencing using the dideoxy chain termination method was used to detect and monitor mutations in codons 221 to 500 of the BCR-ABL kinase domain in CML and ALL patients. Of 15 evaluable patients with CML, 9 had a T315I BCR-ABL mutation as did 3 Ph + ALL patients. Of 14 currently evaluable patients with CML, 11 had an objective (hematologic, cytogenetic, and/or molecular) response, including all 9 patients with the T315I mutation. Two patients with T315I-mutant ALL had an objective response, one achieving a complete hematologic and cytogenetic response as well as resolution of 2 out of 3 mutations present prior to treatment on study. Six of 8 currently evaluable patients with JAK-2 positive refractory MPD have achieved an objective response. Downregulation of BCR-ABL and CrkL phosphorylation in leukemia cells from CML and ALL patients treated on study has been documented - the possible role of aurora kinase inhibition in these clinical responses requires further investigation. Accrual to the study is ongoing at the 40 mg/m^2/hr dose level as well as utilizing both single 24-hour and single 6-hour infusions to sequentially determine the maximum-tolerated dose of MK-0457 using shorter infusion durations. *Conclusions*. The currently reported cases are the first observed clinical activity of a kinase inhibitor against the T315I positive CML/ALL and JAK-2 positive MPD. The observation of responses in these patients to doses of MK-0457 associated with no significant toxicity warrants further study.

Epigenetics, transcription and signalling

0928

TRANSCRIPTIONAL REGULATION OF HEMO-OXIGANASE-1 AND A SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE (SPARC) IN BORTEZOMIB-TREATED ADULT T-CELL LEUKEMIA CELLS

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Background and Aims. Adult T-cell leukemia (ATL) is a fatal neoplasia derived from HTLV-1 infected T lymphocytes frequently exhibiting nuclear factor-kappaB (NF-κB) activation. Despite the development of various treatment regimens, the prognosis of ATL is poor, and new treatment strategies need to be determined. The aim of this study is to investigate the effect and the molecular mechanism of a proteasome inhibitor, bortezomib, in ATL cells. *Methods.* An IL-2 dependent ATL cell line, TaY, two IL-2 independent ATL cell lines, MT-2, and MT-4, and an HTLV-1 negative T cell line, Jurkat, were used. After obtaining informed consent, peripheral blood mononuclear cells (PBMCs) isolated from a patient with ATL were also used. We explored gene expression profiling as well as gene network-based analysis using a pathway-focused oligonucleotide assay (GPL 3837). Quantitative RT-PCR and Western blotting was done to confirm the microarray results. *Results.* We found bortezomib-induced cell death in ATL cell lines with decreased activity of NF-κB. Differential gene expression analysis of an ATL cell line, TaY, revealed up-regulation of oxygen-dependent gene, hemo-oxigenase-1(HMOX-1) which is known as a target gene of hypoxia-inducible gene -1 α (HIF-1 α). Induction of HMOX-1 by cobalt protoporphyrin (CoPP) increased apoptosis of TaY cells in a pharmacologically effective dose of bortezomib, while CoPP did not affect the cell growth in the absence of bortezomib. Gene network analysis by a Bayesian statistical framework extracted a secreted protein acidic and rich in cysteine (SPARC), a tumor-invasiveness related gene. Inhibition of SPARC by siRNA enhanced the apoptotic effect of bortezomib on TaY cells. *Conclusions.* Targeting SPRC may be challenging to treat ATL patients exhibiting extra-nodal lesions including skin invasion. The enhanced sensitivity of TaY cells to bortezomib by CoPP is also intriguing and suggests that HMOX-1 may be an attractive target for treating ATL patients. Applying network-based analysis in addition to gene expression profiling may be new option to provide a novel insight into proteasome inhibitor, bortezomib in ATL.

0929

LACK OF MHC-II MOLECULE EXPRESSION IN T-LEUKAEMIA IS ASSOCIATED WITH DISTINCT EPIGENETIC DNA AND HISTONE MODIFICATIONS AT PROMOTER III CHROMATIN OF MHC2TA

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Previously we have shown that, unlike normal peripheral T cells, *in vitro* stimulation of T-leukaemia cell lines with well-known T-cell activation agents did not result in the induction of class II transactivator (CIITA) and MHC-II molecule expression. The co-activator CIITA, encoded by the MHC2TA gene, is essential for transcriptional activation of all MHC-II genes. Because other T-cell activation markers like IFNγ, IL-4, CD69, and CD45RO were readily induced in the stimulated T-leukaemia cells, we eliminated the possibility that general T-cell activation pathways were corrupted. Additionally, we showed in a transient promoter-reporter assay that promoter III of MHC2TA (CIITA-PIII), which is the principal T-cell employed MHC2TA promoter, was readily activated in CIITA-deficient T-leukaemia cells to levels similar as observed in MHC-II expressing T-lymphoma cells. These observations reveal that all essential transcription factors for CIITA-PIII activation are expressed in the leukaemia T-cells. However, *in vivo* genomic footprint analysis revealed lack of transcription factor binding to CIITA-PIII and hyper-methylation at CpG of CIITA-PIII in the CIITA-deficient T-leukaemia cells. Subsequent inhibition of DNA methyltransferase activity with 5AZA-2'-deoxycytidine resulted in re-expression of the CIITA-PIII isoform in these leukaemia T-cells. Moreover, we also found hyper-methylation of CIITA-PIII in HLA-DR-deficient primary leukaemia T-cells. Therefore, the defect in CIITA expression in leukaemia T-cells correlates with hypermethylation of promoter DNA, which blocks factor assembly on CIITA-PIII resulting in impairment of its activation. Since epigenetic DNA and histone modifications work in concert in promoting accessibility of the transcriptional machinery to regulatory elements of genes, we have extended our research towards the epi-

genetic mechanisms involved in the silencing of MHC2TA transcription in leukaemia T-cells also to histone modifications at the CIITA-PIII region. Using chromatin immunoprecipitation (ChIP) assays we found that the level of acetylated histone H3 in CIITA-PIII chromatin in T-leukaemia was strongly reduced when compared with CIITA-expressing T-lymphoma cells. The opposite was noted for the triple-methylated lysine 27 modification in histone H3. This modification is associated with compact chromatin and transcriptional silent genes. Subsequently, we also established by ChIP that the enhancer of zeste homolog 2 (EZH2), which has intrinsic histone methyltransferase activity, is recruited into CIITA-PIII chromatin in T-leukemia cells. Together our data reveal that, in addition to DNA methylation modifications, histone methylation modifications are associated with transcriptional silencing of MHC2TA in T-leukaemia and provide a link with components of the polycomb group family of proteins.

0930

UNCOVERING THE EPIGENETIC PATHOMECHANISM IN 13Q14

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Introduction. Deletions in chromosomal band 13q14.3 distal to RB1 occur in a variety of human neoplasms like B-cell chronic lymphocytic leukaemia (CLL), indicating a tumor suppressor mechanism in this region. Intriguingly, several characteristics of the region of interest point to an epigenetic pathomechanism: i) candidate genes lack point mutations, yet ii) these genes are downregulated in tumors, iii) the presence of large non-coding RNA genes in 13q14.3 is reminiscent of imprinted regions where only one gene copy is active. The data we show here led us to propose a novel oncogenic mechanism where already in healthy tissue only one gene copy is active while one gene copy is randomly chosen for silencing. Loss of the single active copy is then sufficient for complete loss of gene function in tumor cells. Currently we are trying to identify the (epi-)genetic element that controls the whole locus. *Aims*. Identification of the epigenetic regulatory mechanism localized in 13q14.3. *Methods and Results*. We performed FISH analyses of hematopoietic and non-hematopoietic cell lines to assess replication timing and chromatin packaging of the critical region. In line with an imprinting mechanism, we find that the two copies of the critical region replicate asynchronously and/or show delayed chromatid segregation, suggesting differential chromatin packaging of the two copies of 13q14.3. Next, we found by sequencing of SNPs that 13q14.3 candidate genes are expressed from one copy only in healthy probands. However, expression originated from either the maternal or paternal copy, excluding an imprinting mechanism. In order to identify the regulatory element, we performed DNA methylation analyses and could show that one of the CpG islands of the region is methylated. We could also show a functional interconnection of DNA methylation and gene expression, as demethylating agents and histone hyperacetylation induced biallelic expression. However, replication timing was not affected. Currently we are employing array- and capillary electrophoresis-based analysis of DNA-methylation (aPRIMES and bio-COBRA) and chromatin-immunoprecipitation on arrayed CpG-libraries (chIP on chip) with antibodies specific for histone modifications in order to identify the epigenetic element regulating the critical region. *Conclusions*. We propose that differential replication timing represents an early epigenetic mark that distinguishes the two copies of 13q14.3, resulting in differential chromatin packaging and monoallelic expression. This has profound effects for the tumor suppressor mechanism localized in 13q14.3: Deletion of the single active copy of the region at 13q14.3, which is detected in more than 50% of CLL tumors, will suffice for complete loss of tumor suppressor function, as the remaining gene copies are epigentically silenced. In addition, we are currently identifying the locus control region that orchestrates gene expression in the critical region. Thus, we provide a model for the pathomechanism of 13q14.3 in CLL by the interaction of genetic lesions and epigenetic silencing.

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β-TRCP MEDIATES UBIQUITINATION AND DEGRADATION OF THE ERYTHROPOIETIN RECEPTOR AND CONTROLS CELL PROLIFERATION

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Control of intensity and duration of erythropoietin (Epo) signalling is necessary to tightly regulate red blood cells production. After Epo stimulation of erythroid cells, 2 types of signal are transduced via the Epo receptor (Epo-R): positive signals involved in survival and proliferation, and negative signals involved in signal arrest. We have recently shown that the ubiquitin/ proteasome system plays a major role in the control of Epo-R signalling duration and desensitisation processes. Indeed, after Epo stimulation the Epo-R is ubiquitinated and its intracellular part is degraded by the proteasome, preventing further signal transduction. The remaining part of the receptor, together with associated Epo is internalised and degraded by the lysosomes (Walrafen *et al.* 2005 Blood, 105, 600-608). Our aim was to identify the E3 ubiquitin ligase involved in Epo-R ubiquitination. The Epo-R contains a putative β-Trcp binding site in its intracellular domain. Interestingly, this putative binding sequence is located in a region of the Epo-R that is deleted in erythroid progenitors from patients with familial polycythemia. We show that β-Trcp is responsible for Epo-R ubiquitination upon Epo stimulation. After Epo stimulation, β-Trcp binds to the Epo-R and this binding is dependent on Jak2 activation. Mutation of the Ser 462 residue of the Epo-R, located in the consensus β-Trcp binding site abolished β-Trcp binding, Epo-R ubiquitination and EpoR cleavage by the proteasome. Activation of the mutated Epo-R is prolonged in comparaison with Epo-R WT and BaF3 cells expressing this mutated receptor unable to bind β-Trcp are hypersensitive to Epo. Whether the removal of the β-Trcp binding site contributes to the hypersensitivity to Epo in familial polycythemia is currently under study.

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IDENTIFICATION OF PROTEIN TYROSINE KINASES THAT CAN SUPPORT THE PROLIFERATION AND SURVIVAL OF HEMATOPOIETIC CELLS

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Introduction. Protein tyrosine kinases are an important family of signaling proteins involved in the proliferation and survival of cells. They are frequently activated in leukemia through mutation or their involvement in chromosomal translocations often leading to fusion of kinase domains with homodimerization domains present in the partner genes. Examples of this are the BCR-ABL fusion in CML, the ETV6-PDGFRβ fusion in CMML, and the NPM-ALK fusion in ALCL. *Aims/Methods*. For many tyrosine kinases it remains unclear whether they can be activated by enforced homodimerization and whether these activated kinases would stimulate proliferation and survival pathways, leading to transformation of hematopoietic cells. To test this we generated fusions between the homodimerization domain of ETV6 and a variety of tyrosine kinase domains, and determined if expression of these constructs resulted in the transformation of Ba/F3 cells to IL3 independent growth. *Results*. As a first method, we performed a retroviral insertional mutagenesis screen for which we used a modified retroviral vector containing an exon encoding the homodimerization domain of ETV6 followed by an artificial splice donor site. When inserted in the host genome, this vector would drive the expression of a fusion transcript consisting of the exon encoding the homodimerization domain, and some exons of the gene in which the retrovirus was inserted. Using this screen, we obtained 423 independent Ba/F3 clones that were able to proliferate in the absence of IL3. 271 of these clones contained insertions in 8 different tyrosine kinase genes: Abl1, Fgfr1, Hck, Jak2, Lck, Mertk, Mst1r and Tnk1. In all these cases, the fusion between the homodimerization domain of ETV6 and the kinases were in-frame and the kinase domains were completely included in the generated fusion genes. In the other 152 Ba/F3 clones, no meaningful fusion transcripts could be detected. In a second method, we directly generated expression plasmids containing fusions between the homodimerization domain of ETV6 and different tyrosine kinase domains. So far, our results show that AXL, EPHA2, EPHB4, FGFR1, KIT, SRC, SYK, TYRO3, YES1 and ZAP70 all can be activated by homodimerization and support IL3-independent growth of Ba/F3 cells. When analyzing the activation of downstream signaling proteins such as PI3K, Akt, ERK, SRC kinases and STATs, significant differences were observed between these different oncogenic kinases. *Conclusions*. Our results identify a number of tyrosine kinase proteins that can be activated by homodimerization and lead to stimulation of proliferation and survival pathways in hematopoietic cells. Some of these tyrosine kinase genes are novel candidate oncogenes. The study of specific signaling pathways activated by these activated tyrosine kinases may be usefull for the design of novel therapies.

Publication Only

0933

CLINICAL FEATURES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION-ASSOCIATED ORGANIZING PNEUMONIA

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In this study, we describe the clinical course and outcomes of allogeneic hematopoietic stem cell transplantation-associated organizing pneumonia (HOP) observed in our institution over the past 20 years. Charts and chest radiographs of 603 allogeneic transplant recipients were retrospectively reviewed for HOP. Total 12 cases of HOP were observed (2.0%) at a median interval of 148 days after transplantation (range, 53-475 days). They presented low-grade fever, non-productive cough and dyspnea at the onset of HOP. The initial antibiotics treatment did not ameliorate these symptoms, but most patients well responded to 0.5-1 mg/kg of prednisolone. However, in 9 out of 12 patients, HOP flared-up after discontinuing treatment or while decreasing the doses, but responded to the retreatment with the initial dose of steroids. Although 3 patients died, there was no death due to pulmonary failure. In remaining 9 patients, there was no relapse of primary disease and 5-year survival was 74.1%. The clinical features of 12 patients were similar in that they all received an irradiation containing conditioning and most patients had a prior history of acute graft-versus-host disease (GVHD) and cytomegalovirus (CMV) infection. Furthermore, 8 patients had active chronic GVHD at the onset of HOP. These suggest that several factor such as irradiation containing regimen, previous CMV infection and allogeneic immune reaction may contribute to the occurrence of HOP. Moreover, the patients with HOP might enjoy a relatively good prognosis due to low rate of relapse of primary disease possibly through graft-versus-leukemia reaction, even though facing multiple episodes of disease exacerbation of HOP.

Table 1. Clinical features of HOP.

1	Day 105	+	+	-	-	88	68	71/68	N	M	No	No	+	-	-	0.28	98.6	87.8	ND	ND
2	Day 53	+	+	-	-	96	33	33/531	A+I	M	No	No	+	-	-	0.38	130.3	78.0	68.2	73.2
3	Day 173	+	+	+	-	91	14.3	35/541	A	F	No	Yes	+	-	+	0.06	133.9	72.1	85.7	88.7
4	Day 203	-	-	+	-	96	2.3	ND/ND	N	M	No	No	+	-	+	ND	109.2	79.3	81.7	67.3
5	Day 140	+	+	-	-	ND	2.2	ND/ND	A	F	No	Yes	-	-	+	ND	109.2	77.6	ND	ND
6	Day 259	-	+	-	-	97	1.9	ND/ND	N	M	No	No	-	-	+	0.14	103.9	88.4	94.2	93.7
7	Day 475	+	-	+	-	81	35.1	6/134	A+I	D	No	Yes	+	-	+	ND	122.0	83.5	ND	ND
8	Day 124	+	+	-	-	92	8.4	31/551	A+I	D	No	Yes	+	-	+	0.36	96.0	86.1	57.1	87.4
9	Day 156	-	-	+	-	96	8.0	6/372	A+I	M	No	No	-	+	+	0.51	118.9	83.9	107.0	86.5
10	Day 118	-	+	+	-	94	4.7	19/525	A+I	M	No	Yes	-	+	+	ND	120.7	83.9	ND	ND
11	Day 266	+	+	-	-	92	1.1	90/765	I	D	No	Yes	-	+	-	0.10	104.9	81.3	51.0	76.0
12	Day 122	+	+	-	-	91	14.4	ND/230	A+I	M	No	Yes	-	+	-	ND	89.5	87.6	83.1	93.9

0934

B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA-DERIVED DENDRITIC CELLS STIMULATE ALLOGENEIC T-CELL RESPONSE AND EXPRESS CHEMOKINES INVOLVED IN T-CELL MIGRATION

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Background. Despite discovery of new therapeutic agents, including nucleoside analogs and monoclonal antibodies, the B-cell chronic lymphocytic leukemia (B-CLL) remains incurable. In recent years, some effort has been made in developing T-cell specific immunity against neoplasmatic cells. Reconstitution of effective costimulation and immunological response of host T-cells against CLL cells could be a potential approach in immunotherapeutic trials. CD40/CD40L system is involved in the survival and proliferation of normal and neoplasmatic B-cells. Some preclinical studies have shown that CD40 stimulation can differentiate leukemic cells into dendritic cells (DCs) and result in host response. *Aims*. In this study, we sought to determine whether B-CLL cells could be turned into efficient and functional antigen presenting cells, as well as to assess the type of allogeneic T-cell response against B-CLL - derived DCs. *Methods*. B-CLL cells from 25 patients were stimulated or not with CD40L and IL-4 for 96 hours and then cultured in mixed lymphocyte reaction (MLR) with allogeneic T-cells. The expression of costimulatory and adhesion molecules at mRNA (real-time RT PCR) and protein level (flow cytometry) was assessed before and after the culture of B-CLL cells with or without CD40L/IL-4. The expression of activation molecules on the surface of T-cells before and after MLR was assessed with flow cytometry. The mRNA levels for chosen chemokines was determined by real-time RT-PCR. *Results*. 1) after CD40 stimulation B-CLL cells achieved phenotypical and functional characterization of DCs (i.e. upregulated co-stimulatory and adhesion molecules at mRNA and protein level) 2) leukemia-derived DCs expressed higher amount of mRNA for chemokines involved in T-cell migration (MDC, TARC and CCR7) 3) the proliferating response of T-cells against leukemia-derived DCs consisted of CD4 and CD8 cells (upregulation of HLA-DR and OX40). *Conclusion*. Our experiment confirm that B-CLL cells can be turned into dendritic-like cells, additionally, these cells express chemokines involved in T-cell migration and stimulate allogeneic response.

0935

TRANSCRIPTIONAL SILENCING AND FREQUENT ABERRANT DNA METHYLATION OF ADAMTS-1, AN ANTIANGIOGENIC FACTOR WITH DIRECT INHIBITORY EFFECTS ON LEUKEMIC CELL GROWTH IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Angiogenesis plays an increasingly important role in carcinogenesis and is a prominent feature in the bone marrow (BM) in childhood acute lymphoblastic leukemia (ALL). ADAMTS-1 (A Disintegrin And Metalloproteinase with ThromboSpondin-like motifs-1) is a potent endogenous angiogenic inhibitor and its expression is down-regulated in certain human solid cancers. *Aims*. To examine the involvement of ADAMTS-1 in the pathogenesis of childhood ALL by expression and DNA methylation studies. *Methods*. Semi-quantitative RT-PCR was used for examining ADAMTS-1 expression. Bisulfite DNA sequencing and combined bisulfite restriction analysis for determining ADAMTS-1 methylation. Nucleofection, annexin V and propidium iodide staining for studying the effects of exogenic ADAMTS-1 expression on apoptosis and cell cycle regulation. *Results*. We first demonstrated aberrant DNA methylation associated loss of expression of ADAMTS-1 in 2/3 B-lineage ALL cell lines (Reh and RS4:11) and in 4/7 BM samples from pediatric patients with B-lineage ALL, and reactivation of this gene expression by DNA demethylation in the Reh cells. We also first revealed aberrant DNA methylation of ADAMTS-1 in 31 out of 42 (74%) childhood patients with B-lineage ALL. Exogenic ADAMTS-1 expression significantly induced G2/M cell cycle arrest and apoptosis in the Reh leukemic cells. *Conclusions*. Our findings indicated that aberrant ADAMTS-1 DNA methylation associated transcriptional silencing of the gene might be involved in the leukemogenesis of childhood ALL. More importantly, in addition to the antiangiogenic effect, for the first time, we demonstrated the direct inhibitory effects of ADAMTS-1 on leukemic cell growth, which represents an endothelial cell-independent action of this molecule in cancer growth control. These findings may have critical impacts in molecular targeting strategy in the management of B-lineage childhood ALL.

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0936

THE DIFFERENCES OF PROTEIN EXPRESSION BETWEEN CML-CP AND CML-BC BY COMPARATIVE PROTEOME ANALYSIS

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Background. The clinical course of chronic myeloid leukemia (CML) is characteristically triphasic, comprising chronic and accelerated phases and blast crisis. Chronic phase (CP) is characterized by the Ph chromosome as the sole genetic abnormality and blast crisis (BC), which is the terminal phase of CML, often associated with additional chromosomal and molecular secondary changes. Although CML is probably the most extensively studied human malignancy, the mechanisms of CML blast crisis are still poorly understood. *Aims*. The aims of current study are to screen and identify CML-BC related proteins and explore the mechanism of CML-BC. *Methods*. The changes of protein expression between CML-CP (25 cases) and CML-BC (20 cases) were analyzed by Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), and the proteins were identified by peptide mass fingerprint in combination with database searching. *Results*. Compared with that of CML-CP, 33 proteins' intensities of CML-BC were found to have significant difference including 23 increasing intensity and 13 decreasing. 15 proteins were identified including proteasome activator complex, heterogeneous nuclear ribonucleoprotein, annexin A4, serine proteinase inhibitor, annexin A1, glyceradehyde-3-phosphate dehydrogenase, RhoGDI, enolase, proteasome subunit 6a, GTP binding protein, leukotriene A4 hydrolase, Rac-RhoGDI, thioredoxin, proteasome subunit 4, and DJ-1 protein, and the functions of these proteins involve cell signal transduction, apoptosis, proliferation and transcription. *Conclusions*. Our study provided a profile of protein expression difference between CML-CP and CML-BC and contributed to understand the mechanisms of CML blast crisis.

0937

FACTOR V LEIDEN AND G20210A PROTHROMBIN MUTATIONS IN PATIENT WITH RECURRENT PREGNANCY LOSS: DATA FROM SOUTHEAST OF TURKEY

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Background and Aim. The pathophysiology of pregnancy loss is complex and poorly understood. Historically, the causes of recurrent pregnancy loss (RPL) have been classified as genetic, infective, anatomical, endocrine, immune and idiopathic. However, even after detailed investigation, as many as 80% of all cases remain unexplained. Based on the histologic findings of extensive infarction and necrosis in the placentas of women with antiphospholipid syndrome, researchers postulate that utero-placental thrombosis may lead to placental infarction and eventual fetal death. Therefore, within the past 10 years interest in associations between thrombophilia and RPL has increased remarkably. Actually, thrombophilias have been included in the causes of RPL. In the recent years various studies have examined the incidence of genetic prothrombotic mutation in women with unexplained pregnancy loss. Some of these studies confirmed an association between thrombophilic gene mutations and recurrent miscarriage but other studies detected no association. We performed a case-control study because of the conflicting results of studies and in view of lack of data on Southeast of Turkey population. Moreover, we know that there may be major racial variation in gene polymorphisms. The aim of this case-control study was to investigate the prevalence of FV-Leiden and FII G20210A mutations, and their impact on the development of early recurrent miscarriage in patients with history of unexplained early fetal loss on Southeast of Turkey population. *Methods*. This case-control study was performed in 114 women out of 403 patients with a history of two or more consecutive spontaneous abortions of unexplained etiology during the first trimester. The control group consisted of 185 age-matched women without previous miscarriage or pregnancy complications. Factor V Leiden and FII G20210A were studied with LightCycler PCR. *Results*. In this series of 114 patients, 11 cases (9.6%) were found to have hereditary thrombophilia, and this prevalence was not higher than that found in the control group. In addition, in the group of patient with RPL we found higher prevalence of both genetic prothrombotic mutation in comparison with controls. However, these prevalences did not reach statistical significance in comparison with controls. *Conclusion*. The most common causes of hereditary thrombophilia (FV-Leiden and FII G20210A) was not found to be associated with first trimester recurrent pregnancy loss.

0938

RAPID INFUSION OF RITUXIMAB OVER 90-MINUTES FROM SECOND INFUSION ONWARDS ON AN OUT-PATIENT BASIS IS SAFE AND IMPROVES RESOURCE UTILIZATION

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Background. Administration of rituximab can be associated with substantial infusion-related toxicity, including allergic/hypersensitivity reactions and rarely a fatal cytokine release syndrome. These adverse reactions (AR) are more commonly seen with first infusion (risk of grade ≥3 is 7%) compared with subsequent infusions where the risk of grade ≥3 infusion reactions is 2%. To minimize the risk of AR, rituximab has been traditionally infused over prolonged period of time (average 5-6 h for first infusion and 3-4 h for subsequent infusions). With ever increasing indications for rituximab therapy, day therapy units are expected to accommodate more patients. *Aims*. The aim of this study is to assess the safety of rapid rituximab infusion (over 90 minutes) and to evaluate the impact of this strategy on resource utilization in day therapy units. *Methods*. This is a prospective, single institution study started in April 2005 for one year at the Ulster hospital, Northern Ireland. Eligibility criteria included patients with $CD20^+$ NHL and meet one of the current indications for rituximab therapy, age >16, all patients had received previous treatment with a first infusion of rituximab according to drug monograph and did not develop grades ≥3 infusion related syndrome or cytokine release syndrome, no bulky disease (disease mass <10 cm in diameter) and peripheral blood lymphocyte count $<10\times10^9$/L. Second and subsequent infusions were given over 90-minutes in a total volume of 500 mL; initially at a rate of 200 mL/hr (20% of the total; 100 mL) for the first 30 minutes and then, if no AR, at 400 ml/hr (the remaining 80%, 400 mL) over 1 hour. All patients received antihistamine, corticosteroids and acetaminophen 30 minutes prior to rituximab. All patients were monitored closely and any AR documented using the NCI Common Terminology Criteria for Adverse Events, December 2005. *Results*. 17 patients consented and have been enrolled for a total of 73 rapid infusions (average: 4 infusions per patient). Patient characteristics are as follows: median age when received rituximab 75 years (44-87 years); 59% male (n=10); 41% female (n=7), 71% stage III/IV (n=12). Twelve patients (71%) had diffuse large B-cell lymphoma (DLBCL), four patients (23%) had follicular lymphoma (FL) and one patient (6%) had transformed lymphoma (TNHL). The median total white cell count at the time of rituximab therapy was 6.9×10^9/L. Patients with DLBCL and TNHL were treated with CHOP or CHOP-like chemotherapy plus rituximab (n=13). Patients with FL received CVP chemotherapy plus rituximab (n=4). The 90-minute rituximab infusion schedule was extremely well tolerated with no AR observed. Introduction of this shortened infusion schedule has enabled us to cut rituximab infusion times in half, allowing more patients to be conveniently treated. It also enabled us to administer chemotherapy and rituximab in one day. *Conclusions*. Rapid (90-minute) rituximab infusion schedule in combination with a steroid containing chemotherapy regimen is well tolerated and safe when administered from the second infusion onward. This shortened infusion schedule has resulted in a substantial reduction in resource utilization and allowed us to accommodate more patients for treatment.

0939

INVESTIGATION OF ANTI-LEUKEMIC ROLE OF ACUTE GVHD AFTER UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR INTERMEDIATE- TO HIGH-RISK ACUTE MYELOGENOUS LEUKEMIA

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Background. Allogeneic hematopoietic stem cell transplantation (HSCT), using siblings or alternative donors, is currently the best therapy for high-risk acute myelogenous leukemia (AML). Although the results with transplants from HLA-matched unrelated donors on the basis of high-resolution typing have been reached to those of HLA-identical sibling transplants, graft-versus-host disease (GvHD) has remained a still going problem in patients after either related or unrelated donor HSCT, together with the serial incidence of relapse in each setting. *Aims*. A role of acute or chronic GvHD, based on the concept of graft-versus-leukemia effect in patients transplanted for intermediate- to high-risk AML in complete remission or incomplete remission, after unrelated donor HSCT has not been revealed much, particularly in association with acute GvHD. *Methods*. We present the results of the 74 unrelated

HSCT from the available Asian as well as Caucasian donors for AML. Overall, 56 patients received unrelated donor bone marrow cells, and 18 patients received G-CSF mobilized peripheral blood stem cells. *Results*. The median age of enrolled patients and donors was 36 (16-53) and 38 (20-51) years, respectively. The median follow-up duration was 24 months (range, 6-61). The majority of patients had intermediate (N=45) or unfavourable (N=29) cytogenetic features. The main conditioning regimen consisted in cyclophosphamide plus TBI with our standard GvHD prophylaxis containing tacrolimus plus short course methotrexate. Instead, some of patients (N=19) received additional 2-day course ATG (thymoglobulin, Sangstat) in addition to the standard regimen. All transplanted patients were engrafted. The incidence of acute GvHD was 52%, with grade I (21%), grade II (20%), grade III (10%), and grade IV (1%). Chronic GvHD developed in 52% of evaluable patients, and 17% had extensive disease. Eight (11%) patients were relapsed so far. The 2-year non-relapse TRM was 16%. In order to compare the results of DFS, EFS by the development of GvHD, we compared the survival curves according to the presence or absence of acute or chronic GvHD. Thus, in contrast to improved overall survival results, notably improved DFS was noted when acute GvHD was developed (p=0.0444), but there were no statistical significance for EFS in this study. The comparison of overall estimated probability of DFS and EFS at 5-year were 84%, 67%, respectively. *Conclusions*. These results showed that multinational mismatched unrelated donors for Korean AML patients were available to be performed as possible. Our data revealed that development of acute GvHD after unrelated donor HSCT for AML patients was closely related to better long-term DFS and EFS.

0940

GENOTYPE SPECIFICITIES OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS IN HLA-MATCHED SIBLING DONOR-RECIPIENT PAIRS

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Background. NK cell alloreactions against AML cells were first actively investigated in a clinical setting of allogeneic HLA-mismatched hematopoietic stem cell transplantation (HSCT). More recently, the novel concept of infusing NK cells to promote and consolidate engraftment after haploidentical HSCT has been introduced. The inherent heterogeneity of the multigene killer cell immunoglobulin-like receptor complexes (KIRs) may also be a key factor for the outcome of HLA-matched sibling HSCT. Therefore, it is expected that other researchers will also examine the role of ethnic differences in the KIR genotype in association with NK alloreactivity. *Aims*. There is limited information on the influence of donor-derived NK cells on various outcomes after HLA-matched sibling allogeneic HSCT. *Methods*. We investigated the KIRs, based on the genotypes of inhibitory or activating KIRs in 76 consecutive pairs of stem cell recipients with AML, and their HLA-matched sibling donors. All donor'recipient pairs were typed for the presence or absence of specific KIRs genes. Evaluation for 19 different KIR genes and pseudogenes was performed using the Pel-Freez kit, according to the manufacturer's protocol. PCR data representing KIR genotypes from the recipients and donors were compared. *Results*. Genotyping performed prospectively was perfectly matched in 38% of pairs. Unlike to Caucasians, the single 2DL3 allele without 2DL2 was the predominant pattern for the Korean C1 allotype, either in donors or in recipients. 2DS2 (19% vs 11%, p=0.044) and 2DS4 (75% vs 68%, p=0.044) were at a higher frequency activating KIRs genes in the recipient group. Analysis of KIRs gene numbers revealed that the recipient group usually had two to three more genes than donors. *Conclusions*. Taken together, these factors may be helpful to understand an immunogenetic specificity in different races in association with a specific disease AML.

0941

GENOTYPES OF INHIBITORY AND ACTIVATING KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS IN HLA-MATCHED SIBLING DONOR-RECIPIENT PAIRS ARE IMPORTANT DETERMINANTS OF ACUTE GRAFT-VERSUS-HOST DISEASE IN HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKEMIA

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Background. Natural killer (NK) cell alloreactivity is regulated by killer cell immunoglobulin-like receptors (KIR). Several studies have demonstrated variable expression of KIRs in allogeneic hematopoietic stem cell transplantation (HSCT) donor'recipient pairs. *Aims*. The influence of KIR genes, in stem cell recipients with acute myelogenous leukemia and their HLA-matched sibling donors, on acute graft-versus host disease (aGvHD) after HSCT was investigated. *Methods*. We studied 53 donor'recipient pairs to determine the impact of both donor and recipient KIR genotypes, and their bidirectional KIR interactions. Evaluation for 19 different KIR genes and pseudogenes was performed. PCR data representing KIR genotypes from the recipients and donors were compared. Various clinical factors associated with development of aGvHD in the univariate analyses were used in the multivariate Cox proportional hazards regression analysis. *Results*. All activating KIR genes in donors were important factors for determining outcome in a manner distinctive for each gene studied. Specifically, the 2DS2 gene and the 2DS4*003 allele were closely correlated with aGvHD. The 2DS1 gene was associated with a better long-term survival, even if present only in the donor and not the recipient. The 2DS3-2DS5 dual genes were more often involved in a variety of transplant-related complications. Lastly, when we compared the specific association of the donor 2DS2-recipient 2DL2 genes with acute and chronic GvHD, presence or absence of these genes showed important correlations. *Conclusions*. As a pilot integrative NK-KIR immunogenetic study in association with a variety of clinical outcomes, even in the setting of sibling allogeneic HSCT in patients with AML; our findings suggest that the genotypes of the inhibitory/activating NK-KIR in donor cells, together with the interaction of the recipient NK-KIR characteristics, may be, at least in part, critical for understanding immunogenetic specificity. Further understanding of this process may allow us to better predict transplant outcome, and aid in identifying the best available donor in a specific transplant setting.

0942

PHASE II STUDY OF YTTRIUM-90 IBRITUMOMAB TIUXETAN (ZEVALIN) FOR PATIENTS WITH UNTREATED STAGE I-II FOLLICULAR OR MARGINAL ZONE LYMPHOMA

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Background. Yttrium-90 (90Y) ibritumomab tiuxetan (Zevalin) is an effective treatment for patients with relapsed follicular lymphoma. There is no standard treatment for previously untreated stage I - II indolent lymphoma. *Methods*. Patients with untreated CD20 positive lymphomas including follicular lymphoma grade 1 - 2, and marginal zone B-cell lymphoma of the mucosal (MALT) type were included in the study. Staging included computed tomography (CT) of neck, thorax, abdomen and pelvis, PET-CT and bone marrow biopsy. Eligibility criteria were performance status of 2 or less, white blood count greater than 1500/mL, platelet greater than 100,000/mL, and at least one lesion measuring 1.5 cm in transverse dimension. Response was assessed 3 months after infusion. Response evaluation of bowel disease required repeat biopsy of involved tissues after completing therapy. Patients were treated with Zevalin, (0.3-0.4 mCi 90Y/kg according to initial platelet count, caped at 32 mCi). *Results*. Nine patients have been enrolled with a median age of 60 years (range 37-71). Five are male, and 7 have follicular histology. With a median follow-up of 5 months (range 3 to 9), seven patients have more than 3 months of follow up and are evaluable for response. Of these, 6 (85%) have achieved a complete remission and one has stable disease. Two of two patients with less than 3 months follow up have already achieved a partial response and may continue to respond. Patients experienced a nadir median platelet count of 50,000 (range 20,000-170,000) and a medium neutrophil count of 1,323 (range 560-1,566) at four weeks from the infusion of Zevalin. Nonhematologic toxicity included a rash associated with rituximab/90Y ibritumomab tiuxetan administration. *Conclusions*. This early preliminary data with Yttrium-90 ibritumomab tiuxetan is encouraging; however long term follow-up will determine the clinical utility of this simple convenient treatment.

0943

BONE MARROW TRANSPLANTATION FOR ALL

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Introduction. Multiple myeloma (MM) is a clonal disorder of hematopoietic stem cell. Treatment is based on supportive care, single or combination chemotherapy and autologus or allogenic stem cell transplantation (SCT). We are doing in-patient SCT in Iran since 1991 but this is the first out-patient SCT. *Material and Method*. all patients who were candidate for SCT received out-patient or in-patient SCT according to protocols. In out-patient group, patient were discharged and followed by out-

patient SCT team include a general physician, staff nurse and care giver during their neutropenic period. All data collected and analyzed by SPSS software. *Results*. 60 patients received in-patient or out-patient autologus SCT. In the in-patient group20, 20 patients received 200 mg/m^2 and 140 mg/m^2 melphalan respectively as conditioning regimens. In out-patient group 20 patients received 140mg/m^2 melphalan. Median ages were 50±7.5 and patients were in complete (85%) or partial (15%) remission. Median hospital stay were 28 days (19-54) and 6.5 days (1-8) in in-patient and out-patient groups respectively. Median home visit by team were 10.5 days. There were not significant difference (p<0/1) between these groups in apheresis days, GCSF requirement for mobilization, number of mononuclear cell (MNC) or CD34$^+$ cell .parameters. There were significant difference (p<0/001) between two groups in blood product consumption parental antibiotic uses, overall survival and relapse rate during follow up period. There were also significant decrease in total cost of SCT in out-patient group by 70% (p<017 including visit cost 80% (p<0/001), drug cost 50% (p<0/002), laboratory cost 70% (p<0/02) and hospital cost 70% (p<0/04). *Discussion*. Results show that out-patient autolgous SCT in multiple myeloma patient is feasible and its complication is manageable. Significant reduction in cost and bed requirement also occurs.

0944

HALOFUGINONE INDUCES APOPTOSIS MEDIATED BY FOXO3A IN MANTLE CELL LYMPHOMA CELL LINE

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Background. Halofuginone (HF), a low molecular weight plant alkaloid, inhibits collagen α1 gene expression, and also has been shown to inhibits angiogenesis and tumor progression, mediated by transforming growth factor-β (TGF-β) inhibition. We previously demonstrated that mantle cell lymphoma (MCL) aberrantly over express the TGF-β pathway in comparison to normal naïve B cells. *Aims*. To evaluate the phenotypic and molecular changes caused by HF treatment in a MCL cell line and normal peripheral blood mononuclear cells (PBMC). *Methods*. Granta-519 cell line and PBMC were grown in 90% DMEN medium supplemented with 10% fetal bovine serum, glutamine, penicillin and streptomycin. Cells were kept in culture for 0 (control), 12, 24 and 48 hours with HF at different concentrations (10^{-8}M, 4×10^{-8}M and 1.6×10^{-7}M). Apoptosis was evaluated by flow cytometry (anexin V-FITC and propidium iodide), in whole cell population (cell line and PBMC), and in CD19$^+$ and CD3$^+$ PBMC cells. Gene expression profiling was performed with Amersham CodeLink UniSet Human I BioArrays, containing 10,000 probes for Granta cell line treated with 4×10^{-8}M, in all points of treatment (0, 12, 24 and 48 hours) in duplicate. Gene fold change was evaluated by comparing with cells without treatment. Real-time PCR with TaqMan probes was carried out for CCDN1, GAL3, IL21, IL12B, IL10, ITGB7, COL4A3, FOXO3A, SPARC, AKT1, CTNB1, GSK3B, TOSO, PTEN, TLR2, BCL2, CASP3, CASP9, NFKB1 and NFKB2, the results were expressed as 2e-DeltaDeltaCt using the median DeltaCt value of each untreated sample as a reference. *Results*. Low dosis (10e-8M) of HF induced apoptosis in 23% cells of the MCL cell line, in comparison to normal cells (11% for CD19$^+$; 7% for CD3$^+$). Apoptosis increased to 38% of MCL cells with a higher dosis of HF (1.6×10^{-7}M), in comparison with 6% for CD19$^+$ and CD3$^+$ normal cells. Microarray data revealed that 410 genes were altereted in at least one time of treatment [10x fold (positive or negative), p<0.001]. Real time PCR demonstrated that FOXO3A was significantly induced after 24 and 48 hours of treatment in the cell line (positive fold change of 40 and 57 times, respectively) compared to PBMC (mean change: 4 times, range: 1.6 - 6.6). Interstingly, HF was able to inhibts the cyclin D1 expression in MCL (negative fold change: 2 times), however, in PBMC CCDN1 was induced (mean positive fold: 6 times, range 1.4-10.5) in all points of treatment. Only high dosis of HF induced the expression of NFKB1 (fold change: 5-19 times) and NFκB2 (12-27 times) in both MCL and normal PBMC. *Conclusions*. Pathway analysis indicates that CCDN1 downregulation is probably mediated by FOXO3A over expression. Our results suggest that NFκB inhibition is dosis dependent, and that halofuginone, a well tolerate anticoccidial drug, induces a selective apoptosis in MCL cell line, strengthening its potential as a new therapeutical target for MCL.

0945

MEASUREMENT OF HUMAN CYTOMEGALOVIRUS (HCMV)LOADS BY QUANTITATIVE REAL-TIME PCR(RQ-PCR) FOR MONITORING CLINICAL INTERVENTION IN BONE MARROW TRANSPLANTATION (BMT)RECIPIENTS

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HCMV is well-known cause of morbidity and mortality in blood and BMT patients. Monitoring of its reaction and preemptive or prophylactic treatment using gancyclovir are critical for BMT recipients. We could detect HCMV DNA in this patients by RQ-PCR For monitoring of HCMV reaction. If copy number of HCMV was increased, preemptive therapy will be initiated. 51 recipients of BMT (9-51 years) were monitored as weekly intervals until day 100 after transplantation. For amplification of the pp65 gene (UL83) RQ-PCR assay and pp65 Antigenemia method were preformed in parallel with 415 samples. By cloning of this region, we made standards for RQ-PCR. The results obtained by the two techniques were significantly correlated (p<0.01). We could detected 10 copies of HCMV DNA/capillary. Detection limits of this method were 13×10^1-15×10^7copies/2×10^5 cells by RQ-PCR.76% of patients developed more than one episode of HCMV replication. First positive result of RQ-PCR 13 days earlier than first positive of pp65 Antigenemia. After preemptive therapy 16 days (7-21 days) needed to become negative result of RQ-PCR. There was no relationship between death and increase of HCMV copy(p<0.419). RQ-PCR was more sensitive than pp65 Antigenemia. After preemptive therapy, negative results of RQ-PCR were the best indicator for determining of successful treatment. Reaction of HCMV in our patients mostly endogenous and depend on kind of immunosuppressive therapy. If copy number of HCMV increased one log, HCMV reaction developed 1.22 fold.

0946

JAK2 MUTATIONS IN ESSENTIAL THROMBOCYTHAEMIA PATIENTS OF ASIAN ETHNICITY

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Background. JAK2 mutation is well established to occur in approximately half of Caucasian patients with essential thrombocythaemia (ET). A single point mutation (Val1617Phe) identified in JAK2 confers proliferative and survival advantages on haemopoietic precursors. ET patients with the mutated JAK2 gene had significantly longer disease duration, higher rate of complications and need for treatment with cytoreductive therapy than patients with wild type JAK2. Much of the literature on JAK2 in ET has centred on Caucasian patients with little study on Asian patients. *Aims*. To determine the incidence of the JAK2 gene mutation in ET patients of Asian descent and if the disease profile of JAK2 mutated Asian ET patients is different from that of Caucasian ET patients. *Methods*. A diagnosis of ET was made based on PVSG criteria or WHO criteria, depending on the date of diagnosis. Patient demographics and disease profile were captured in the Myeloproliferative Disease Registry and informed consent obtained. Patient genomic DNA was amplified by PCR and products were sequenced using the Genetic Analyser with primers to amplify coding exons 13 and 14 of the JAK2 gene. Patients were examined in 2 groups (JAK2 gene mutated and wild type) with respect to the disease profile and compared to that of Caucasian patients. *Results*. 80 patients of Asian ethnicity (81.3% Chinese, 13.5% Malay, 2.5% Indian, 2.5% of other Asian ancestry) were studied. JAK2 mutation was detected in 22/80 (27.6%); 21 (26.3%) were heterozygous and 1 (1.3%) homozygous for the mutation. JAK2 mutated patients had higher bone marrow cellularity (p=0.01). There was a trend towards increased age (p=0.067), higher leukocyte counts at diagnosis (p=0.055) and high risk disease (p=0.086). There was no difference in follow up duration, presenting platelet count, haemoglobin, haemocrit, use of cytoreductive therapy and risk of bleeding or thrombosis. *Conclusions*. Our series of JAK2 mutation in 80 Asian ET patients is the largest reported so far, with a Korean paper and a Chinese paper reporting on 26 and 68 patients respectively. The incidence of JAK2 mutation detected in this group of Asian patients (27.6%) is lower compared to the Caucasian population. Asian JAK2 mutated patients may have a different clinical profile to that of the Caucasian population as they do not have an increased risk of bleeding and thrombotic complications. They have increased marrow cellularity and may have increased age and higher leukocyte counts at presentation and high risk disease. We conclude

that the incidence of JAK2 mutation in Asian ET patients is lower than in Caucasian ET patients. Their disease profile may be similar to that of their wild-type counterparts and different from the Caucasian patients.

0947

THE PREPARATION OF MONOCLONAL IMMUNOGLOBULIN LOADED DENDRITIC CELLS VACCINE FOR MYELOMA PATIENTS UNDER GMP CONDITIONS: PRECLINICAL AND FIRST CLINICAL RESULTS OF A PHASE I/II CLINICAL TRIAL

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Background: Adjuvant immunotherapy with antigen-loaded dendritic cells (DCs) represents a novel and relatively non-toxic treatment modality for multiple myeloma (MM). Malignant cells in MM produce a monoclonal immunoglobulin (idiotypic protein) which is considered a tumor-specific antigen and can be used for the induction of T lymphocytes. To enhance the anti-myeloma immune response, the idiotypic protein (Id-protein; Idiotype) can be loaded into autologous DCs and used for vaccination. *Aims*. The aim of this study was preclinical evaluation of to evaluate DC-based vaccine and clinical demonstration of the safety and immune response of the vaccine in patients with MM. *Patients and Methods*. Pre-clinical testing was performed in 8 patients with MM. DC loaded with autologous myeloma cells were used for autologous T cell stimulation *in vitro*. After successful preclinical testing, we have vaccinated 4 patients with stable disease or asymptomatic slow progressive disease after prior stem cell transplant (SCT) according to EBMT criteria. DC precursors were isolated as an adherent fraction from peripheral blood of the myeloma patients. DCs were prepared *in vitro* and loaded with Id-protein under GMP conditions as previously described (Ocadlikova *et al.* Med Oncol 2006, 23: 377-384). Patients were vaccinated every 4 weeks subcutaneously with 6 doses, each containing 1,46-18,1×10^6 (mean 9,52×10^6) DCs. The immune response was evaluated by flow cytometry, Elispot and the skin test of hypersensitivity. *Results*. IFN-gamma production of T cells stimulated with autologous myeloma cell loaded DC was observed. After successful pre-clinical testing a clinical phase I/II trial was initiated. A total of 24 vaccines were applied to 4 patients so far (January 2007). The viability, number and functional characteristics of *in vitro* matured DCs loaded with Id-protein were satisfactory with 50,10-99,3% (mean 87,08%) of HLADR/CD86+ cells. Each vaccination was well tolerated with only mild fever in 1 patient. No grade II-IV toxicity appeared. The clinical trial is ongoing and a total of 12 patients is planned to be enrolled. *Conclusions*. Feasibility and safety of vaccination with Id-protein loaded autologous dendritic cells was proved in patients with multiple myeloma.

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0948

ISOLATION AND EXPANSION OF ALLOGENEIC MYELOMA-SPECIFIC T CELLS PRODUCING INTERFERON-GAMMA

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Background. Multiple myeloma (MM) is a malignant disease characterized by clonal proliferation of plasma cells in the bone marrow. High-dose chemotherapy with autologous stem cell transplantation is recently considered a standard therapy for myeloma. Despite of the high number of complete remissions achievement is the median relapse-free survival 3 years and median overall survival of 4 to 5 years. Adoptive immunotherapy is a promising approach in the treatment of multiple myeloma in the last 20 years. *Aims*. The aim of this study was to identify and characterize autologous myeloma-reactive T cells *in vitro* and to evaluate their cytotoxic effect. *Methods*. Irradiated myeloma cell line ARH 77 has been used as tumor antigen to stimulate allogeneic $CD4^+$ and CD8+ T lymphocytes. Peripheral blood mononuclear cells of 8 healthy volunteers and 10 MM patients were used for repeated stimulation of T lymphocytes. Activated myeloma-specific T cells that produced interferon-gamma (IFN-γ) were isolated using immunomagnetic separation (Miltenyi Biotech) and further expanded *in vitro* by phytohemaglutinin and high concentrations of interleukin 2. Cytotoxicity against the original myeloma cells has been tested after the T cell expansion with propidium iodide or 7-amino actinomycin D. Activated T cells were labeled by a 5-(6-) carboxyfluoresceine diacetate succinimidyl ester. Allogeneic T cells and IFN-γ negative fraction of T cells served as controls. *Results*. Myeloma-reactive IFN-γ positive T cells in healthy donors were enriched from 2.83% (1.97%; 4.58%) to 48.57% (15.14%; 82.98%) and from 1.91% (1.14%; 3.4%) to 73.14% (3.9%; 88.75%) (median, min., max.) for $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells after immunomagnetic separation. IFN-γ positive T cells were further expanded *in vitro* from 0.5×10^6 (0.5×10^6; 0.6×10^6) to 160×10^6 (150×10^6, 420×10^6) (median, min., max.) cells within 4 weeks. The percentage of killed ARH 77 myeloma cells was 69.17% (38.04%; 78.23%) (median, min., max.). Cytotoxicity against the third-party PBMC was negligible. IFN-γ negative fraction of T cells demonstrated also negligible cytotoxicity against ARH 77 myeloma cells. The percentage of myeloma-reactive IFN-γ positive cells in MM patients was enriched from 1.12% (0.27%; 6.2%) to 7.85% (0.42%; 12.6%) and from 1.9% (0.37%; 14.4%) to 14.7% (1.28%; 71.4%) (median, min., max.) for $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells after immunomagnetic separation. IFN-γ positive T cells were expanded *in vitro* from 0.12 ×10^6 (0.05 ×10^6; 0.4 ×10^6) to 88.5 ×10^6 (35 ×10^6; 226 ×10^6) (median, min., max.) within 8-12 weeks. The percentage of killed autologous multiple myeloma cells was 41,5% (15,6-60,5%) (median, min., max.). For the 2:1, 10:1 and 50:1 E:T ratios, the median killing of ARH 77 myeloma cells was 21.78%, 34.31% and 62.33% after 4 hours; 60.84%, 69.82%, and 73.49% after 24, and 57.75%, 71.85% and 59.69% after 48 hours, respectively. Third-party reactivity of myeloma-reactive T cells from patients with MM was negligible. *Conclusion*: This study demonstrates the feasibility of identification and isolation of myeloma-specific T cells in healthy donors as well as in patients with MM. Myeloma-specific T cells can be further expanded and used as adoptive immunotherapy.

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0949

ASSOCIATION OF ZAP-70 EXPRESSION WITH PLASMA LEVELS OF ANGIOGENIC ACTIVATORS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukaemia (CLL) is a disease with an extremely variable clinical course. Several studies have shown that angiogenesis is increased in CLL and may potentially serve as a new prognostic factor. Zeta-associated protein of 70 kilodaltons (ZAP-70) is an intracellular tyrosin kinase belonging to modern powerful prognostic markers in CLL with significant impact on clinical course. *Aims*. to assess potential relationship of ZAP-70 and angiogenic signaling in CLL. *Methods*. We analyzed ZAP-70 expression using flow cytometry in CLL cells from peripheral blood of 32 patients. Furthemore, we quantified plasma concentrations of angiogenic activators (vascular endothelial growth factor - VEGF, basic fibroblast growth factor - bFGF) in peripheral blood plasma of the same CLL patient group and 80 healthy donors. Commercially available sandwich ELISA kits (RD Systems) were used for bFGF and VEGF measurement. ZAP-70 expression was quantified using PE-conjugated ZAP-70 monoclonal antibody (Caltag); cut-off level of 20% positivity was used as recommended by literature. *Results*. Both angiogenic cytokines were significantly increased in CLL patients when compared to controls (bFGF, $p<0.0001$; VEGF, $p=0.0004$). Twenty patients were ZAP-70 negative and eleven ZAP-positive. Interestingly, bFGF and VEGF correlated inversely with percentage of ZAP-positive cells (bFGF, r=-0.43, $p=0.014$; VEGF, r=-0.39, $p=0.025$) and were significantly elevated in ZAP-negative versus positive patients (bFGF, $p=0.021$; VEGF, $p=0.035$). *Conclusions*. In the present study, angiogenic activators were elevated in CLL; furthemore, ZAP-70 expression correlated inversely with plasma levels of bFGF and VEGF. Our findings underline the importance of angiogenic signaling in CLL and point to possible association with ZAP-70 expression. Further investigation in terms of impact on clinical course and survival is clearly warranted. Supported by grant NR/8373-3 and research project MZO 00179906 from Ministry of Health of Czech Republic.

0950

HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT IN ADOLESCENT PATIENTS WITH RELAPSED OR REFRACTORY HODGKINS LYMPHOMA: CLINICAL OUT COME AND IMPACT OF PROGNOSTIC FACTORS IN 53 PATIENTS

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Background . High dose chemotherapy and autologous stem cell transplantation (HDC ASCT) is well-accepted therapy for most patients with hodgkin's lymphoma (HL) who have persistent disease or relapse after multi-agent chemotherapy / combination treatment. Literature is limited in adolescent patients in this setting. *Aims.* To review clinical outcome and prognostic factors for over all survival (OS) and event free survival (EFS) in adolescent patients with recurrent and/or primary refractory HL (PR-HL) after HDC ASCT. *Methods*. From 1996 to May 2006, 113 consecutive patients with HL had HDC ASCT, 53 of these were between the ages of 14-21 years. Impact of various prognostic factors (Table 1) prior to the initiation of salvage chemotherapy on EFS and OS was evaluated using multivariate regression analysis. Primary refractory HL (PR-HL) is defined as patients who failed induction chemotherapy i.e. only partial response (PR), no response , stable disease (SD), progressive disease (PD) or relapsing within 3 months. Patients with progressive disease on salvage chemotherapy were not eligible for HDC ASCT. BEAM was used as HDC. *Results*. 26 male (49%), 27 female (51%), Median age at diagnosis 15 years (6 to 20) and at ASCT 16.6 years (14 to 21). Prior to salvage chemotherapy, stages I:II:III:IV were 4:18:9:22, bulky disease (≥8 cm) in 15 (28%), involvement of mediastinum in 35 (66%), spleen in 10 (19%) and extranodal involvement in 23 (43%) patients. Relapsed disease in 23 (43%) and PR-HL in 30 (57%) in patient. 37 patients (70%) had tissue confirmation at relapse/progression. ESHAP as first line salvage in 47 patients (89%); median cycles administered were 3. Disease evaluation post ASCT showed overall response in 44 patients (83%); total CR / CRu 40 patients (75%), PR 6 (11%), NR/SD 2 (4%), PD 8 (15%) patients. 24 out of 39 patients (61%) with no CR prior to ASCT achieved CR after HDC ASCT. 13 (32%) out of 40 patients in CR post ASCT relapsed. Median followup: 53 months from diagnosis and 28 months from ASCT. EFS is 49%; 29 (55%) remain in CR, 8 (15%) are alive with disease and 16 (30%) died of disease. Prognostic factors for EFS and OS are shown in the Table 1, only B symptom at relapse was a significant negative factor for OS (p=0.031). EFS was 71% and 34% respectively in relapsed and refractory disease (p=0.049). *Conclusions*. In this group of adolescent patients with 57% PR-HL, ESHAP + BEAM combination resulted in 83% response rate with 75% CR rate. Despite this high responsiveness, almost quarter of these patients with CR have relapsed. At a median follow up of 28 months, EFS is only 49%. Negative prognostic factor was B symptom at relapse for OS (p= 0.031) and refractory disease for EFS (p=0049). Better treatment strategies are needed for this group of patients.

Table 1. Impact of various prognostic factors on EFS on OS.

	Event free survival	Overall survival
Prognostic Factors	P value, multivariate	P value, multivariate
B symptoms	.653	***.031*** *
Prior XRT	.887	.813
Relapsed vs PR-HL	***.049*** *	.440
Bulky disease	.320	.537
Mediastinal involvement	.319	.482
Spleen involvement	.248	.624
Extranodal involvement	.838	.694
HL IPI, good(0,1,2)vs bad (≥3)	.272	.890
Elevated LDH	.856	.923

* P value significan

0951

DELETERIOUS EFFECTS OF KIR LIGAND INCOMPATIBILITY ON CLINICAL OUTCOMES IN HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT IN VITRO T CELL DEPLETION

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Backgrounds. KIR ligand incompatibility in graft versus host direction is associated with natural killer (NK) cell alloreactivity. The effect of this incompatibility on outcomes of haploidentical or mismatched unrelated hematopoitic stem cell transplantation (HSCT) remains controversial. In recent years, we have successfully established a novel conditioning protocol that includes antithymocyte globulin followed by haploidentical hematopoietic stem cell transplantation without *in vitro* T cell depletion, and we have found that this protocol can achieve outcomes comparable to those obtained with HLA-matched transplantation. *Aims*. Because of inconsistent NK cell-mediated alloreactivity in haploidentical or mismatched unrelated HSCT, we analyzed the relationship of the KIR ligand mismatch with clinical outcomes in our cohort of 116 patients. *Methods*. This analysis included 116 recipients of haploidentical donor HSCT. Cases were divided into those with (n=30) and those without KIR ligand incompatibility (n=86), as described by Ruggeri *et al.*, based on known KIR ligands (HLA-C alleles with Asn77-Lys80; HLA-C alleles with Ser77-Asn80; and HLA-Bw4 alleles). *Results*. Multivariate analysis showed that both KIR ligand mismatch (HR 2.484, CI 1.241-4.973, p= 0.01) and *high* dose T cell categorization (>1.48×108/kg) (HR 4.099, CI 1.899-8.849, p= -0.0003) were independent risk factors for aGVHD. There was a higher cumulative incidence of aGVHD in patients with KIR ligand mismatch compared to those patients without KIR ligand mismatch (p=0.013), or in patients in the *high* T cell group compared to those in the *low* T cell group (p=0.001). Meanwhile, KIR ligand mismatch significantly increased the incidence of aGVHD in the *high* T cell group (p=0.039), but had little effect in the *low* T cell group (p=-0.213). We found a significant and striking difference in the cumulative incidence of aGVHD between the patients with KIR ligand mismatch in the *high* T cell group and those without KIR ligand mismatch in the *low* T cell group (p<0.00001). In HLA-C allele mismatch group, KIR ligand mismatch worsened the adverse effect of HLA-C allele mismatch on aGVHD (p= 0.059). Multivariate analysis demonstrated that both high-risk leukemia and KIR ligand mismatch were independent predictors for OS (HR 3.544, CI 1.396-8.995, p=0.008; and HR 2.230, CI 1.003-4.960, p= 0.049, respectively) and relapse (HR 8.080, CI 1.427-45.75, p= 0.018 and HR 4.771, CI 1.315-17.312, p=0.017, respectively). In the standard-risk group, KIR ligand mismatch increased aGVHD incidence (p=0.001), possibly eliciting a higher TRM (p= 0.011), and then resulted in poorer patient survival (p=0.030). In contrast, KIR ligand mismatch further worsened the high relapse rate in the high-risk group (p= 0.003). When analyzed separately, patients with KIR ligand mismatch had comparable TRM rates compared with their KIR ligand-compatible counterparts, but had a higher cumulative relapse rate and inferior OS rate in the AML (n=34) and ALL (n=40) groups (relapse: p=-0.007 for AML, and p= 0.003 for ALL) (OS: p= 0.040 for AML, and p=-0.044 for ALL; respectively). However, KIR ligand mismatch had little effect on other myeloid disease patients, including those with CML, MDS, or AMLL. *Conclusions*. These findings for haploidentical HSCT without *in vitro* T cell depletion show that KIR ligand mismatch is associated with higher aGVHD, a greater relapse rate, and inferior survival.

0952

FACTOR V G1691A AND PROTHROMBIN G20210A IN ANGIOGRAPHICALLY DOCUMENTED CORONARY ARTERY DISEASE PATIENTS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

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Background. The development of Type 2 Diabetes Mellitus (T2DM) increases the risk of coronary artery disease (CAD) and CAD is the most complication and major cause of mortality in T2DM. Epidemiological studies have indicated an association between T2DM and CAD. Factor V Leiden and prothrombin G20210A mutations are two frequent genetic risk factors involved in venous thromboembolism. The role of factor V 1691G:A (FV Leiden) and the prothrombin 20210G:A in arterial thrombotic disease are still controversial. *Aims*. The aim of present study was to investigate the role of factor V G1691A and prothrombin G20210A as risk factors for coronary artery disease (CAD) in patients with or

without type 2 diabetes. *Methods*. This case-control study consisted of 100 CAD patients (55 males and 45 females) with angiograhically documented coronary artery disease (at least 30% stenosis) which among them 65 were diabetic patients with CAD, aged 57.6±8.4 and 35 were CAD patients without type 2 diabetes, aged 53.3±9.1 and 59 (26 males and 33 females) unrelated controls with angiographically normal coronary and without diabetes, aged 54.3±3. All patients and controls were from the Kermanshah province of Iran. Genotyping was done by PCR-RFLP using Mnl I and Hind III for factor V Leiden and prothrombin G20210A, respectively. *Results*. Heterozygous factor V Leiden mutation was found in 3 out of 65 (4.6%) diabetic patients with CAD, 2 of 35 (5.7%) CAD patients without diabetes and 2 out of 59 (3.4%) control subjects (p=0.6). Only 2 diabetic patients with CAD (around 3%) were found to be heterozygous for both the 20210G and 20210A alleles of prothrombin gene. No homozygous carrier for these mutations was found. *Summary/conclusions*. The present results indicate that although the prevalence of FV Leiden and the prothrombin 20210G:A are increased in CAD patients compared to healthy individuals but these differences are not significant. Neither FV Leiden nor prothrombin G20210A were associated with type 2 diabetes mellitus or CAD.

0953

NEURAL CELL ADHESION MOLECULE (CD56) EXPRESSION ON PLASMA CELLS IN MONOCLONAL GAMMOPATHIES

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It was shown that absence of CD56 on malignant plasma cells (PCs) is hallmark of plasma cell leukemia (PCL), special subset of multiple myeloma (MM) (Leukemia 1998; 12: 1977) and may play role in pathogenesis of central nervous system MM (Br J Haematol 2005; 129: 539). There was also found that expression of CD56 correlates with presence of osteolysis in MM and distinguishes MM from MGUS and lymphomas with plasmacytoid differentiation (Am J Pathol 2002; 160: 1293). Aim of this study was to evaluate intensity of CD56 expression on bone marrow (BM) myelomatous PCs and to assess clinical correlations. The study group consisted of 204 MM patients (112M 92F, median age 63, range 32-89yr ;30 at stage I, 50-II, 124 -III; 153 had osteolysis; monoclonal protein IgG was in 128 patients, IgA-50, IgD-1, IgM-2, Bence Jones'20, NS-3) and 26 PCL 8 IgM lymphoplasmacytic lymphoma and 14 MGUS patients. Controls were 10 healthy subjects. Immunophenotyping was done on freshly collected BM samples using triple staining combination of CD138/CD56/CD38 monoclonal antibodies analysed by flow cytometry. Plasma cells were identified as cells showing high-density expression of CD38 and CD138 (syndecan-1). Antigen expression intensity was calculated as relative fluorescence intensity (RFI) and for direct quantitative analysis the QuantiBRITE test was applied. Mean channels of phycoerythrin fluorescence were defined and antibody bounding capacity (ABC) was then calculated using QuantiCALC software. *Results*. In 135 patients (66%) PCs showed CD56 expression. Out of all $CD38^{++}$/$CD138^{+}$ BM cells mean proportion of PCs with CD56 expression, was 82±20%, median 91%. RFI values ranged from 7,6 to 27,4 in particular patients (18,0±4,5, median 17,8) and the number of CD56 binding sites (ABC) on MM plasma cells ranged from 2255 to 58469 (14199±15038, median 8866). A correlation was found between RFI and ABC values (r=0,76; p<0,001)). In 69 MM patients considered as CD56 negative myeloma mean proportion of all BM $CD38^{++}$ cells with CD56 expression was 5,2±4,9%, median 4,0%. A correlation was found between proportion of all BM $CD38^{++}$ cells with CD56 expression and ABC (r=0,60) and RFI (r=0,62) indices (p<0,001). Normal PCs did not express CD56. Osteolytic lesions were found in 80% of $CD56^{+}$ MM patients and in 60% of patients with CD56 negative myeloma. When comparing other clinical and biological disease characteristics e.g. monoclonal protein isotype, β2M, LDH, stage of disease, calcium, creatinine, response to chemotherapy, survival time of CD56 positive and CD56 negative cases, no significant differences were found. CD56 expression on monoclonal PCs showed 14 of 26 PCL, 6 of 14 MGUS and 1 of 8 lymphoplasmacytic lymphoma cases. *Conclusions*. In two thirds of MM patients malignant PCs show CD56 expression. Intensity of CD56 expression on PCs varies among particular CD56 positive MM patients. There is relationship between proportion of BM CD56 positive PCs and density (ABC) and intensity (RFI) of expression of this molecule. In half of PCL and half of MGUS cases monoclonal PCs show CD56 expression.

0954

NF-κB ACTIVATOR INHIBITOR EFFECTIVELY INHIBITS CELLULAR GROWTH OF U266 MYELOMA CELL VIA BLOCKAGE OF IL-6 MEDIATED JAK-STAT CELL SIGNALING PATHWAY

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Background. Il-6 plays a pivotal role in the pathogenesis of multiple myeloma(MM), leading to the activation of JAK/STAT and MAPK pathway. However, IL-6-induced molecular pathways of downstream in these pathways. *Aims*. The role of NFκB in MM was examined in order to investigate the downstream pathway of JAK/STAT and MAPK pathway. *Methods*. Cellular proliferation was measured by [3H]thymidine incorporation. IL-6, sIL-6R, IL-8 levels were determined by ELISA. Expression of molecules was done by wetern blot, and transcriptional activity of NFκB was measured by luciferase assay. *Results*. When U266 MM cells were treated with IL-6, cell growth rate was increased via activation of JAK/STA signals. NFκB activation inhibitor was most effective on the blockage of JAK/STAT cell signaling among four different types of signaling inhibitors (AG490, JAK2 inhibitor II, JAK inhibitor I, and NFκB activation inhibitor). Expression of IL-6-induced p-STAT-1,-3, and p-Erk was dramatically inhibited by treatment of NFκB activation inhibitor. In addition, NFκB activation inhibitor effectively reduced the activation of transcription factor NFκB (p65 and p-IkB-a). Furthermore, NFκB protein bound to NFκB DNA binding site was dramatically diminished following treatment of NFκB activation inhibitor as evidenced by lufciferase assay. NFκB activator inhibitor suppressed the production of IL-6 and sIL-R, which affected on the proliferation of MM cells by autocrine fashion. *Summary/Conclusions*. Our data suggested that blockage of JAK/STAT mediated NFκB activation was useful to control the growth of MM cells.Consequently, inhibitor for inactivating IL-6 mediated NFκB cell signaling pathway may possibly be used as an anti-cancer drug in patients with multiple myeloma.

0955

NOVEL COMPLEX T(V;9;22) REARRANGEMENTS IN THREE CASES WITH CHRONIC MYELOID LEUKEMIA AND A RARE TRANSLOCATION IN A CASE WITH CLASSICAL PH-CHROMOSOME

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According to literature in 2-10% of the cases with chronic myeloid leukemia (CML) a fusion gene BCR/ABL arises in connection with complex translocation events. Such chromosomal rearrangements involving one or more additional chromosomes were described in over 600 cases with CML by now. Here we report three additional cases with complex aberrations never observed previously, i.e. t(1;22;9)(p32;q11;q34), t(2;9;22)(q11;q34;q11), t(6;9;22)(q11;q34;q11), plus one case with a rare translocation t(3;8)(p22;q22) along with classic Philadelphia (Ph) translocation; the cases were characterized in detail by molecular cytogenetic methods. All patients with complex aberrations were young (median age 26). The patient with atypical translocation was even an 8-year old girl. Cytogenetically prepared bone marrow samples were used for GTG-banding and FISH analysis and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995). In order to confirm the presence of the BCR/ABL fusion gene, LSI BCR/ABL ES (Abbott/Vysis) and M-BCR/ABL probes (Oncor/Q-BIOgene) were applied. In the case with a t(2;9;22) two cells clones with different variants of the rearrangement were found. M-FISH and MCB (Multicolor Chromosome Banding) revealed the exact breakpoints and the karyotypes were described as: Case 1 : 46,XY,t(1;22;9)(p32;q11;q34). Case 2 : 47,XY,t(6;9;22)(q11;q34;q11),+8,i(17)(q10) Case 3 : 46,XY, t(2;22)(q11;q11)[17]/46,XY,t(2;9;22)(q11;q34;q11q12)[16]. Case 4: 46,XX,t(3;8)(p22;q22),t(9;22)(q34;q11). According to the literature variant rearrangements of Ph chromosome do not confer any specific phenotypic or prognostic impact as compared to CML with a standard Ph chromosome. However, additional aberrations like trisomy 8 and isochromosome 17 have a poor prognosis. The detection of a breakpoint 3p22 was not described previously in CML-however, this region is known to be involved in solid tumor. In summary, our study demonstrates that appli-

cation of LSI-probes, M-FISH and MCB allows to comprehensively characterize complex chromosomal rearrangements that were not identified by banding cytogenetics alone. Supported in parts by the Ernst-Abbe-Stiftung, the INTAS (AISbl 03-51-4060), the IZKF Jena (Start-up S16), the DFG (436 ARM 17/2/04, 436 ARM 17/5/06), the UICC (ICR/05/030), the Stiftung Leukämie, and the IZKF together with the TMWFK (TP 3.7 and B307-04004).

0956

TWO INTRA-ARTERIAL TREATMENT PROTOCOLS FOR STEROID REFRACTORY GRAFT VS. HOST DISEASE OF THE LIVER

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Introduction. It has been previously shown by us, that intra-arterial targeted steroid therapy may be useful even in steroid refractory hepatic graft vs. host disease (GVHD). In the 1st protocol, an intermediate dose steroid with low dose methotrexate was used (protocol I). Due to suspected toxicity of methotrexate, it was omitted and substituted with high dose steroids (protocol II). *Patients and Methods*. 7 patients with steroid resistant/dependent GVHD were treated with protocol I and 13 with protocol II at time of analysis. Serum bilirubin level was followed for the evaluation of efficacy. *Results*. Median time from SCT to IA and from GVHD to IA tended to be longer in the patients treated with protocol II. No life threatening event was seen with protocol II. Four out of 7 and 10/12 patients responded to the treatment (protocol I and protocol II respectively Figure 1). There was a trend toward faster time to response in the patients treated with protocol II. one out of 7 and 7/12 patients are long term survivors in the 2 protocols respectively. *Conclusions*. Intra-arterial catheter guided steroid therapy for steroid resistant/dependent GVHD with high dose steroids is at least as good as intermediate dose steroids with methotrexate and may be safer.

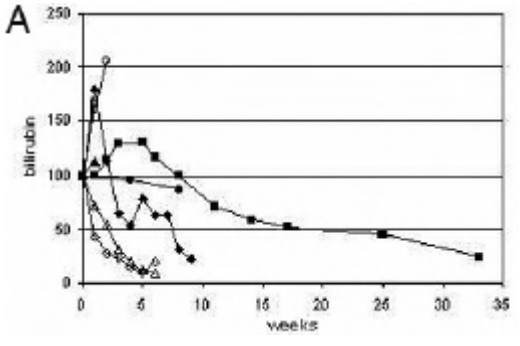

Figure 1. A-B.

0957

BRAF MUTATIONS IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Background. Approximately 75% of patients with juvenile myelomonocytic leukemia (JMML) harbour mutations in PTPN11, NF1 and RAS genes. The remaining cases presumably carry somatic mutations in other genes in the RAS pathway. BRAF plays a central role in this pathway between RAS and downstream molecules including MEK and ERK. BRAF mutations frequently occur in cancer and recently, BRAF mutations were described in leukemia. *Aims*.The aim of the study was to investigate whether BRAF mutations play a role in the pathogenesis of JMML. *Methods*. In 65 JMML patients screening for BRAF V600E mutations in exon 15 was performed from mononuclear cells. In a subset of 15 patients, without RAS or PTPN11 mutations, and no clinical signs of NF1, the entire coding sequence of BRAF was analyzed. Sequence analysis was performed by direct, bidirectional sequencing of purified polymerase chain reaction products. *Results*. In none of the 65 cases a V600E mutation of the BRAF gene was found. In the subset of patients in which the entire coding sequence of BRAF was analyzed, no mutations were identified either. *Summary*. Mutant proteins of the RAS-RAF-MEK-ERK pathway play an important role in the pathogenesis of JMML, resulting in G-CSF hypersensitivity. In about 75% of the JMML cases these mutations affect RAS, NF1 or PTPN11 genes. The hypothesis for this study was that BRAF might play an important role in JMML as it is an important downstream effector of RAS. Our data show that BRAF mutations do not occur frequent in JMML. Therefore, additional analysis of genes of the RAS pathway will be necessary to identify genetic aberrations in cases without known mutations.

0958

BCR-ABL DUPLICATION REVEALED BY FISH ANALYSIS IS FREQUENTLY ASSOCIATED WITH IMATINIB RESISTANCE IN CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA PATIENTS

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Background. BCR-ABL mutation analysis and bone marrow conventional cytogenetic studies are classically employed to study patients (pts) resistant to imatinib mesylate (IM). However, the systematic use of FISH has not been applied to large series of resistant pts. Another drawback in studying resistant pts is that most studies report a mixed population of chronic phase (CP), accelerated phase and blastic phase chronic myelogenous leukaemia (CML). In order to estimate more precisely the molecular and chromosomal abnormalities associated with imatinib resistance, we have systematically studied CP CML patients by caryotype, FISH and BCR-ABL TKD sequencing. *Patients and Methods*. CP CML pts resistant to imatinib and eligible for second line tyrosine kinase inhibitors therapy were recorded in three centers. All pts had conventional cytogenetic, metaphase FISH and BCR-ABL mutation screening at entry. *Results*. 54 CP CML pts were studied (median age 54 years) with a median time from diagnosis of 74 months (13-199). 12 pts were only treated with IM first line, 14 received 2 lines of therapy and 28 three lines or more. Median time of IM exposure was 42 months (8-67). 33 pts were primary resistant to imatinib and 21 were secondary resistant. 11 different BCR-ABL TKD mutations were found in 22 pts (40.7%). 17 pts (31.5%) had additional cytogenetic abnormalities (ACA) including der(22) in 6 cases and der(9) in 2 cases. FISH analysis revealed that 15 pts (27%) had metaphases with more than one BCR-ABL fusion spot, including the 6 cases with der(22). A true BCR-ABL amplification was demonstrated in only 2 cases (3.7%). Deletion of the derivative der(9) was evidenced in 4 cases (7.4%). Overall, FISH analysis revealed additional abnormalities in 21

pts (38.8%) that were isolated (absence of ACA and absence of mutation) in 6 pts. As duplication could be a consequence of genetic instability, we then tested the link between BCR-ABL duplications and BCR-ABL TK mutations. No correlation could be evidenced with the Fisher's exact test (p=0.55) and only 5 pts were found to have both BCR-ABL duplication and mutation, suggesting that the two mechanisms are independent. Duplications were not associated with primary or secondary resistance (p=1) whereas mutations are more frequent in the context of secondary resistance (p=0,01). *Conclusions*. We have identified an unexpected high frequency of BCR-ABL duplications (27%) using FISH analysis in samples from pts resistant to IM. In 2/3 of the cases, these duplications were not associated with gain of Philadelphia chromosome on conventional cytogenetic. BCR-ABL duplications were present independently to the detection of BCR-ABL TKD mutations. Whether this underestimated mechanism of resistance is correlated with a particular response to second line TKI remains to be determined.

0959

EXPRESSION OF WT-1, PRAME, RHAMM AND BAALC GENES IN PERIPHERAL BLOOD OF AML PATIENTS AT DIAGNOSIS

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Background. Several genes, including WT-1, PRAME, RHAMM have been suggested as possible markers of minimal residual disease (MRD) or prognostic factors in AML patients. Also expression of the BAALC gene has been proposed as a possible prognostic factor of medical outcome in AML patients with normal cytogenetics. *Aims*. The goal of our study was to test expression of genes mentioned above and to find an alternative suitable marker of MRD especially for those patients who had a relatively low expression of the WT-1 gene at presentation. *Methods*. The expression of WT-1 and PRAME was measured by the quantitative real-time PCR with the specific TaqMan probes on the Rotor Gene thermocycler in peripheral blood of patients and healthy controls; and their expression was related to expression of the control gene ABL. The expression of BAALC and RHAMM was quantified using TaqMan® Gene Expression Assays and was related to the expression of the control gene ABL-1 - TaqMan® Gene Expression Assay. The informed consent was obtained from all patients.

Figure 1. The expression of BAALC in the peripheral blood of AML patients at diagnosis.

Results. The cDNA level of WT-1 was detected in peripheral leukocytes from 52 AML patients at diagnosis. The expression of WT-1 was significantly lower in 8 AML patients with t(8,21), p=0,0004. An elevated expression of the PRAME and RHAMM genes was found in AML patients at diagnosis (n=51 and n=70, respectively) compared to their expression in peripheral blood of normal healthy donors (n=21) p<0,0001. The expression of the PRAME gene seems to be higher in AML patients at diagnosis with t(8;21) compared to the rest of AML patients at diagnosis p=0,0029. Similarly, the expression of the BAALC gene was significantly elevated in 18 patients with t(8;21) and inv.16 (i.e. CBF leukemias), compared to AML patients with normal cytogenetics (n=82), p<0,0001. On the other hand, the BAALC expression was surprisingly low in 9 AML patients with the translocation t(15,17) and in 13 leukemic cell lines. *Conclusions*. Our results propose the alternative MRD marker PRAME and possibly BAALC in the subset of AML patients with the low expression of WT-1 at presentation i.e. AML1/ETO+ and some others. Unexpectedly, the high expression of BAALC gene, which is a negative prognostic factor in AML patients with normal cytogenetics, was found in CBF leukemias, which are generally associated with a good clinical outcome.

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0960

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

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Background. Patients with hemophilia may be at risk for developing reduced bone mineral density for a number of reasons such as recurrent hemoarthrosis and immobilization. *Aims*. To assess the bone mineral density (BMD) in children with hemophilia and, to correlate bone mineral density with findings regarding the joint disease (hemophilic arthropathy). *Patients and Methods*. Thirty hemophilic patients aged 4.97+3.64 years and 30 control healthy individuals (had no joint disease) aged 5.09+3.64 years were selected from the hematology unit and outpatient clinic of MUCH respectively. Anthropometric measurements was done to all cases. Z score was used for weight, height, and Body Mass Index (BMI). Joint evaluation for hemophilic patients and controld was done using Colorado PE-0.5: Half Point Instrument before using Dual Energy X-ray Absorptiometry(DEXA). DEXA scanning was performed to all hemophilic patients and controls focusing on L2-L4. *Results*. There was no significant difference between hemophilic patients and controls as regard anthropometric measurements and their z-score. There was a significant difference between hemophilic patients and controls as regard BMD and BMD z-score (mean + SD) (BMD: 0.48 + 0.13 gm/m^2 for hemophilic patients versus 0.55+0.14 gm/m^2 for control, p= 0.05, BMD z-score: -0.68+0.44 for hemophilic patients versus 0.19+0.14 for controls p=0.003). There was a significant difference between severe hemophilic patients (factor level assay less than 1%) ans controls as regard BMD and BMD z-score (BMD: 0.41+0.15 gm/m^2 for hemophilic patients versus 0.55+0.14 gm/m^2 for controls, p=0.01, BND z-score: -1.49+0.12 for hemophilic patients versus 0.19+0.14 for controls p=0.001). Also, in hemophilic patients, there was an inverse significant correlation between total joint evaluation scores and BMD z-score (r=-0.365, p=0.04). *Conclusions*. Children with hemophilia could have reduced bone mineral density compared with age and gender matched controls. This reduction in bone mineral density was independent on difference in age and body size.Children with more established hemophilic arthropathy exhibited the lowest BMD and BMD z-score. *Recommendations*. 1. Early detection of osteopenic hemophilic children using DEXA scanning. 2. Bisphosphonates plus calcium for hemophilic children with reduced bone mineral density. 3.Evaluation of the effect of on demand versus prophylaxis replacement therapy in hemophilic patients on BMD and hemophilic arthropathy.

0961

THE BENEFICIAL EFFECT OF CHRONIC GRAFT-VERSUS-HOST DISEASE ON THE CLINICAL OUTCOME OF TRANSPLANTATION WITH FLUDARABINE-BUSULFEX-BASED REDUCED-INTENSITY CONDITIONING FOR PATIENTS WITH *DE NOVO* MYELODYSPLASTIC SYNDROME

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Background. The increased severity and frequency of transplant-related morbidity and mortality in conventional stem cell transplantation (CST) in treating MDS are major problems in improving disease-free survival (DFS) after transplantation, even though the results of CST have improved in the past decade. We have also reported our experiences regarding CST in treating MDS, and have drawn attention to the necessity of approaches to minimize TRM for improving DFS after transplantation for advanced MDS (Int J Hematol 2005;82:66-71). *Aims*. We tried to define the role of RIST in patients with *de novo* MDS, classified by WHO criteria. *Methods*. We performed RIST for 22 consecutive patients

with *de novo* MDS, who received an allograft using fludarabine/busulfex or fludarabine/busulfex/antithymocyte globulin (ATG). *Results*. Nineteen patients (86.4%) achieved engraftment. At a median follow-up of 18.9 months (range, 13.1-24.8 months), the estimated 2-year overall survival (OS), event-free survival (EFS), transplantation-related mortality, and relapse incidence were 78.7, 67.7, 12.6, and 22.5%, respectively. Acute graft-versus-host disease (GVHD) greater than grade II developed in three patients (15.8%). Chronic GVHD developed in 10 patients (55.6%) and none of these patients received ATG as a conditioning regimen. Variables influencing EFS were chronic GVHD, marrow blasts before transplantation, and the WHO criteria. *Conclusions*. The present study clarifies the benefits of the fludarabine/busulfex-based conditioning regimen for *de novo* MDS diagnosed according to the WHO criteria, and shows that chronic GVHD appears to have a beneficial effect on survival rates, which are strongly associated with graft-versus-tumor effects.

0962

STUDY OF APOPTOSIS AND EXPRESSION PATTERN OF TNF RECEPTOR FAMILY MEMBERS IN HUMAN BONE MARROW MESECHYMAL STEM CELLS

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Background. There is currently great interest in exploring the use of bone marrow (BM) derived mesenchymal stem cells (MSCs) for the repair of tissue damage. The expression of tumor necrosis factor receptor (TNFR) family members and survival characteristics of MSCs in inflammatory microenvironment is entirely unknown *Aims*. The aim of the study was to investigate (a) the expression of the apoptosis-related TNFR family members in human BM MSCs and (b) the apoptotic characteristics of BM MSCs in standard culture conditions and following incubation with TNF family members. *Methods*. Normal human BM cells were isolated from posterior iliac crest aspirates and MSCs were expanded according to a standard protocol. MSC identification was based on immunophenotypic characteristics ($CD45^-$, $CD14^-$, $CD34^-$, $CD90^+$, $CD73^+$, $CD44^+$, $CD29^+$, $CD105^+$, $CD146^+$) and the potential of cells to differentiate towards the adipogenic (Oil red O stain and aP2 and PPAR-Á expression by RT-PCR), osteogenic (ALP/Von Kossa stain and ALP and CBFA1 expression by RT-PCR) and chondrogenic (Masson and Alcian blue stain and Collagen II and Aggrecan expression by RT-PCR) lineages. Flowcytometry and RT-PCR was used for evaluation of the expression of the apoptosis-related TNFR family members namely Fas, TNFRI, TNFRII, TNF related apoptosis-inducing ligand (TRAIL) R1, TRAILR2, TRAILR3, TRAILR4, time-course from Passage (P) 1-6 The survival characteristics of MSCs under different culture conditions were evaluated using flowcytometry and 7-aminoactinomycin D (7AAD) stain. *Results*. Spontaneous apoptosis of MSCs at P2 was elevated to 10.02%±6.48% and this percentage remained stable time-course. As expected, the presence of 10% fetal calf serum (FCS) in the culture medium protected MSCs from spontaneous apoptosis compared to FCS deprived cultures ($p<0.05$). MSCs highly expressed cell surface Fas (85.6±5.0 at P2) and TNFRI (69.1±10.2) and this expression remained high time-course. TNFRII and TRAILR1-4 were expressed at levels lower than 10% time-course. Fas and TNFRI mRNA expression was also confirmed by RT-PCR. In the presence of recombinant human Fas Ligand (rhFasL) (100ng/mL) the proportion of apoptotic cells dramatically increased in both serum deprivation and 1%FCS culture conditions compared to baseline ($p<0.01$ and $p<0.01$, respectively) whereas 10% FCS protected MSCs against FasL mediated apoptosis. On the contrary, addition of rhTNFα (5-500 ng/mL) did not affect the survival characteristics of MSCs or their proliferative potential as judged using the Methyl Triazolyl Tetrazolium (MTT) colorimetric assay. *Summary/Conclusions*. BM MSCs display low levels of spontaneous apoptosis. Despite the high functional surface Fas expression they might be used under appropriate culture conditions (optimal FCS concentrations) even in inflammatory microenvironment(s) for the repair of tissue damage. Interestingly, TNFα does not affect MSC survival or cell proliferation despite the high expression of TNFRI. The effect of TNFα on the differentiation or immunomodulatory potential of MSCs is a field for further investigation.

0963

DIFFERENTIAL EXPRESSION OF THE FLICE-INHIBITORY PROTEIN, C-FLIP, IN $CD34^+$ CELLS IN CHRONIC MYELOGENOUS LEUKEMIA, POLYCYTHEMIA VERA AND ESSENTIAL TROMBOCYTHEMIA

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Background. Apoptosis-inducing systems, including death receptor pathways, are deregulated in hematopoietic neoplasms. FLIP (FLICE-inhibitory protein) was identified as an inhibitor of FAS and TRAIL signals in apoptosis extrinsic pathway. *Aims and Methods.* In this study, c-flip gene expression was evaluated on hematopoietic progenitor cells ($CD34^+$) and peripheral blood mononuclear cells (MC) from healthy individuals and from patients with chronic myeloproliferative diseases (CMD) without treatment. $CD34^+$ cells were obtained from bone marrow of 10 healthy subjects, 4 polycythemia vera (PV), 6 essential trombocythemia (ET) and 10 chronic myelogenous leukemia (CML) patients

by using the Miltenyi CD34 isolation kit. MC were isolated from peripheral blood of 15 healthy subjects, 4 PV, 6 ET and 20 CML patients by Ficoll-Hypaque density gradient protocol. Total RNA was extracted from MC and CD34+ cells using Trizol method, cDNA was synthesized by using High capacity Kit (Applied Byosystems, EUA) and results were given as relative expression (amplicon ratio: c-flip gene / β-actin housekeeping gene). Lymphocyte resistance to apoptotic inducers (etoposide, cytarabine and cycloheximide) was also evaluated through cell death quantification measured by flow cytometry (annexin and hypotonic-fluorescent-solution assays). *Results*. c-flip mRNA levels in CD34+ cells were increased only in CML patients (expression median, 45.1) in comparison to PV (3.23), ET (1.02) patients and controls (1.43) (p<0.001 for all comparisons). In contrast, c-flip expression in MC was found to be higher in all CMD studied [CML (4.3), PV (7.85) and ET (5.12)] in comparison to controls (1.43) (p<0.001 for all comparisons). c-FLIP levels in MC from CMD patients were positively correlated with lymphocyte resistance to apoptosis stimulated by etoposide (p=0.03). *Conclusions*. The data suggest that is differentially expressed in CD34+ and MC in CML, PV and ET patients. Regarding CMD physiopathology, the findings also suggest that c-flip up-regulation is a primary alteration in CML patients, but a secondary event in PV and ET patients. In fact, it appears that this molecule is involved in CMD apoptosis deregulation and may contribute to myeloid cells proliferation. c-FLIP is probably a promising target to be explored in new therapeutical strategies in CMD, particularly in CML. Supported by FAPESP (06/50094-8) and CNPq. *EPLG and RVC contributed equally for this work.

0964

EARLY PROGENITORS AND APOPTOSIS IN MYELODYSPLASIA AFTER LOW-DOSE RADIATION EXPOSURE

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Background. Ineffective hematopoiesis and stem cell function with an increased apoptosis are characteristic for myelodysplastic syndromes and also were demonstrated in healthy radiation-exposed. *Aim*. To study possible modifying role of low-dose radiation exposure on the progenitor cells and apoptosis in myelodysplastic syndrome. *Patients and Methods*. Bone marrow (BM) and peripheral blood (PB) were studied in 49 MDS patients (RA) - 25; RAEB- 16; RAEB-t-6; CMML-2 cases. Age was between 30-77 years (mean-55,7). In 14 patients MDS was initiated at the late period after low-dose irradiation during clean-up works at Chornobyl accident. Control group included 20 healthy donors (mean age 51,0). Expression of CD 34, CD13, CD33, CD71, CD117, HLA-DR and bcl-2 protein was studied by flow cytometry. Spontaneous and verapamil-induced apoptosis was measured by Annexin V assay and CD95 expression. *Results*. RAEB, RAEB-t and CMML were characterized by high CD34+ (68,0±7,08%), CD71+, CD117+ or CD117+34+ cell counts associated with poor prognosis and transformation to acute leukemia. BM CD34+ cell subset demonstrated the prevalence of the lineage committed progenitors. In RA a statistically significant increase of mean values of fluorescence intensity of CD117, CD33, CD34 and CD71 antigens was observed together with a significant decrease of SSC parameters in granulocytes population of PB and BM in MDS patients compared with healthy donors group (p<0,01). Hypogranularity in granulocytic region in BM is more marked in comparison with PB. High level of apoptosis compared with healthy donors group was observed in RA cases, whereas a switch to a low level of apoptosis was detected in the RAEB, RAEB-t and CMML blasts.

Figure 1. CD95+ and bcl2 cell fractions in myelodysplasia patients exposed to low-dose irradiation after Chernobyl accident.

Cells demonstrated an increase of verapamil-induced apoptosis in the Annexin-V/PI test as comparing with the spontaneous apoptosis levels. For granulocytes a significant increase of the verapamil-induced apoptosis was shown *in vitro*. Patients with MDS had significantly increased expression of CD95 antigen (Figure 1). Positive correlations were found between over-expression of CD 95 and the expression of CD34-receptor in RAEB, RAEB-t, CMML groups. Levels of CD 95 and Bcl-2 expression in RAEB, RAEB-t, CMML groups show negative correlations for lymphocytic (r=-0,72; <0,03) and granulocytic (r=-0,90; p<0,001) populations. In RA such correlations were not shown for Fas-receptor and Bcl-2 positive cell counts in lymphocytes to the contrary to granulocytes, in which the statistically significant correlation between these values (r=-0,84; p<0,005) was detected. Radiation exposed showed the highest CD95+ and low bcl2+ cell counts. *Summary*. This study demonstrated some correlations of between early progenitors and increased apoptosis in myelodysplasia patients. D95 apoptotic cells fraction and verapamil-induced apoptosis seem to be substantially higher in radiation exposed than in other MDS cases indicating possible influence of exposure on the course of disease.

0965

COMBINATION CHEMOTHERAPY IN MDS: A LIMITED IMPACT ON SURVIVAL AS A SINGLE THERAPY, A BENEFICIAL EFFECT ON OUTCOME OF SUBSEQUENT TRANSPLANTATION

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Aims. An effect of combination chemotherapy on survival in comparison to other treatment approaches was studied in a group of 142 adult patients with advanced forms of primary MDS (RAEB>10% blasts, RAEB-T). *Methods and results*. Median survival of patients treated with combination chemotherapy only was 8,0 months compared to low dose chemotherapy (5,5 months) or supportive care only (3,0 months)-p=0.005. Univariate statistical analysis using Kaplan-Meier curves and log-rank2 test revealed achievement of complete (CR) or partial (PR) remission (according to Cheson criteria) after chemotherapy as a significant variable affecting survival (χ^2=31,9, p=0.001 for subsequently transplanted and χ2=27,8, p=0.001 for non-transplanted patients), however, treatment by combination chemotherapy itself regardless to the outcome was also a significant parameter beneficially affecting survival (χ^2=13, p=0.001). On the other hand, estimated 3 years survival (ES3y) of patients treated with combination chemotherapy as a single treatment was only 4,3%. Allogeneic stem cell transplantation (SCT) performed in a subgroup of 31 patients was the most important parameter affecting survival in univariate analysis regardless to the number of bone marrow blasts at the time of conditioning (χ^2=31,9, p<0.0001). A multivariate analysis revealed SCT as the only independent variable determining survival in the whole group of patients, SCT performed in CR/PR was a significant variable affecting survival in patients <55 years (χ^2=3,9, p=0.04). Median survival of patients transplanted in CR was 30,8 months compared to 19,5 months in patients transplanted with > 5% bone marrow blasts at the time of conditioning (p=0.04). ES3y, ES5y, transplant related mortality {TRM) and relapse rate (RR) in both the groups are shown in Table 1, in patients transplanted not in CR, estimated 5 years survival was only 10% an the RR was 45,5%. *Conclusions*. Our results show that combination therapy may play a significant role in the treatment of advanced MDS when combined with other treatment modalities. In this regard, combination chemotherapy followed by treatment with demethylating agents may be a promising approach to prolong survival in patients with advanced MDS who are not candidates for SCT. In the same way, a maintenance treatment with demethylating agents after SCT may improve outcome of patients transplanted with excess of blasts in bone marrow.

Table 1.

Group	Number of patients	Estimated 3 years survival (%)	Estimated 5 years survival (%)	TRM %	RR %
SCT in CR	18	68,5	44,0	19,0	12,5
SCT not in CR	13	27,0	10,0	27,0	45,5

0966

EXPRESSION OF CYTOKINE RECEPTORS ON CD34+ BONE MARROW CELLS 100 DAYS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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After reinfusion, autologous stem cells repopulate bone marrow promptly. However recovery of their proper function is still poorly studied. The aim of our investigation was evaluation of expression of cytokine receptors that are important for haematopoietic progenitors' engraftment. We compared expression of those receptors in patients' and normal bone marrow samples. *Materials and methods*. The study group consisted of 14 patients 100 days after autologous transplantation. Normal bone marrow control samples were obtained from healthy bone marrow donors. $CD34^+$ cells were evaluated according to standard ISHAGE protocols. Expression of CD184 (CXCR-4 receptor for SDF-1 chemokine), CD117 (c-Kit, receptor for c-Kit ligand) and CD114 (G-CSF receptor) on $CD34^+$ cells was studied with CANTO BD flow cytometer. Commercial monoclonal antibodies stained with fluorochromes were used. *Results*. 1/ Higher percentage of $CD34^+$ $CD117^-$ cells was found in patients' samples in comparison to normal bone marrow (p=0.003), which indicates more progenitors with lymphoidline commitment. 2/ Higher expression intensity of CXCR-4 receptor on myeloid progenitors was detected in patients' samples than in normal bone marrow (p=0.04) 3/ Stem cell recipients showed lower $CD4^+$ lymphocyte counts in the blood 100 days after transplantation in comparison to normal blood samples and as a result lower $CD4^+/CD8^+$ ratio (0.1-1.4 vs. 1.1-2.8) *Conclusion*. Our results of cytokine expression analysis indicate that 100 days after autologous transplantation regulation of hematopoiesis, and in particular lymphopoiesis, is not completely stabilized. That may influence abnormal proportion of T cell subsets in peripheral blood.

0967

CHANGING FROM A FIRST TO SECOND GENERATION IFOSFAMIDE-BASED CHEMOTHERAPY REGIMEN (FROM IMVP TO ICE) DOES NOT IMPROVE OUTCOME OF PATIENTS WITH RELAPSED OR REFRACTORY AGGRESSIVE NHL

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Background. There are no randomized trials comparing different chemotherapy regimens used for treatment of relapsed or refractory aggressive NHL. Some years ago very good results have been reported with a combination of ifosfamide, carboplatinum and etoposide (ICE). Consequently, a lot of centers started using this regimen. Five years ago we changed from our previous regimen, consisting of ifosfamide, methotrexate and etoposide (IMVP), to ICE. *Aims*. Here we report our results with ICE and compare them to the results obtained during the previous five-year period with IMVP. *Methods*. Patients with relapsing or refractory aggressive NHL received two cycles of ICE or IMVP. Responders continued with stem-cell mobilization, high-dose chemotherapy and autografting. Areas that were not in complete remission pretransplant were irradiated after hematopoietic recovery. *Results*. Forty-five patients were treated with ICE and 28 with IMVP. The percentage of patients with refractory disease was similar in both groups (22/45 vs. 12/28), while more patients treated with IMVP had unfavorable IPI scores (16/45 vs. 17/28, p=0,033, t-test). The response rate (RR) to ICE was 47% (6 patients achieved CR and 15 PR), 2-year overall survival (OS) 30% and 2-year event-free survival (EFS) 21%. These results were similar to those obtained with IMVP (RR 39%, 2-year OS 23%, 2-year EFS 13%, all differences non-significant using t-test and log-rank test) (Figure 1). Toxicity of both regimens was similar. Sixteen of 45 patients treated with ICE had significant hematologic toxicity, 12 febrile neutropenia or serious infections and 3 other serious toxicity (2 neurologic disturbances and one case of supraventricular tachycardia). Serious hematologic toxicity occurred in 9 out of 28 patients treated with IMVP, febrile neutropenia and serious infections in 9 and 1 patient had a tumor-lysis syndrome. One patient in each group died of treatment-related toxicity. *Conclusions*. Changing from a first generation ifosfamide-based regimen, such as IMVP, to a second generation regimen, such as ICE, does not substantially improve outcomes of patients with relapsed or refractory aggressive NHL.

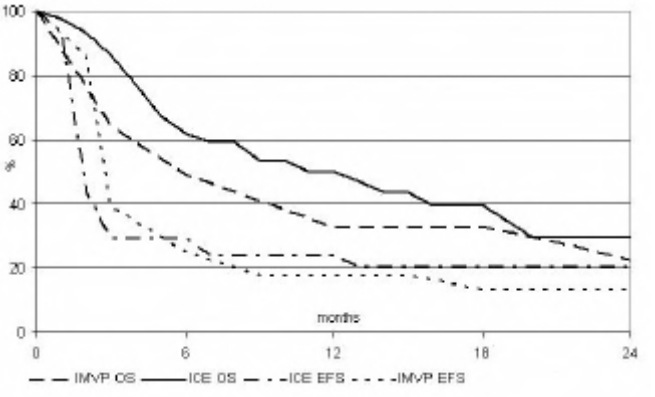

Figure 1.

0968

COMPLEX CHROMOSOMAL REARRANGEMENTS IN ADULT PATIENTS WITH AML ARE INDEPENDENT PROGNOSTIC FACTOR

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Background and aims. Acquired cytogenetic aberrations are detected in 55-70% of newly diagnosed patients with AML. Most of karyotypic abnormalities are associated with specific disease subtypes, characteristic morphologic and immunologic profiles and distinct therapeutic and/or prognostic implications. However, approximately 10-15% of AML with abnormal karyotype have no specific aberrations, but do have complex chromosomal rearrangements (CCR). The aim of the study was a comprehensive analysis of CCR found in bone marrow cells of patients with AML or MDS RAEBt at diagnosis by molecular cytogenetic methods and to evaluate the significance of complex aberrations for prognosis of these patients. *Methods*. Karyotypes of all patients were analysed by conventional cytogenetic method. FISH analyses were performed according to the result of G-banding using locus-specific and/or centromeric commercially available probes (Abbott-VysisTM). Structural and/or complex chromosomal aberrations were proved by multicolor FISH (mFISH) with the *24XCyte* probe kit with combinatorially labeled painting probes specific for all autosomes and sex chromosomes (MetaSystemsä). In some cases multicolor banding (mBAND) was carried out using XCyte-color kits (MetaSystemTM) for chromosomes 3, 5, 7 and 11. *Results*. During the years 1998-2006 we examined 392 patients with AML or MDS RAEBt. We found complex chromosomal rearrangements at the time of diagnosis in 58 of them (14,7%). This group included 32 females and 26 males with an average age 61,2 years, median of overall survival was just 3 months. Only three patients are living, one after bone marrow transplantation, one in partial and one in complete remission. The majority of structural abnormalities were unbalanced. In 50 patients (86%) loss or rearrangements of chromosome 5, 7 and/or 11 was proved. Deletion of critical regions 5q31 was determined in 35 (60,3%) and deletion 7q31 in 16 (27,5%) patients. Aberration of MLL gene was found in 11 cases (19%). Trisomy of chromosome 8 was the most frequent numerical change (11 patients). *Conclusions*. Our study demonstrates the clinical importance of cytogenetics in adult patients with AML. We proved that complex chromosomal rearrangements are one of the most important prognostic factors, are associated with very poor prognosis and poor response to antileukemic treatment. Precise identifications of these aberrations and delineation of breakpoints in bone marrow cells of patients with AML at the time of diagnosis could lead to a better understanding of genetic events during leukemogenesis as well as quiding further molecular studies of genes involved in evolution of leukemia. We believe that these findings could provide clinically relevant information that can assist in the development of risk-adapted therapeutic strategies.

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0969

COMPARISON OF CD25 IMMUNOHISTOCHEMISTRY AND CD25 FLOW CYTOMETRY IN SYSTEMIC MASTOCYTOSIS

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Background. Systemic mastocytosis (SM) is a clonal myeloid disorder characterized by an abnormal accumulation and growth of mast cells (MC) and their progenitors in one or more extracutaneous organs. In most cases, the bone marrow is involved and thus is examined in suspected disease. Expression of CD25 in bone marrow MC, with or without co-expression of CD2, is an important minor criterion of systemic mastocytosis. So far, most studies have examined CD25 expression on MC by fow cytometry. *Methods*. We examined the expression of CD25 by bone marrow MC in patients with SM (n=30) by immunohistochemistry (IHC) and compared these results with those obtained from flow cytometric assessement of CD25 expression. *Results*. In the majority of all patients (27/30; 90%), CD25 was detectable in MC by both staining techniques. In one patient, CD25 was only detectable by IHC, but not by flow cytometry, whereas in 2 patients, in whom no compact MC infiltrates were detectable in the bone marrow, only the flow cytometric evaluation revealed aberrant expression of CD25 in MC. *Conclusions*. CD25 IHC is equally diagnostic and sensitive in SM compared to flow cytometry and thus can be recommended as diagnostic test to document the phenotype-related minor SM criterion. Our data also suggest, however, that optimal assessment of CD25 expression in neoplastic MC in all patients requires the application of both techniques, i.e. IHC and flow cytometry.

0970

SELF-RENEWAL CAPACITY OF STROMAL PRECURSOR CELL IN MESENCHYMAL STEM CELLS HIERARCHY IN MICE

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Background. Adult mesenchymal stem cell (MSC) as any other stem cell is capable for self-renewal and differentiation. In the mice bone marrow (BM) there are cells able to create hematopoietic microenvironment *de novo* after transplantation of the BM plug under the renal capsule of syngeneic animal. In hematopoietic ectopic foci formed 6 weeks after the transplantation the stromal cells belong to a donor of BM and hematopoietic cells are of recipient origin. The capability of these cells to differentiate within all stromal cell types forming functional hematopoietic microenvironment completely fits stem cells differentiation criteria. MSC could be retransplanted 9 times without changes of foci size formed *de novo*. It is possible to calculate the approximate number of MSC per femur which comes to 5-10 per 106 BM cells. In irradiated recipients the size of the foci enlarged significantly, but this phenomenon is not due to MSC number increase as this effect doesn't apply to the next retransplantation to the non-irradiated recipients. So the category of precursor cells, capable to differentiate to the cells of functional stromal microenvironment, but not capable of self-renewal exists. *Aims*. The position of CFU-F in hierarchy of MSC is still obscure. The aim of the study was to investigate the capability of CFU-F to transfer hematopoietic microenvironment. *Methods*. Çone marrow plugs were transplanted under the renal capsule of syngeneic mice. In 6 weeks the foci formed were either retransplanted under the renal capsule of secondary recipients or nucleated cells in the foci were resuspended and counted. CFU-F frequency was measured by standard protocol. Cloning was performed using 3 concentrations of BM cells (30000, 40000 and 50000 cells per well of 96-well plate). Clones of fibroblasts were passaged to 24-wells plate, than to 6-well and finally to 25 cm^2 flask in media containing 20% FCS and 5 ng/mL bFGF. The cells from the flask were transplanted under the renal capsule both of non-irradiated and irradiated mice. *Results*. CFU-F frequency in murine BM is 47,6±1,2 per 106 cells if fider of irradiated guinea pig BM cells was added to system and 36,2±9,5 per 106 when the media with 5 ng/mL bFGF was used. Limiting dilution analysis showed that the frequency of CFU-F equals 1 per 30000 cells, in flasks it was estimated as 1 per 21000 cells on fider cells and 1 per 27000 cells in bFGF presence. CFU-F possesses limited proliferative potential. Hundred percent of clones had grown up in 24-well plate (i.e. 5 mitoses were performed), 60% filled the well of 6-well plate (two more mitoses) and only 45% reached the square of the flask (i.e. could undergo 9 mitoses). After transplantation of these layers under the renal capsule of both non-irradiated and irradiated syngeneic mice no ectopic foci developed. So progeny of CFU-F are not able to transfer hematopoietic microenvironment. *Conclusions*. The data suggest that CFU-F has limited proliferative capacity and do not contain the MSC. CFU-F position in MSC hierarchy seems to be lower than cells capable to develop large foci in irradiated recipients.

0971

SPONTANEOUS FACTOR V AUTOANTIBODY-A CASE REPORT AND SYSTEMIC REVIEW OF ITS NATURAL HISTORY AND MANAGEMENT

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Background. Factor V(FV) inhibitors are infrequently encountered. They often arise as alloantibodies after topical bovine thrombin exposure, or may develop in congenital FV deficiency patients after fresh frozen plasma(FFP) transfusion. Less commonly, they are autoantibodies that develop spontaneously in non-hemophiliac patients. FV autoantibody tends to be associated with more serious haemorrhagic complications compared to alloantibody associated with bovine thrombin exposure. *Aims*.We report the spontaneous development of FV autoantibody in an elderly lady who presented with melaena. We also performed a systematic review of published cases of spontaneous FV autoantibody and our patient, to attain a better understanding of the patient characteristics, natural history and management of this condition. *Methods*. Published cases of spontaneous FV autoantibody from 1950 till December 2006 were searched with no language restriction on Ovid MEDLINE. Additional articles were identified by reviewing reference lists. Cases associated with topical thrombin exposure and congenital FV deficiencies were excluded. Analysis was done on 71 cases(including our patient) with available information. *Results*. A 92-year old female with diabetes mellitus and dementia presented with haemetemesis and melaena. She was usually on tolbutamide, chlorpromazine, carbamazepine and haloperidol. Her prothrombin(PT) and activated partial thromboplastin time(aPTT) were >100secs, and were not correctable on 1:1 plasma mixing studies. There was no demonstrable lupus anticoagulant(LA) activity. Her FV level was <1% and the rest of the clotting factors were normal or elevated. She did not respond to FFP transfusion, and was started on IV immunoglobulins and hydrocortisone. This was followed by improvement in her clotting profile from 3rd day onwards and complete resolution of bleeding. She subsequently passed away from hyperglycaemic hyperosmolar non-ketotic state without recurrence of bleeding. The patients' median age was 68 (3-95)years. Unlike acquired factor VIII inhibitor which has equal gender distribution, the male to female ratio was 2:1. FV autoantibody had been reported to be associated with medication especially antibiotics (53.5%); surgery (36.6%); infection including HIV (29.2%); malignancy (21.1%), transfusion (15.5%); autoimmune diseases (16.6%). 22.5% of the cases had no associated factors. More than one condition may coexist and it is difficult to prove causality.

Table 1. Success rate of various FV autoantibody treatment.

All patients, except one, had prolonged PT and aPTT with median values of 53.9seconds and 127seconds respectively. Median FV level was low at 1.6%. The median inhibitor level was 8.45 Bethasda units, and the median duration of its presence was 40(5->900) days in all treated and untreated patients. 48 patients(67.6%) had bleeding complications

while 19(26.8%) did not. 4(5.6%) patients developed venous or arterial thrombosis instead of bleeding. 1 had normal clotting profile while the rest had low FV and prolonged PT and aPTT (2 of which also had LA activity). Among those who bled, 81.4% achieved haemostasis and 20% died from haemorrhage. Univariate analysis showed that bleeding manifestation was significantly associated with low FV, prolonged PT and aPTT, but not with age, gender, level and duration of inhibitor. Mortality from haemorrhage was only significantly associated with haemostatic failure and not with other characteristics. Hence, appropriate therapy must be started promptly for bleeding patients to rapidly achieve haemostasis and decrease mortality. Table 1 showed the success rates of various treatment. The most effective therapy was immunosuppression and immunoadsorption. Platelet transfusion also helped as platelets contain 20% of the body's FV which might be shielded from the inihibitor. In 15.5% of the cases, the condition was self-limiting and resolve without treatment. *Conclusions*. Spontaneous FV autoantibody development is unpredictable and often unavoidable, with variable manifestation and course. It can be life-threatening in cases with bleeding complications. Treatment should be instituted as soon as possible in such cases to avoid mortality.

0972

COMPREHENSIVE VIEW OF THE HAEMOPOIETIC STEM CELL PROGRAM: THE PRE-TRANSPLANT PHASE

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Background . The pre-transplant phase of the Stem Cell Transplantation (SCT) may influence survival and quality of life, and efficiency of health expenses. However, there is a lack of published data on it. *Aims*. To analyze the pre-transplant stem cell program phase of a tertiary Institution, in order to identify pre-transplant variables influencing relevant clinical outcomes. *Patient and methods*. Pre-transplant data on 172 consecutive patients evaluated by the SCT Committee of a tertiary Institution (Paediatric and adult programs) were prospectively collected and analyzed. Period: April 21th 2005, through December, 31th 2006. Variables were codified in a computer software tool developed as support for the management of SCT programme (Gesthronica®) allowing us to register social-demographic, and clinical variables. Statistical analysis was performed with the SPSS® statistical package. Comparison between groups of patients by type of SCT, disease characteristic and referring centre were performed (Kruskal-Wallis Test). *Results*. One hundred and seventy two consecutive patients entered the study: 152 were referred for the first time -acute leukemias (56), lymphomas (43), plasma cell disorders (22), myelodysplastic syndromes (9), chronic myelogenous leukemias (6), inherited disorders (4), chronic lymphocytic leukemias (3), solid tumours (3%), aplastic anemias (3), hemoglobinopathy (2), non clear diagnosis established (1). Additionally, 20 patients, previously treated with SCT, were re-considered as candidates to SCT because of relapse (10), graft failure (8) secondary malignancy (1) or multiple-graft program (1). One hundred and thirty nine (80.8%) were accepted for transplant while 25 (14.5%) were not, and 8 (4.7%) were delayed because of additional courses of treatment required before SCT. Scheduled procedures were autologous SCT (75), allogeneic STC (58) and DLI (6). At the time of the analysis, 83 (59.7%) patients had already been treated, 24 (17.3%) were still awaiting SCT and 32 (23%) were secondarily excluded from the SCT program because of: chemotherapy related death (11; 34%), poor stem cell mobilization (7; 22%) patient's refusal (5; 15,%), relapse/progression (3; 9%), acquired co-morbidity (2; 6%), donor's refusal (2; 6%) and other reasons (2; 6%). Median time between inclusion in the program and transplant was 3,81 months (range 0,27-13,43), being 5,7 months (p<0.05) for unrelated allogeneic transplants. No significant differences were found by diagnosis or hospital of origin. *Conclusions* . 1. There is a need for an agreement on SCT indications between the referring doctors and centres and the tertiary hospital performing the transplant. 2. The time from the inclusion in the SCT program and transplant was adequate, being longer in the allogeneic unrelated transplant setting. 3. The causes of not performing the scheduled transplant were deaths, inadequate mobilization and refusal of the patient. These causes should be further analysed. 4. Reaching an insight into the pre-transplant phase may help to improve survival and quality of life of patients and the efficiency and quality of the health assistance provided.

0973

TRANSCRIPTIONAL FEATURES OF MULTIPLE MYELOMA PATIENTS WITH CHROMOSOME 1Q GAIN

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Background. 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. *Aims*. In order to better characterize the biology of 1q/gain, the purpose of the present study was to provide a comprehensive analysis of the transcriptional profiles and the molecular features associated with 1q/gain on MM patients. *Methods*. Purified plasma cells from 77 MMs at diagnosis were characterized by FISH for the presence of 11 polisomy, the most recurrent IGH translocations, ploidy status, chromosome 13 deletion, and by global gene expression profiling using the Affymetrix U133A arrays. Patients were then stratified accordingly to the proposed Translocation/Cyclin D (TC) classification in five groups: TC1 characterized by the t(11;14) or t(6;14) translocations, with the consequent overexpression of either CCND1 or CCND3, and a non-hyperdiploid status; TC2 showing low/moderate levels of the CCND1 gene in the absence of any primary IGH translocations, and a hyperdiploid status; TC3 including tumors that do not fall into any of the other groups, most of which express CCND2; TC4 showing high CCND2 levels and the presence of the t(4;14) translocation; and TC5 expressing the highest levels of CCND2 in association with either the t(14;16) or t(14;20) translocation. Assessment of 1q/gain by FISH was performed by using three BAC clones specific for the BCL9 (1q21.1), CKS1B (1q22) and ARF1 (1q42.13) loci, and setting the threshold as 10%. *Results*. 1q/gain was identified in 40/77 (51.9%) 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/gain was significantly absent in TC2 group (p<10-4) and present in TC3 (p=0.008), whereas the correlation was not significant in the TC1 (p=0.053) or TC4 (p=0.142) groups; in addition, 1q extra copies significantly associated with Δ13 (p<10^{-4}), and chromosome 11 polisomy (although at a limited significance level of p=0.038), but not ploidy status (p=0.0971). 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. *Conclusions*. These data improve our knowledge concerning the specific genes/pathways deregulated by 1q abnormalities, and provide a promising focus for further studies aimed at defining new therapeutic strategies in MM.

0974

UMBILICAL CORD BLOOD TRANSPLANTATION USING NON-MYELOABLATIVE CONDITIONING: THE MEXICAN EXPERIENCE

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Twenty eight both children and adults were grafted with allogeneic umbilical cord blood cells (UCB), 15 males and 13 females. Seven patients were grafted because a non-malignant condition and 21 for a malignant disease. The median age was 8 years (range 4 months-72 years). Ten UCB were obtained from Mexican cord bloods banks, five cords were from compatible siblings and the remaining 13 cords were obtained from abroad. Median time to recover > 0.5×10^9/ L granulo-

cytes was 24 days (range 8-32 days), whereas median time to recover > 20×10^9/ L platelets was 26 days (range 12-50 days) Twelve recipients never engrafted and recovered subsequently endogenous hematopoiesis. The non-engraftment rate was significantly higher in patients allografted for benign conditions (71%) than in those allografted for malignant conditions (28%). The median overall post-transplant survival (SV) was 33 months and the 73-months overall SV was 39%. The cumulative incidence of grade II'IV acute GVHD and grade III'IV GVHD for the entire cohort of patients were 14% and 7%, respectively. Additional studies are needed to define if non-myeloablative conditioning is preferable over conventional conditioning in the case of UCB allografting.

0975

HOMOGENEOUS AND NON-HOMOGENEOUS CD138 EXPRESSION ON MYELOMA PLASMA CELLS

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Syndecan-1 or CD138, a molecule belonging to the heparan sulfate family, is a marker of normal and malignant plasma cells and differentiation antigen and play a role as a co-receptor for numerous growth factors of myeloma cells (Baff/April, EGF). CD138 also mediates cell-cell adhesion through heparin-binding molecules expressed by adjacent cells in bone marrow microenvironment (Bataille *et al.* Haematologica 2006; 91: 1234-1240., De Vos*et al.* Immunol Rev 2006; 210: 86-104). In the present study the $CD138^+$ cells were analyzed on freshly collected bone marrow (BM) samples of 70 multiple myeloma patients (37 M 33F, median age 62, range 38-81 yr. 8 at stage I, 17-II, 45-III acc. to D.S.; 52 had osteolysis, 7 had hipercalcemia, 9 renal failure; monoclonal protein IgG was in 47 patients, IgA-12, IgD -1, IgM-1, Bence Jones - 8, NS-1; mean proportion of plasma cells in morphological analysis of BM smears was 46±22, median 46%). Controls were 10 healthy subjects. Flow cytometry method using fluorochrome -conjugated monoclonal antibodies was applied. Plasma cells were determined by means of CD45, CD38 and CD138 antigens. Monoclonal antibody against CD138 FITC (Serotec) was applied. There were performed determinations of: CD138 expression intensity using RFI index, CD138 expression range using Cv (coefficient variability) index, size and granularity of investigated CD138+ cells. Following findings were revealed: 1.Heterogeneity of CD138 expression of analyzed patients respecting CD138 expression intensity, with: presence of plasma cell population showing high and homogeneous expression (n=12), presence of plasma cell population with homogeneous low/middle CD138 expression intensity (n=43), presence of plasma cell population with heterogeneous CD138 expression intensity forming two cell subpopulations: $CD138^{+/++}$ cells and CD138± cells (n=15), 2.Occurrence of negative correlation between RFI value and Cv magnitude (r=-0.7422, p=0.0001)., 3.Occurrence of positive correlation between size of $CD138^+$ cells and degree of their granularity (r=0.571, p= 0.0001). No correlation was found between CD138 expression intensity on plasma cells and their size (r=0.537, p=0.65), and correlation between CD138 expression intensity and granularity of plasma cells was close to statistical significancy (r=0.53, p= 0.058). The correlation CD138 expression with clinical picture of the disease will be determined in this study.

0976

PRESENTING CLINICAL FEATURES, BIOLOGICAL PROFILE, IMMUNOLOGIC SUBTYPES AND OUTCOME FOLLOWING TREATMENT IN PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background. There are few reports have analyzed the presentation features in relation to clinical outcome following treatment for subjects with T-cell acute lymphoblastic leukaemia (T-ALL). *Aims*. We analyzed retrospectively the clinical and biological characteristics associated with the outcome following treatment in patients diagnosed of T-ALL. *Patients and Methods*. Forty patients from seven institutions in the area of Canary Islands diagnosed with T-ALL, between August 1983 and November 2006, were enrolled in this study. Demographic data, clinical features (sex; age; presence of hepatomegaly, splenomegaly, mediastinal mass, and lymphadenopathy), haematological data; morphological and immunophenotypic characteristics of the blast cells, and cytogenetic abnormalities were recorded. Immunophenotypic classification of T-ALL according to EGIL scoring system was grouped into two major categories: 1. immature T-ALL, including EGIL TI and TII cases and 2. common thymocytic/mature T-ALL within EGIL TIII and TIV subtypes. The assessment of response to the antileukaemia therapy and the current status of the patients at the present time were reported. The majority of paediatric T ALL patients received BFM-based protocols and most of adults were treated according to PETHEMA LAL protocol of the Spanish Society of Haematology. Ten patients underwent intensification therapy with stem cell transplantation (four autologous and six allogenic) after achieving complete remission (CR). Chi-square and Fisher's exact tests were used for statistical comparison. The survival and duration of response was estimated using the Kaplan and Meier method and were compared by the log-rank test. *Results*. The median age of patients was 24 years (range 4-62); 8 was children under 12 years; sex distribution was 32 males and 8 females; 20 subjects (50%) presented with a mediastinal mass, 5.5% with central nervous system leukaemia and 28 patients had lymphadenopathies as presenting manifestation. Patients' white blood cell counts ranged from 0.5 to 757×10^9/L (median 33.3×10^9/L), anaemia (haemoglobin level <100.0 g/L) was detected in 35% and thrombocytopenia (platelet count $<100\times10^9$/L) in 52.5%. Lymphoblast exhibited a L2 morphology (FAB) in 60%. Thirty-five percent of immunological subtypes were immature T-ALL (2 EGIL TI + 11 EGIL TII) and 65% were mature subtypes (14 EGIL TIII + 11 EGIL TIV). 20% of cases had an abnormal karyotype. Overall 35 patients reached CR after induction chemotherapy. There was no association among the achievement of CR after induction chemotherapy with the main clinical and biological analyzed characteristics at diagnosis. Median overall survival was 52 months (1-168+). Estimated 5 year overall survival for patients achieved no CR was 0% compared with 54.6% for whom reached it. Considering overall survival statistical differences were observed regarding with ECOG >2 (log rank test p=0.02), liver (p=0.003) and spleen enlarged at diagnosis (p=0.009), immature subtypes of T-ALL (p=0.03), CD34 expression (p=0.007) and no achievement of CR after induction chemotherapy (p=0.004). *Conclusions*. Our findings regarding the association between clinical and biological factors at diagnosis with the outcome following treatment are similar to published data. Immature cases of T-ALL, CD34 expression of the lymphoblasts and induction failure to chemotherapy had prognostic significance for shorter survival.

0977

FLOW CYTOMETRIC PERIPHERAL BLOOD SCORE FOR MYELODYSPLASTIC SYNDROMES DIAGNOSIS

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Background. Myelodysplastic Syndromes (MDS) are a heterogeneous group of haematological malignancies which are often difficult to diagnose specially when cytogenetic abnormalities are not found. Cherian *et al.* have recently defined a flow cytometric score (FCs) using peripheral blood (PB) samples that help define MDS. Orthogonal light scatter (SSC) abnormalities and phenotypic changes in neutrophils are taken into account (Cherian *et al.* 200. Cytometry Part B 64B:9-17). The aim of this study was to provide the score usefulness in MDS diagnosis in order to reach the most accurate diagnosis. *Methods*. PB samples from 48 healthy donors and 21 pretreatment patients with suspected MDS or diagnosed MDS were evaluated for the MoAbs combination: CD66FITC/CD10PE/CD45PerCP and CD116FITC/CD45PerCP/CD11aAPC. All MoAbs were purchased from Becton-Dickinson (B-D). SSC abnormalities were also evaluated. Samples were collected in EDTA tubes before 6 hours from collection, stained with panel antibodies mentioned above and later on erythrocyte lysis was performed. Tubes were then washed with a phosphate buffer solution (PBS) and acquired on a FACSort flow cytometer (B-D). Analysis was carried out on the Paint-a-gate software. Corrected mean fluorescent intensity (MFI) was determined for each antibody based on the following ratio: tested antibody MFI - auto MFI / auto MFI. Corrected granulocyte SSC for transformed and non transformed form was calculated by the ratio mean granulocyte SSC/mean lymphocyte SSC. According to WHO criteria, patients were diagnosed with: refractory cytopenia with multilineage dysplasia RCMD

(n=5); refractory anemia with excess blasts type II, RAEB II (n=4); RAEB type I (n=1), refractory cytopenia with multilineage dysplasia and ringed sideroblasts RCMD-RS (n=2); refractory anaemia with ringed sideroblasts RARS (n=2) and refractory anaemia RA (n=1). Six patients with bicytopenia and dysplastic uni - bilineage features were diagnosed with non MDS pathology. Proper cytogenetic studies from 14 patients were obtained and only five of them (35.7%) had cytogenetic MDS characteristic abnormalities. Karyotype was normal in the rest of patients. *Results*. According to Cherian *et al.* indications, standard deviation (SD) and median (M) were calculated for the following parameters in healthy donors (Table 1).

Table 1.

	SCC cRatio	cMFI CD66	cMFI CD10	cMFI CD11a	cMFI CD116
M± SD	7.9±1.2	49.3±14	32±17.3	20.1±4.9	7.89±4.4

cRatio: corrected ratio for non transformed SSC. cMFI: corrected MFI

Score: 1 point was assigned when patient neutrophils had 1-2 SD of the healthy donor values, and 2 points if >2 SD for the SSC diminishing or CD66 and CD11a over-expression. 2 points were scored when patient granulocytes shown CD10 loss expression or showed abnormal expression (over-under) with more than 2 SD of CD116 MFI. A sensibility of 83% and specificity of 66% was found when scores >3 points were assigned to patients. *Conclusions*. According to our results, FCs determined in PB samples is a useful non invasive tool to diagnose different MDS types. However it is necessary to take into consideration that the reproducibility of these results could vary depending on several factors. We would like to highlight that analytical and preanalytical factors may influence surface markers expression on granulocytes. A technique standarization to solve these problems must be reached.

0978

SEROPREVALENCE OF HEPATITIS B, C AND HUMAN -IMMUNODEFICIENCY VIRUS AMONG BLOOD DONORS IN OSOGBO, SOUTHWESTERN NIGERIA

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Background. Screening for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C (HCV) and Human immunodeficiency virus (HIV) was carried out in 2,496 volunteer and replacement blood donors (1988 males and 508 females) at the Ladoke Akintola University of Technology Teaching Hospital, Osogbo between July 2005 and December 2006 after obtaining informed consent from each participant. *Aim*. This is to estimate the seroprevalence of and to identify risk factors of chronic hepatitis B and C and HIV infections among prospective blood donors in our institution. *Methods*. Sandwich immunoassay test strip (Using monoclonal and polyclonal antibodies) was used for the detection of HBsAg while a recombinant double antigen sandwich immunoassay was employed in testing for hepatitis C virus. Antibodies to HIV-1, HIV-2, and HIV-1 O subtypes were detected with the aid of HIV1/2/O Triline Human immunodeficiency virus Rapid Test Device (Qualitative immunochromatographic assay). *Results*. Of the number screened, 496 (19.9%) were positive for HBsAg. 160(6.4%) for anti HCV and 80(3.2%) for anti HIV antibodies. HBsAg and HCV antibodies were found in 40 (1.6%) donors, HBsAg and HIV antibody in 12(0.5%) and anti HCV and anti HIV antibody in 12 (0.5%). There was no case positive for HBsAg, anti HCV and anti HIV antibodies. There was no sex differences in the distribution of single HBV infection (X2=3.287, OR=0.7986, p=0.0698), HCV infection (X2 = 0.6804, OR=1.219, p=0.4095) or HIV (X2 =0.00634, OR=1.023, p=0.9365) among the donors. There was also no significant differences in the age group distribution of HBV infection (X2= 3.043, df =3, p=0.3850), HCV infection (X2=6.474, df = 3, p=0.0907) and HIV (X2=7.544, df= 3, p=0.0564) among the donors. *Summary / Conclusions.* When compared with figures obtained from other parts of the world, the prevalence of isolated chronic hepatitis B, hepatitis C and HIV infections among donors and dual infections of HBV and HCV or HBV and HIV or HCV and HIV are high. There is need to increase public awareness on health care and socio-cultural practices that can predispose to transmission of these viruses in our environment.

0979

MULTISTEP REGULATION OF TELOMERASE DURING DIFFERENTIATION OF HL60 CELLS

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Background. Telomerase plays a key role in maintaining telomere length and in replicative senescence. Telomerase is active in immature somatic cells and is suppressed in differentiated cells, but the mechanism by which telomerase activity is regulated during cell differentiation remains unclear. Several regulatory mechanisms for telomerase have been reported, including transcriptional, translational, and post-translational mechanisms, suggesting that the regulation of telomerase activity is a complex process. *Aims*. To determine the mechanisms modulating telomerase activity during differentiation of the granulocytic and monocytic lineages of hematopoietic cells. *Methods*. A human acute myeloblastic leukemia cell line (HL60) was induced to undergo differentiation into monocytes by exposure to VD3, and into neutrophils by exposure to ATRA or Am80 (an RAR-α/β-selective retinoid that does not bind and activate RAR-Á and RXRs). Changes of several signaling proteins during differentiation were examined by western blotting. To assess the effect of PI3K inhibitors on granulocytic and monocytic differentiation, HL60 cells were preincubated with LY294002 and then differentiation inducers were added before further incubation. Cell cycle progression, esterase staining, flow cytometric analysis, telomerase activity, expression of human telomerase reverse transcriptase (hTERT) protein and mRNA, and epigenetic factors within the telomerase promoter region were examined. *Results*. Telomerase activity and expression of hTERT decreased gradually throughout differentiation of HL60 cells into both lineages. Exposure to any of VD3, ATRA, or Am80 caused a significant increase of various signaling proteins, including AKT, mTOR, and p70 S6K, at 3 days after differentiation. In addition, a dose-dependent increase of telomerase activity was observed after exposure to recombinant AKT and transcription of telomerase was inhibited by LY294002. Pretreatment of HL60 cells with a PI3K inhibitor, LY294002, before induction of differentiation suppressed the phosphorylation of AKT and mTOR. It also decreased the number of α-naphthyl-butyrate-positive cells, without affecting $CD14^-$ or $CD33^-$ positive cells. To further assess the transcriptional regulation of telomerase, a chromatin immunoprecipitation (ChIP) assay was performed. Acetyl-Histone H4 (which binds to the hTERT promoter) underwent deacetylation during differentiation, while trimethyl-Histone H3 remained stable during differentiation. *Conclusions*. There was a decrease of telomerase activity and hTERT (protein and mRNA) expression during granulocytic and monocytic differentiation stimulated by ATRA, Am80 or VD3, respectively. Active forms of AKT, mTOR, and p70 S6K were increased 3 days after the induction of differentiation. It has been reported that AKT promotes transcription of hTERT and post-translationally activates telomerase. The discrepancy between telomerase protein expression and telomerase activity observed during Am80-based differentiation, suggests post-translational regulation of telomerase activity. Changes of acetyl-Histone H4 that regulate transcription of telomerase gene were observed before the activation of AKT, which might mean that epigenetic control of telomerase transcription takes place before AKT activation occurs during differentiation. These results indicate that telomerase activity is regulated by at least two mechanisms during granulocytic and monocytic differentiation, with one being transcriptional and the other being post-translational.

0980

AN ATYPICAL CASE OF A TRANSLOCATION T(8 ;16) (Q12;P13) WITH AN INV(8)(P11Q11) ASSOCIATED WITH A SECONDARY ACUTE MONOCYTIC LEUKEMIA INVOLVES THE MOZ AND CBP GENES

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Background. The infrequent recurrent translocation t(8;16)(p11;p13) is

a cytogenetic hallmark for M4/M5 subtype of poor prognosis acute myeloid leukemia (AML) characterized by blast cells erythrophagocytosis found in both *de novo* and therapy-related AML cases. At the molecular level, translocation t(8;16) fuses the MOZ gene (located on 8p11) and CREB binding protein (Rubinstein-Taybi syndrome; CREBBP, or CBP) gene (located on 16p13), both encoding proteins with histone acetyltransferase activity. Recently, gene expression profiling of AML with t(8,16) reveals a specific pattern of FLT3 and Hox genes expression. We described here an atypical case of a secondary acute monocytic leukemia (M5) with erythrophagocytosis and a t(8;16)(q12;p13) associated with an inv(8)(p11q11) which shown similar gene expression signature. *Materiel and Methods*. A 59-year-old man, initially diagnosed as LMNH has been treated with ESHAP chemotherapy. 2 years later, he developed a secondary AML with peripheral blood analysis showing anemia (Hb 9.9g/dL), hyperleukocytosis (939×10^9/L) and thrombocytopenia (48×10^9/L). Bone marrow aspiration demonstrated hypercellular marrow with 90% blast cells of AML-M5 type of the FAB classification, with erythrophagocytosis feature in 5% of blast cells. According to the ISCN 2005 nomenclature, conventional karyotype analysis at diagnosis was: 46,XY,add(3) (q11), del(8)(q11.1),der(16)t(8;16)(q12;p13)X2,add(20)(q13)[19]/ 46,XY[1]). The patient died four months later. The chromosomal rearrangement and the molecular breakpoint were characterised by FISH, M-FISH and 5' Race molecular analyses. The expression level of Hoxa9/FLT3/MeisI transcripts were performed by quantitative Real Time RT-PCR (RT-RQPCR) in using specific probes for each genes and ABL control gene to normalised results. *Results*. The final karyotype was: 46,XY,del(3) (q11), t(8;16)(q11;p13),+der(16)t(8;16)(q11;p13)X2,der(20)t(X;20)(?;q13)[19]. In order to characterize the sequences fused to CBP, we carried out 5'Race PCR from CBP gene and PCR products were subcloned. As it was unexpected, sequence analysis revealed an in-frame junction between exon 15 from MOZ and exon 4 from CBP genes. The MOZ/CBP fusion transcripts were confirmed by RT-PCR performed with specific primers from MOZ and CBP genes confirming the involvement of MOZ in this rearrangement. As it was recently described by Camos *et al.* in other t(8;16)(p11;p13), RT-RQPCR performed in this case showed an up-regulation of the Hoxa9/FLT3/MeisI expression levels, suggesting a similar leukaemogenesis pathway in all AML with MOZ-CBP involvement. *Conclusions*. In spite of an atypical t(8;16)(q11;p13) associated with a masked inversion inv(8)(p11q12) in a M5 AML, the molecular characterization showed the known breakpoints in 3' part of MOZ and in the 5'end of the CBP genes resulting in a MOZ-CBP in frame fusion transcripts. Moreover, as it was suggested by the Hoxa9/FLT3/MeisI overexpression in this case, this leukaemia would correspond to the specific signature of AML with the recurrent t(8;16)(p11;p13) previously described. These results provide further evidence for the multiple contribution of both MOZ and CBP genes in this particular AML.

0981

CD8+CD28- IN PERIPHERAL BLOOD OF PATIENTS WITH CUTANEUS T-CELL LYMPHOMA (CTLC) IN DIFFERENT CLINICAL STAGES

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Background. Patients with CTLC usually have a poor immune response. $CD8^+CD28^-$ T cells are new types of immune suppressor cells. The study was to analyze the level and changes of them in peripheral blood with CTLC, their effects on immunosupression of CTLC, the influence factors to provide reference for treatment of CTLC patients. *Patients and Methods*. 27 patients with CTLC were enrolled in the study: 8 women and 19 men aged 35-81, medium 57. According to Ann Arbor classification stage I was represented by 1 patient, stage II '6 patients, stage III-5 patients, stage IV-6 patients and stage early stage (premycoticus) was represented by 8 patients. Peripheral blood samples were assessed from all patients with CTLC before treatment and 8 normals. Percentage $CD8^+CD28^-$ T cells was analyzed by flow cytometry. *Results*. Compared with control group percentage of $CD8^+CD28^-$ T cells was significantly higher in patients group p=0,005 (test U Mann Whitney). There were no significant differences of T-cells in among patients with different clinical stages. We revealed the correlation between $CD8^+CD28^-$ cells and advanced clinical stage r=0,54, p=0,035 (Spearman correlation test). *Conclusions*. Percentage of $CD8^+CD28^-$ T cells is increased in peripheral blood in CTLC patients, and correlates with advanced stage.

0982

HIGH FREQUENCY OF CMV-SPECIFIC CD4 AND CD8 IMMUNE RESPONSE AND CORRELATION BETWEEN THEM IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) is characterized by a monoclonal proliferation of lymphocytes mainly B cells (B-CLL) in the blood and bone marrow. Morphological and functional abnormalities of T cells and monoclonality of them have been documented in CLL. Such expanded cells may be specific for recognition of pathogens and cytomegalovirus (CMV) is most likely to be involved in this phenomenon in CLL. CMV infects the majority of the human population in their lifetime and stimulates the generation of a vigorous CMV-specific immune response. In addition patients who are immunosuppressed frequently have supra-normal levels of CMV-specific T cells, which may be a response to subclinical episodes of viral reactivation. However CMV reactivation is an increasing concern with the use of lymphocytotoxic antibodies used in the treatment of B-CLL and is likely to reflect inadequate CMV-specific T cell immunity. *Aims*. Our study hypothesized the relationship between expanded T cells and the CMV-specific CD4 and CD8 immune response in B-CLL. *Methods*. 41 CMV seropositive and 32 CMV seronegative CLL patients were studied. The level of CMV-specific CD4 was detected by intracellular cytokine staining and flow cytometry and the level of CD8 immune response was detected by CMV tetramers. *Results*. The frequency and absolute number of IFNγ and TNFα positive $CD4^+$ T cells were significantly higher in CMV positive CLL patients compared to CMV positive age-matched control group (p=0.0004and 0.0006 for IFNγ ;0. 003 and 0.01 for TNFα respectively) and were higher in patients with a history of treatment compared to untreated subjects (p=0.01 and 0.04 respectively). The level of CMV-specific CD8 immune response was also higher in patients compared to control group. There was a positive correlation between the level of CD4 and CD8 immune response (p=0.009). *Conclusions*. These results show the deep influence of CMV infection and the immune response to it in T-cell abnormalities in CLL patients. This level of immune response is likely to be required to control viral reactivation.

0983

IMMUNOPHENOTYPE OF NK AND NKT CELLS IN PATIENTS WITH CHRONIC NK LYMPHOCYTOSIS

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Background. Chronic natural killer cell lymphocytosis (CNKL) is a persistent state of natural killer (NK) cell ($CD3^-CD16/CD56^+$) excess in the peripheral blood that is not associated with clinical lymphoma. CNKL is a heterogeneous but not well characterized disorder. *Aims*. The purpose of this study was the definition of the phenotypic markers of human NK- and NKT-cells in healthy individuals and patients with CNKL. *Methods*. Peripheral blood lymphocytes of 20 patients with CNKL (defined by more than 500 NK lymphocytes/μL) and 20 healthy individuals were labeled with monoclonal antibodies CD3, CD56, CD16, CD2, CD7, CD8, CD11a, CD57, CD11c, CD45RA, CD45RO, HLADR, TCRÁ‰, perforin, granzyme A by direct whole blood staining and were analyzed by four-color flow cytometry. *Results*. The NK cells percentage increase was mainly due to $CD16^+$ NK cells (p<0,001) in comparison to healthy individuals and not to $CD56^+$ NK cells. This increase was accompanied by a percentage increase of NKT cells (p=0,005) concerning mainly $CD3^+CD56^+16^+$ NKT cells (p=0,005). In addition, NK cells showed higher percentages of CD45RO (p=0,045), CD57 (p=0,021) and HLADR (p=0,027) and higher expression (intensity) of perforin and granzyme A in CNKL patients in comparison to healthy individuals, whereas $CD3^-CD56^+$NK cells showed higher percentages of CD45RO (p=0,046), CD57 (p=0,023) and HLADR (p=0,019) positivity and decreased incidence of CD7 (p=0,015). CNKL patients showed in NKT cells higher percentages of HLADR (p=0,050) and a very significant increase of perforin positive cells percentage in comparison to healthy individuals (9+11% vs 30+32%, p=0,010), whereas $CD3^+CD56^+$NKT cells showed higher percentage of HLADR (p=0,045) expression. *Conclusions*. CNKL patients showed increased $CD16^+$ NK cells and higher percentages of CD45RO, CD57 and HLADR, concerning mainly $CD56^+$NK cells, a phenotype possibly characterizing CNKL. Abnormal functionality of NK cells was

observed, as this was assessed by the determination of perforin and granzyme A expression (intensity). NK and NKT cells immunophenotyping of the CNKL patients, accompanied by clinical and laboratory features, could help in the better definition of this haematological disorder.

0984

A NEW OPTION FOR AN ANCIENT DISEASE: ANGIOGENESIS THERAPY BY BONE MARROW TRANSPLANTATION IN ARTERIAL LIMB ISCHAEMIA

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Background. therapeutic angiogenesis has recently been developed as a new method of treatment for several ischaemic diseases; both experimentally and clinically there are preliminary data suggesting that implantation of bone marrow-mononuclear cells into ischaemic limbs increases collateral vessels formation. *Aims*. to evaluate viability of the therapeutic angiogenesis using hematopoyetic bone marrow progenitors mobilized by G-CSF (granulocyte colony stimulating factor) and safety of the procedure. *Methods*. 40 patients developing critical arterial limb ischaemia (candidates to amputation) were included in this study (International Working Party). 23 men and 17 women. Median age 65 years old (44-86). Mobilized by filgrastim (Neupogen®) 5 µg/kg weight daily (5 days). Bone marrow harvest at 5th day. Local anaesthesia was employed in all the patients. Unmanipulated cells were injected in the affected limb in 2 ml aliquots into the gastrocnemius muscle. All the patients received injections unless in one limb. 1 patient received treatment in both. Each patient was evaluated regularly for rest pain, amount of required analgesia , healing of the ulcers, peack walking time, Doppler and angiographic findings. The mean number of injected mononuclear cells was $1,9\times10^9$/kg. All the patients received low molecular weight heparin (nadroparin, Fraxiparine®) 3800-5600 IU anti-Xa subcutaneously , aspirin 81 mg and pentoxifiline 400 mg daily, as medical treatment after the procedure for at least 60 to 90 days. A control population of 39 vascular patients affected by critical arterial limb isquemia was considered. They don't received angiogenic treatment, only the same medical antithrombotic schedule described. *Results*. there were no secondary effects for the mobilization or injection of cells in the 40 studied patients. There were no dead patients secondary to the procedure. Moreover, in 30 patients with a median follow up of 24 months, 32 patients showed an improvement of all parameters, mainly pain at rest, peack walking time and skin trophic lesions. Two patients suffered amputation of the affected extremity because obstruction of an old by-pass (5%). On the control population, amputation of the affected extremity was necessary in 34 of the 39 patients evaluated with medical treatment only (87,2%). The statistical differences betwen the two groups were highly significant in favor of the angiogenic group. They were evaluated by the chi square test and log rank test ($p<0,05$). *Conclusions*. our preliminary data suggest that autologous bone marrow transplantation can be performed safely and appears to be a benefical therapy for selected patients with severe peripheral arterial disease.

0985

BCR-ABL MUTATED CLONE PROGRESSION AND ELIMINATION IN CML PATIENTS TREATED WITH ABL KINASE INHIBITORS

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Background. Imatinib is an effective tyrosine kinase inhibitor highly efficacious in treatment of CML patients in chronic phase. However, a resistance to imatinib is occurred in some patients. One mechanism of acquired resistance is mutations in BCR-ABL kinase domain, which is the binding site to imatinib. The crossover to dasatinib is promising to overcome the imatinib resistance. *Aims*. The main aim of this study was to monitor the level of mutated BCR-ABL clone in CML patients treated with imatinib and dasatinib in the retrospective analysis and propose the clinical aspects. *Methods*. Hematological and cytogenetic examinations and data from the routine monitoring of BCR-ABL mRNA level were evaluated in 150 patients treated with imatinib. Thirty six patients developed the resistance or presented a suboptimal response to imatinib. These patients were screened for BCR-ABL mutation by sequencing. Twelve mutation positive patients treated with imatinib including 4 patients with crossover to dasatinib enrolled in clinical trials (Bristol-Myers Squibb) were involved in the study (130 samples). The Mutation Surveyor 3.01 program (Softgenetics) was used for the mutation detection in sequenced 914 bp PCR product generated by two steps PCR. The two steps PCR procedure was used for sensitive and specific amplification of a cDNA region encoding the BCR-ABL kinase domain. The percentage of mutated alleles presented in each sample analyzed was calculated by DNA quantification tool using Mutation Surveyor. *Results*. The level of BCR-ABL transcript and the percentage of a mutated allele per each sample were compared. Two groups of patients were formed: A. The group with significant increase of BCR-ABL transcript and hematological relapse correlated with increasing amount of mutated BCR-ABL clone (n=4). B. The group with permanently high level of BCR-ABL mRNA (10-100%) independent from mutation presence and kinetics (n=7). One patient was not involved in either group because only one sample before crossover from imatinib to dasatinib was available. In one patient (group B) mutation E255K was detectable already before imatinib treatment. Two mutations were detected in 4 patients (n=2 group A; n=2 group B). In 3 of 4 patients with crossover to dasatinib (n=1 group A; n=2 group B; n=1 ungrouped), the mutated leukemic clone was reduced or eliminated after the crossover, however in one patient a new BCR-ABL clone with different mutation was multiplied. In all 4 patients the BCR-ABL mRNA level was significantly reduced under dasatinib treatment. *Conclusions*. We found that the mutation development and its impact on resistance to imatinib are individual and not dependent on the type of mutation. In group A, the resistance to imatinib and disease progression was confirmed and mutations might contribute to disease progression. These patients are at high risk and the change of treatment is necessary. In group B, the suboptimal response to imatinib is independent on mutations. Probably another mechanism of resistance is involved. The crossover to dasatinib seems to be very successful, however monitoring of BCR-ABL transcript level and mutation detection is necessary.

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THE ROLE OF TGF-β INDUCIBLE EARLY GENE 1 (TIEG1) IN HUMAN BONE MARROW B LYMPHOPOIESIS

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Background. B lymphopoiesis in the bone marrow (BM) is tightly regulated by the microenvironment, where stromal cells and components of the intracellular matrix provide important factors. Identification of these factors in the B-cell maturation niche is important to understand the regulation of B lymphopoiesis in normal cells as well as in acute lymphoblastic leukemia (ALL). TGF-β and several BMPs which are produced by the BM microenvironment, have been shown to regulate cellular fate and division of several haematopoietic progenitor cells, including B progenitor cells and leukaemias. TGF-β and BMPs initiate signaling by binding to combinations of types I and II serine/threonine kinase receptors. Subsequently, the signal is transferred to their nuclear effectors termed Smads (Kawabata *et al.*, 1998) and a range of target genes are activated, including members of the ID family of transcription regulators. Previously, the TGF-β-responsive protein TGF-β inducible early gene 1 (TIEG1, also called KLF10) has been shown to be rapidly induced in osteoblasts upon stimulation with several BMPs and TGF-β. Overexpression of TIEG1 mimics the TGF-β action in osteoblasts (Hefferan *et al.*, 2000). *Aims*. The aim of this study has been to study expression and regulation of TIEG1 in BM B progenitor cells and leukemia cell lines. Further we want to investigate the involvement of TIEG1 in the TGF-β/BMP effect on human B lymphopoiesis with particular focus on the TGF-β/BMP signaling pathways. *Methods*. Microarray, Realtime PCR, Northern blot, Western blot, Thymidin assay, CFSE assay, siRNA, luciferase reporter assay, transient over-expression of TIEG mRNA by electroporation. *Results*. We have previously demonstrated by microarray analysis that TIEG1, along with other members of the TGF-β/BMP signaling pathway are tightly regulated during early human B lymphopoiesis (Hystad *et al.*, manuscript). Confirmation of these findings by real-time PCR clearly shows that the expression level of TIEG1 decreases dramatically as the cells differentiate from the pro-B to the pre-B stage. TIEG1 is also highly expressed in several pre-B ALL cell lines. This is in line with our findings that TGF-β and BMP6 inhibit proliferation and induce expression of TIEG1 mRNA in several pre-B ALL cell lines. The induction of TIEG1 mRNA is rapid (1 hour) and transient, and parallels the induction of the ID3 and SMAD7 mRNAs. The role of TIEG1 as a mediator of TGF-β/BMP signaling and the functional effect in human B progenitor cells and pre-B ALL cell lines is currently being investigated. *Summary/Conclusions*. We have shown that TIEG1 is tightly regulated in

normal human BM B progenitor cells and in pre-B ALL cell lines. The expression of TIEG1 mRNA is regulated by TGF-β and BMP6, and the role of TIEG1 in mediating the inhibition of proliferation in B progenitor cells is currently being studied.

0987

EVALUATING PULMONARY COMPLICATIONS IN ACUTE CHILDHOOD LEUKEMIA

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Background. Pulmonary complications are frequently encountered in childhood acute leukemias and infectious complications constitute the leading etiology. *Aims*. The purpose of this study was to evaluate the frequency, etiologic factors, and the comorbid conditions affecting the morbidity and mortality of pulmonary complications in acute childhood leukemia. *Methods*. All patients who had been treated in Dokuz Eylül University Department of Pediatric Hematology and who developed pulmonary complications at the time of diagnosis, during and after the treatment between November 1989 and August 2005 were included in this retrospective study. Patients were treated by the BFM protocols. Clinical, laboratory, and radiologic features were evaluated as well as the properties of the acute leukemia. Properties associated with the diagnosis, treatment, clinical course, and outcome were investigated. *Results*. A total of 163 pediatric acute leukemia patients were included in the study. Sixty-six (40.4%) of them developed a total of 79 pulmonary complications. The median age was 6 years (range 1-17). Seventy (88.6%) of the pulmonary complications were observed in ALL and 9 (11.4%) in AML patients. They mostly developed in the consolidation phase of therapy. Of these pulmonary complications, 92.4% had infectious etiology. Noninfectious causes were ARDS in 3, leukostasis in 2, lenfomatoid granulomatosis in 1, GVHD in 1, pneumothorax in 1, pulmonary edema due to capillary leak syndrome in 1, and bronchial hyperreactivity in 1 patient. Twenty-five patients (31.6%) were neutropenic during the pulmonary complication. In 14 (17%) complications microbiological confirmation of the infectious agent could be successed. The most identified agent was Klebsiella pneumonia, followed by Aspergillus flavus and Escherichia coli. Seven (%8.9) patients died because of pulmonary complications, all were neutropenic and 3 of them weren't in remission. Tachypnea, clinical manifestations of shock, requirement of oxygen and mechanical ventilation, disseminated intravascular coagulation, involvement of other organs or systems, neutropenia, anemia, thrombocytopenia, and requirement of modification in antimicrobial drugs were found in association with increased mortality risk ($p<0.05$). In the maintainance phase, patients with pulmonary complications had better hematologic values and the pulmonary complications could be more successfully and rapidly taken under control with oral antibiotics in the most of the patients ($p<0.05$). *Conclusions*. Pulmonary complications are frequent in children with acute leukemia, and infections are the leading cause. Gram (-) bacteria and fungi are the most identified agents. If the patient is neutropenic and have multisystemic involvement, the prognosis gets worse. In the maintainance phase, in which the treatment intensity decreases, the frequency of pulmonary complications is lower and may be treated more successfully and rapidly even with oral antibiotics.

0988

ALLOGENEIC MARROW TRANSPLANTATION IN CHILDREN WITH ACQUIRED APLASTIC ANEMIA : THE TUNISIAN EXPERIENCE FROM A SINGLE CENTER

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Introduction. Acquired severe aplastic anemia (ASAA) is a rare disease which can be cured with bone marrow trasplantation (BMT) from an HLA-matched sibling donor, resulting in 60-92% survival rates. The aim of our retrospective study was to analyse the outcome in 50 children with newly ASAA, receiving BMT from HLA-identical sibling between 1998 and 2006. *Patients and Methods*. Patients were 2 to 18 years of age (median, 13 years). Conditioning regimen consisted of cyclophosphamide (CY) and antithymocyte globulin (ATG). Marrow cells (MC) was infused 48h after the last dose of CY. The median no. of MC was $3,07\times10^8$/kg (range, 0.67-5,5). Cyclosporine A and short methotrexate were administred as graft-versus-host disease (GvHD) prophylaxis. *Results*. Forty-seven patients (94%) were evaluable for engraftment. Forty-three patients (91%) had sustained grafts, whereas four patients (8%) rejected grafts. Two are alive after second allogeneic stem cell transplantation and two died from infection. Acute grade 2-4 GvHD was seen in only 6% of patients. An extensive chronic GvHD was seen in one patient. After a median follow-up of 46 months (range, 2-92 months), the actuarial survival rate at 5 years was 85%, with a Lansky performance score of at least 90%. *Conclusion*. We conclude that CY-ATG conditioning is well tolerated and effective in children with ASAA. Allogeneic BMT should be the first-line therapy for such patients.

0989

TRANSCRIPTIONAL REGULATION OF HUMAN DENDRITIC CELL SUBLINEAGE DIFFERENTIATION BY PU.1, RELB AND NOTCH

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Epithelial Langerhans cells (LCs), interstitial type dendritic cells (intDCs) and plasmacytoid dendritic cells (pDCs) represent three major DC subsets. All three lineages can be generated efficiently from a myeloid pathway, and tissue-specific signals are critical for their differentiation from precursors. We established *in vitro* differentiation conditions for all three DC subset pathways from human $CD34^+$ progenitors and performed retroviral transduction experiments to modulate transcription factor expression levels. We analyzed PU.1, RelB and Notch signalling in response to Delta-like 1 (DL1), since these factors were previously implicated in both DC subset development and myelopoiesis. How these factors take part in a transcriptional network regulating three-lineage human DC subset specification is not known. We found that monocyte-derived intDC development depends on RelB and is enhanced by DL1/Notch signaling. Conversely, pDC development occurs independently of RelB, but is similarly promoted by DL1. We further observed that RelB inhibition promotes pDC generation in the presence of DL1. In contrast to these two lineages, TGF-β1-induced LC generation occurs independently of RelB and is not significantly modulated by DL1. Ectopic PU.1 promoted $CD1a^+$ intDC and LC generation depending on presence or absence of TGF-β1, but failed to increase percentages of generated pDCs. These data suggest that RelB regulates lineage decisions of a common pDC/intDC progenitor and both lineages are positively stimulated by DL1. Furthermore, PU.1 upregulation might restrict lineage options of shared DC progenitors.

0990

EFFICACY AND SAFETY OF 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 such as corticosteroids, rituximab and splenectomy. Recently there are same report 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 31 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 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 concomitant > 2.0 g/dL rise in Hb concentration. All three patients received 30 subcutaneous administration of alemtuzumab(total dose 300 mg). 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, with mild (grade I) haematological and extra-haematological side effects. 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. Regarding clinical responses, one patient obtained SD, the other two patients achieved PR. All three patients underwent further treatment because of disease progression after 9,10 and 26 months from the end of alemtuzumab therapy. We show considerable efficacy in the treatment of transfusion-dependent and resistant AIHA in pretreated BCLL patients. Our study demonstrates that an identical AIHA response can be obtained with a lower dose of subcutaneous alemtuzumab respect to the standard dose with a similar duration of response, less hemato/extrahematological complications even if a prolonged treatment was required to achieve the same response. Alemtuzumab may be worthwhile to consider before rituximab if AIHA is accompanied by progressive CLL in need of cytoreductive therapy. Therefore, further studies with alemtuzumab in the setting of CLL/AIHA are warranted considering that this monoclonal antibody appears effective in the treatment of both the leukaemia and the autoimmune complication.

0991

PROHYLAXIS OF INVASIVE FUNGAL INFECTIOTNS (IFI) WITH HIGH DOSE LIPOSOMIAL AMPHOTERICINE-B (L-AMB) IN ACUTE NON LYMPHOID LEUKEMIA (ANLL): A SINGLE CENTRE EXPERIENCE

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The efficacy of L-AmB seems to be related both to improved tissue penetration and to sustained bioactivity of drug levels in lung, brain, kidney, liver, spleen. (Walsh *et al*. 2001; Anaissie *et al.* 2004). On the basis of this issue, we have planned a pilot study for IFI prophylaxis in adult ANLL with a single dose of L-AmB (15 mg/Kg) during post induction neutropenia. Primary endpoint: to valuate the incidence of fungal infection according to International Consensus (Ascioglu *et al.* 2002) during and up to four weeks after prophylaxis. Protocol prophylaxis: patients received a single dose of L-AmB at 15 mg/Kg i.v on the day after the end of induction. A single dos was repeated 15 days later in those cases with persistent neutropenia. L-AmB PK profile was tested at following times: 1st day (0,1,4,24 hours), 7st and 14st days from drug administration. Inclusion criteria were: 1) neutropenia (PMN <0.5×10^9/L) longer than ten days; 2) surveillance cultures and mannano and galattomannano antigens negative; 3) no fever and/or clinical signs of infection. From October 2004 to July 2006, 28 consecutive adult ANLL-18 m, 10F, median age 63 yrs (39-78), 18 AML *de novo*, 10 secondary AML-received median dose of L-AmB 1050 mg (range 750-1300); 6 pts received a second dose. Toxicities >2 WHO have not been reported ;overall median duration of neutropenia was 21 days (range 11-42). 17/28 pts (60%) achieved complete haematological remission, 5 was resistant and 6 died during induction aplasia; 3 cases had proven IFI (2 Aspergillus Flavus, 1 Candida Albicans) and 1 probable infection. The median L-AmB PK results are available in 15 pts: h0: 0.0; h1: 5.57; h 4: 16.72; g 2°: 10.08; g 7° : 0.43; g 14°:0.0. The results obtained in this study showed that L-AmB single large dose is an effective and safe approach in IFI prophylaxis. Further studies might demonstrate the correlation between PK, plasmatic clearance, uptake in reticulo-endothelial system and IFI prophylaxis.

0992

EVALUATION OF THE COMBINATION EFFECT OF FACTOR V G1691A, FACTOR II G20210A AND HYPERHOMOCYSTEINEMIA ON THE INCIDENCE OF DEEP VENOUS THROMBOSIS

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Backergound. Inherited predisposition to venous thromboembolic disease are the mutations in gene encoding factor V (G1691A) and G20210A variation of the factor II gene. Hyperhomocysteinemia is a recognized independent risk factor for occlusive vascular disease. *Aim*. The aim of this study was to evaluate the combined effect of factor V G1691A (FV-Leiden, FVL), factor II G20210A (PTH) and hyperhomocysteinemia on the incidence of deep venous thrombosis (DVT). *Patients and Methods*. We enrolled 102 patients (aged 41.59±14.11, 54 male-48 female) with first episode of deep venous thrombosis and 46 healthy individuals (aged 45.99±14.98, 20 male-26 female). Factor V and factor II genotypes were analyzed using PCR amplification. Total homocysteine (tHcy) levels were determined with ADVIA Centaur® GP Immunoassay System (Chemiluminescence, Bayer). Total homocysteine levels above the upper limit of the laboratory ranges (>13.9 μmol/L) were considered as hyperhomocysteinemia. We calculated the genotypes frequency, odds ratio (OR) with 95% confidence intervals (CI) and we performed logistic regression analysis. Statistical significance was set at $p<0.05$. *Results*. The frequency of FV-Leiden genotype (G/A or A/A) was significantly higher among patients than healthy individuals (25.5% vs 6.5%, $p=0.007$, OR=4.904, 95% CI=1.402~17.153). Hyperhomocysteinemia were observed among patients in higher frequency versus healthy individuals (41.1% vs 23.9%, $p=0.043$, OR=2.227, 95% CI=1.017~4.878). The prevalence of factor II G20210A genotype was similar between patients and healthy individuals (10.8% vs 4.3%, $p=0.2$, OR=2.65, 95% CI=0.565~12.517). In order to evaluate the combination effect of the above mutations and hyperhomocysteinemia on the incidence of DVT, we divided the entire cohort into three groups according to the presence of one or two mutations and hyperhomocysteinemia. The combination of the two mutations and hyperhomocysteinemia was not detected in controls. The group without any mutation and with normal tHcy levels was used as reference group. The combination of FVL with hyperhomocysteinemia significantly increased the risk for DVT compared to the reference group (OR=6.572, 95% CI=0.833~51.83, $p=0.023$). In contrast, the combination of PTH G/A with hyperhomocysteinemia does not increase significantly the risk for DVT compared to the reference group ($p=0.293$, OR=2.811, 95% CI=0.329~24.035). *Conclusions*. Our results suggest that hyperhomocysteinemia combined with FV-Leiden genotype and not with factor II G20210A genotype increases the risk for deep venous thrombosis.

0993

EFFECTIVE INTRAMYOCARDIAL IMPLANTATION OF AUTOLOGOUS BONE MARROW-DERIVED MONONUCLEAR CELLS IN PATIENTS WITH END STAGE ISCHEMIC CARDIOMYOPATHY

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Background. Ischemic myocardial damage is an increasing cause of heart failure in the western world and has long been considered irreversible because adult cardiomyocytes are terminally differentiated and do not proliferate. Stem cells are undifferentiated cells capable of self-renewal, proliferation, and differentiation into multiple lineages permitting tissue regeneration. A number of types of stem cells are now recognized, as well as partially differentiated progenitor cells that are capable of proliferation and differentiation to multiple lineages. Reversal of heart failure would require myocardial revascularization, remodelling of the left ventricle and replacement of damaged myocyte. *Patients and Methods*. We investigated the safety of transplanting un-manipulated autologous bone marrow into infracted myocardium in 16 patients with NYHA class III and IV heart failure. These patients underwent coronary bypass using the pi-circuit technique and external reshaping of left ventricle in off-pump surgery. We evaluated the efficacy of this combined technique in the improvement of cardiac function. Autologous bone marrow (400ml) was obtained by bilateral posterior iliac bone aspiration at the time of surgery. Bone marrow mononuclear cells (BM-MNCs) were isolated by means of a density Ficoll-Paque gradient. Then the cells were exhaustively washed and re-suspended in a normal saline solution containing 5% human serum albumin. Cell count, viability and cultures were appropriately performed. Following the operation the BM-MNCs (30ml, 1.5±0.8 ×10^9 BM-MNCs) were injected directly to the myocardium of the left ventricle. *Results*. No significant complications were observed. The left ventricular ejection fraction (EF) at rest was improved significantly almost in all patients from 21.7±7.4 to 37.7±4.2%, ($p<0.0001$), 12-18 months following the operation. Furthermore, we observed significant reduction of the end diastolic volume of the left ventricle from 66.1±4.2 to 59.3±4.2mm, ($p<0.001$). Thallium scanning and MRI, revealed hypokinesia or reduced distribution of radio-opaque contrast in areas, where previously akinesia or lack distribution were observed. Along with the reperfusion assessment we measured consecutively biochemical markers of immuno-response such as the chemotactic proteins IL-8 and MCP-1 as well as the protein of innate immunity and energy balance leptin. We observed a significant reduction of these proteins levels immediately after the injection of BM-MNCs, while months later these proteins were increased in parallel with the amelioration of patients' clinical status. *Summary and Conclusions*. It remains possible that BM-MNCs do confer some beneficial effects, possibly by secreting paracrine factors that are cardioprotective and/or angiogenic. These findings suggest that transplantation of un-manipulated autologous bone marrow into the peri-infract areas of heart in combination with myocardial revascularization and remodelling of the left ventricle is safe, effective and ameliorates patients' cardiac function and quality of life.

0994

INCIDENCE OF INVASIVE ASPERGILLOSIS (IA) IN A TERTIARY HEMATOLOGICAL CENTRE IN CZECH REPUBLIC, EVALUATION OF THE GALACTOMANNAN MONITORING

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Background. IA is a major opportunistic infection in patients with hematological malignancies. The incidence of IA has increased in the past years. The high mortality due to IA reflects both the invasivity of the pathogen and the profound immunosuppression of the host organism. *Aims*. We retrospectively evaluated the monitoring of galactomannan (GM) - twice a week sampling using a commercial kit (one-stage immunoenzymatic sandwich microplate assay) in a tertiary hematological centre. The aim was to assess the burden of IA and to evaluate the benefit of GM monitoring. *Methods*. In the study period (5/2005-12/2006) 330 patients at risk of IA (407 patients were hospitalized in the same period in total) were repetitively sampled for GM. Median number of samples per patient was 12. The spectrum of underlying diseases for the 330 sampled patients was: 33% AML, 19% MDS, 21% lymphoproliferative diseases and multiple myeloma, 14% myeloproliferative diseases, 6% high dose corticosteroid treatment, 7% others. The same population of 330 patients divided according transplant status: 35% after allogeneic stem cell transplant (SCT), 3% after autologous SCT and 62% nontransplanted. For the IA case-definition we used the EORTC/MSG criteria, but as clinically relevant we considered only the *proven* and *probable* categories. We omitted the *possible* EORTC/MSG category from our analysis. For the defining positivity of GM we used the dynamic cut-off (index > 0,5 in two samples) for serum samples and static cut-off (index > 0,5 in a single sample) for bronchoalvelar lavage (BAL) samples. *Results*. None of the GM non-monitored patients developed IA in our centre in the study period and there was no autopsy or biopsy proven case of IA in the study period in the GM monitored group, which would escape early diagnosis. 23 patients in the sampled group (n=330, all hospitalized) fulfilled the criteria of proven (2 cases) or probable IA (21 cases). Of the 23 proven and probable cases: 15 patients were treated for hematological malignancies - nontransplanted, 7 patients were after allogeneic SCT and 1 suffered from severe aplastic anemia. As a microbiological criterion for diagnosis served in 19 cases positive serum GM, in 2 cases positivity of GM from BAL, in 1 case cultivation from blood culture and concomitantly positive serum GM and in 1 case positive histology of pulmonary tissue and concomitantly positive serum GM. Only two of the 23 patients diagnosed to have IA are alive on follow-up (at 2/2007). The incidence of IA among hospitalized patients in our centre was in the study period 5,7% (23/407). The overall mortality (both IA related and IA non related) in patients with diagnosed IA reached 91% on follow up. *Summary*. We conclude that the incidence of 5,7% in our cohort is in concordance with published numbers for its the risk profile. GM is of considerable help in the diagnosis of IA (although we could not validate its performance in terms of sensitivity and specificity). The mortality in patients with IA is very high on follow-up.

0995

THE SIZE OF THE ACTIVE HEMATOPOIETIC STEM CELL POOL FROM BIRTH TO ADULTHOOD

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Background. Hematopoietic stem cells (HSC) are the targets of a variety of genetic disorders, both inherited and acquired that can lead to either marrow failure or neoplastic proliferation. The risk of acquiring mutations is related to the replication rate, mutation rate and the population of stem cells at risk. It is thought that only a fraction of the HSC are actively contributing to hematopoiesis and it is this fraction that is at highest risk of acquiring mutations. *Aims*. To determine the size of the active HSC pool (NSC) from birth to adulthood. *Methods*. HSC contribute to formation of all types of blood cells. Hence, the total circulating reticulocyte count is a marker of the size of the active HSC pool for any adult mammal. We determined the total number of circulating reticulocytes in adult mammals across 40 species and correlated this with their average mass. NSC should scale in the same way with mass. Utilizing the known size of the active stem cell pool in cats, we can determine the size of the active HSC pool in any mammal including adult humans. We utilize a similar relationship for humans during growth to estimate how the HSC pool expands from birth to adult life. *Results*. In adult mammals, including humans, the active stem cell pool scales with mass as NSC ~ M 3/4 while during human growth, NSC ~ M. Using the known size of NSC in cats, we estimate that an adult human (M~70kg) has an active stem cell pool, NSC ~385 cells, while a newborn (M~3.5kg) has NSC~22 cells. In addition, using data obtained from transplantation in cats suggests that in adult humans, the average size of NSC~116 after a bone marrow transplant. We estimate that HSC replicate approximately 1/year. Our results are in excellent agreement with published reports on informative patients with chronic granulomatous disease and after marrow transplantation respectively. *Summary/Conclusions*. The active hematopoietic stem cell pool is small in humans and it expands linearly with mass during ontogeny growth. HSC replicate slowly and this may be one mechanism to decrease the risk of acquiring mutations in a pool of cells that contribute to hematopoiesis for many years.

0996

PRE-TRANSPLANT MYELOFIBROSIS IS INVERSELY CORRELATED WITH THE DEVELOPMENT OF EXTENSIVE CHRONIC GRAFT-VERSUS-HOST DISEASE IN PATIENTS WITH MYELOID MALIGNANCIES AFTER REDUCED INTENSITY STEM CELL TRANSPLANTATION

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Background. Secondary myelofibrosis (MF) frequently occurs in patients with hematologic malignancies and successful treatment of underlying disease has resulted in the reversal of bone marrow fibrosis. MF in patients with acute leukemia has been regarded as an adverse prognostic factor, attributable to the possible interference with marrow regeneration after transplantation. However, the clinical significance of secondary MF in reduced intensity stem cell transplantation (RIST) has not been fully evaluated. *Aims*. We evaluated the effects of pre-transplant MF on the transplantation outcomes in patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS) undergoing RIST. *Methods*. We evaluated pre-RIST bone marrow MF grade using conventional method (N= 39, median age 42.0). Donors were HLA-matched siblings in 30 cases (76.9%) and HLA-matched unrelated volunteers in 9 (23.1%). Eleven (28.2%) patients received bone marrow and 28 (71.8%) received G-CSF-mobilized peripheral blood. All patients received fludarabine-based conditioning regimens combined with busulfan (82.0%), cyclophosphamide (10.3%) or melphalan (5.1%). Cyclosporin A or FK506 was used for graft-versus-host disease (GVHD) prophylaxis. Patients were divided into two groups according to the existence of pre-transplant MF (20 as non-MF group and 19 as MF group). *Results*. Number of total nucleated cells and $CD34^+$ cells infused was not different between two groups. Engraftment was documented 11 days after RIST in non-MF and 13 days after RIST in MF group (p=0.528). The platelet recovery was not different according to the MF. Graft failure was totally documented in 3 cases (7.7%) which all had advanced disease status in the absence of MF. There were no differences in the incidence of acute GVHD, bacterial or fungal infections, and cytomegalovirus (CMV) infection according to the MF. However, the incidence of extensive chronic GVHD was significantly lower in the MF group compared to the non-MF group (11.2% vs 40.7%; p=0.035). Relapse rate and non-relapse mortality were not different according to pre-transplant MF (p=0.060 and p=0.350, respectively). Extensive chronic GVHD significantly correlated with increased event-free survival rate (p=0.01). Multivariate analysis revealed that pre-transplant MF significantly associated with reduced risk of extensive chronic GVHD (odds ratio 0.107, 95% CI 0.014'0.838, p=0.033). *Conclusions*. Pre-transplant MF was significantly associated with reduced risk of extensive chronic GVHD after RIST. The underlying immunologic mechanisms should be further evaluated.

0997

BONE MARROW TRANSPLANTATION IN A GIRL WITH HERMANSKY-PUDLAK SYNDROME AND LEUKEMIA

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Introduction. Hermansky-Pudlak syndrome (HPS) is a rare, autosomal-recessive disorder characterized by oculocutaneous albinism and bleeding tendency caused by a platelet storage pool disease. The patient reported here also suffered from acute myeloid leukemia (AML). To our knowledge a correlation between HPS and leukemia has not been reported. As the patient was transplanted for her AML we could also study the effect of BMT on the bleeding disorder associated with HPS. *Case report*.

A 4-year old girl, child of non-consanguineous parents, presented with easy bruising and recurrent nosebleeds starting at the age of ambulation. On physical examination the girl had pale skin and blond hair, pale-blue irises with occasional outbursts of horizontal nystagmus and many hematomas mostly on legs and arms. Both parents had normal pigmented skin and hair. Ophthalmic examination revealed reduced visual acuity. Laboratory investigations showed normal platelet counts and morphology, normal prothrombin time (PT) and activated partial thromboplastin time (APTT), normal platelet aggregation with ADP and collagen-arachidonic acid. Bleeding time however, was prolonged (8-8-9.25 minutes) and ADP in platelets was decreased (0.9 nmol/10*8 platelets). Platelet Function Analysis (PFA)performed at a later stage showed delayed closure time with epinephrine (>300seconds). She was diagnosed with Hermansky-Pudlak syndrome. Three years later, at the age of 7, our patient presented with persisting fever, malaise and rash. The peripheral blood showed pancytopenia with 95% blasts. Examination of the bone marrow aspirate with morphology, immunophenotyping and cytogenetics revealed acute myeloid leukemia (AML), FAB classification M1. There were no good risk cytogenetics (t(8:21), t(15:17) or inv(16)). There was no CNS involvement. Our patient was treated according to the MRC-AML 12 protocol. After the first course she attained complete remission. Two-and-a-half years later however a relapse of AML occurred, again without CNS involvement. Treatment according to the DCOG Relapsed AML-protocol 2000/2001 was commenced. After achievement of a second complete remission our patient received BMT with T-cell depleted marrow from a matched unrelated donor. Conditioning regimen consisted ofATG, cyclophosphamide and total body irradiation of 2‡6 Gray. The transplantation procedure was uneventful. Six weeks post-transplantation coagulation screening was repeated. Platelet counts were normal, as were PT, APTT, platelet aggregation and PFA. ADP in platelets has normalized to 3.1 nmol/10*8 platelets. Until one year after BMT the patient was well without sign or symptom of a bleeding disorder. One year after BMT a second relapse of AML occurred and she died 3 weeks later. *Conclusion*. HPS is a rare disorder causing oculocutaneous albinism and bleeding tendency. It is considered to be a defect in intracellular vesicle formation in platelets, melanocytes and lysosomes. There is no evidence suggesting a link between HPS and leukemia, as was the case in our patient. We have shown that the bleeding tendency can be successfully corrected by bone marrow transplantation, which may be an option for those few patients with serious bleeding problems. However, whether serious complications like lung fibrosis can be prevented by bone marrow transplantation remains yet to be seen

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SIMULTANEOUS DETECTION OF FLT3, NPM1 AND WT1 MUTATIONS USING HIGH-RESOLUTION CAPILLARY ELECTROPHORESIS

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Background. FLT3-Internal Tandem Duplication (FLT3-ITD) and Nucleophosmin (NPM1) mutations are the most frequent genetic alterations in normal karyotype AML. FLT3-ITD results in inframe duplications within the juxtamembrane region (exon 14 and 15). In contrast mutations in NPM1 result in frameshift mutations in exon 12. Both FLT3-ITD and NPM1 mutations are strongly associated with NK-AML prognosis (adverse and favourable respectively). We have recently shown that the mutations in Wilms' Tumor-1 (WT1) cluster to exons 7 and 9 and typically result in short regions of insertion or deletion. The change in PCR product size makes these suitable for high throughput mutational analysis using high-resolution Capillary Electrophoresis (CE). We have set out therefore to establish a reliable screening method for simultaneous detection of FLT3, NPM1 and WT1 mutation. *Patients and Methods*. FLT3, NPM1 and WT1 mutation status was determined by DNA amplification and direct sequence analysis of 83 NK-AML patients. Multiplex PCR (QIAGEN Multiplex PCR Kit) for all 3 genes were performed using 5' end dye labelled reverse (R) primers [FLT3 (HEX), NPM1 (HEX), WT1 exon 7 (FAM), WT1 exon 9 (TAMRA)], followed by CE using a Genetic Analyser. *Results and Conclusions*. Twenty samples were selected for analysis based on their FLT3 (10 cases), NPM1 (10 cases) and WT1 (6 cases - exon 7 and 2 cases - exon 9) mutation status. These included cases with all mutation permutations and included heterozygous and homozygous events. There was complete concordance with CE profile and the mutation pattern obtained by direct sequencing. We can conclude therefore that CE of fluorescently labelled PCR products provides a sensitive approach for the simultaneous detection of DNA fragment size variation up to and including 1bp difference. It is semi-quantitative, can discriminate between low, intermediate and high mutation load and allows the analysis of several fragments simultaneously.

0999

FINAL CLINICAL RESULTS OF A NEW INTRAVENOUS BUSULFAN DOSING STRATEGY AS PART OF BUCY CONDITIONING REGIMEN IN CHILDREN AND ADOLESCENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT): LOW MORTALITY AND LONG TERM DISEASE FREE SURVIVAL IN MYELOID LEUKEMIAS

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Background. In children and adolescents allogeneic (allo-) hematopoietic stem cell transplantation (HSCT) is a standard therapeutic option, but its use remains limited by the risk of transplant-related mortality (TRM). Busulfan (Bu) is often included in conditioning regimens but under- or overdosing may have a fatal outcome. We published that instead of an age-based dosing a body-weight- (BW) based calculation of IVBu can successfully target a therapeutic blood exposure without any dose adjustment (Vassal G *et al.* EHA 2006). This approach was applied prospectively in 36 patients (pts) and we report here the final clinical results. *Patients and Methods*. 36 children (21 boys/15 girls); median age 7.5 y (range 0.3 to 17.2 y) and weight 27 kg (range 5.0 to 62.0). IVBu (Busilvex®) was given over 2 h q6h x 16 doses according to BW: 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg, and 0.8 mg/kg for patients(pts) with <9 kg, 9 to <16 kg, 16-23 kg, >23-34 kg, and >34 kg BW, respectively. Cy was administered at 50 mg/kg qdx4. Clonazepam was used as seizures prophylaxis. Graft-versus-host disease (GVHD) prophylaxis was given according to local practice. Indications for HSCT were: AML (n=17: 15 CR1/2 CR2), ALL (n=1, CR2), CML (n=3), MDS (n=1), SAA (n=1); SCD (n=7), thalassemia (n=1), Wiskott-Aldrich Syndrome (WAS, n= 3), LAD (n=1), and HLH (n=1). Pts received bone marrow (n=35), and PBPC (n=1) containing 6.4 ×10^6 CD34+/kg (range 1.1-29) from matched (28/36) mismatched (1/36) related or unrelated (7/36, 2 mismatched) donors.

Figure 1.

Results. All pts achieved sustained engraftment at day 19 (range 9-47) for ANC > 0.5×10^9/L, and day 30 (range 16-111) for platelets >50×10^9/L. Complete chimerism (>99%) was seen in 31/36, and 5/36 were mixed chimeras but had mainly donor cells(>85%). IVBu was well tolerated: no grade (G) IV toxicity, G III (mainly stomatitis) occurred in 14 pts. VOD occurred in 8% (all in pts with genetic diseases) but none was severe. G I-II and III-IV acute GVHD rates were 50% and 6%, respectively. The median follow-up of the entire cohort is 36.7 months (range 3.9-49.1) Two pts died of treatment-related causes (hemorrhage=1, c-GVHD=1)and

3 of disease relapse.TRM at day+100 was 3% while the cumulative incidence (Ci) of TRM was 6% . Five relapsed (1 WAS and 4 AML) and 2 of them (1 AML and 1 WAS) received second transplant. 31 pts remain alive and disease free. For all pts Ci of relapse, EFS, and OS were: 15% ±12%, 82% ±13, and 85% ±12%, respectively. For pts with myeloid leukemias (mainly AML): Neither VOD nor TRM, and low relapse rate: Ci of 12%±11%. EFS and OS rates were 82% and 86%, respectively (Figure 1). *Summary/Conclusions*. IVBu-containing regimen allows sustained engraftment coupled with a reduced toxicity and mortality. Favourable outcome was seen in pts with myeloid leukemias as this regimen offers significantly reduced TRM and > 80% long-term curative potential.

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BLOOD DONATION AND IRON DEFICIENCY: ANOTHER POSSIBLE FACE OF CELIAC DISEASE

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Background. Blood donation, above all if done frequently and without a well balanced diet, can induce a latent or clear iron deficiency. Annual serum ferritin measurement, mandatory in Italy since 2005, allows early iron deficiency diagnosis before anaemia onset. *Aims*. Aim of our study is to evaluate, testing periodic blood donors for IgA and IgG antibodies antitransglutaminase, if iron deficiency could be a sign of underlying celiac disease in periodic asymptomatic blood donors. *Methods*. Periodic blood donors of National Cancer Institute Transfusion Service of Aviano (Italy) were checked annually for serum ferritin level from February 2004 to January 2006 (range ng/mL: male 30-350, female 10-190), and identified as iron deficient male donors with serum ferritin < 40 ng/mL and female donors with serum ferritin <20 ng/mL. All sideropenic donors were screened for antibodies anti transglutaminase IgG and IgA and donors with a positive screening sent to our Gastroenterology Service for further evaluation (examination and endoscopy with biopsies of duodenum second part). *Results*. Among the 1679 periodic blood donor screened, iron deficiency were found in 579, a percentage of 34.4%. There is no difference between male (290 subjects) and female (289 subjects) with a median age of 39 years (range 19-65). 13 donors were positive for antibodies anti transglutaminase, 2.3% of the studied group. In all the subjects it has been found a villous atrophia of the duodenal second part and celiac disease were the final diagnosis. Neither difference were found in the median age between subjects positive and negative for antibodies anti transglutaminase (median 32 years, range 21-54) nor very low serum ferritin level was associated with increased risk for celiac disease (serum ferritin median among celiac donors 14.7 with range 3-37, among non celiac donors 15.8 with range 1-40, Wilcoxon test: p not significative).

Table 1.

	Total	Male	Female	Age (medium)	Age (range)	Ferritin ng/mL Median (eange)
Donor screened	1679	1092	587	41	19-65	40 (1-859)
Iron deficient donors	579	290	249	39	19-65	15.2 (1-40)
Anti transglutaminase negative	566	284	282	39	19-65	15.8 (1-40)
Anti transglutaminase negative	13	6	7	32	21-54	14.7 (3-37)

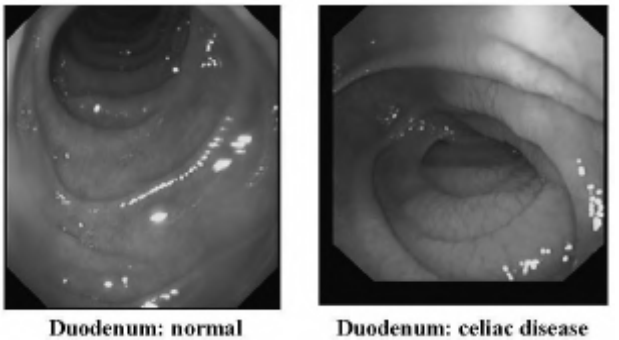

Figure 1.

Summary/Conclusions. Annual serum ferritin measurement in periodic blood donor enables to detect frequent situations of latent iron deficiency in subjects moreover without clinical symptoms. Iron deficiency, however, can be a marker of underlying diseases and, above all, it has not to be considered blood donation-related a priori. In our study we have shown that the prevalence of celiac disease in clinically asymptomatic, but iron deficient periodic blood donor is 2.3% (0.6% in normal population). All subjects began a gluten-free diet and were allowed to donate blood again as long as serum ferritin level returned in normal range. Asymptomatic iron deficient periodic blood donors are a group of population worth being studied for an early diagnosis of celiac disease, because this is an often-underestimated disease and, unknown and not treated, possible cause of severe systemic pathologies.

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IMPACT OF EXPERT PATHOLOGICAL REVIEW ON DIFFUSE LARGE B-CELL LYMPHOMA IN A SAUDI ARABIAN COHORT

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Background and Aims. Diffuse Large B-cell lymphoma (DLBCL) is the commonest tumour in males in Saudi, although the incidence is only about one third of that seen in Europe. We aimed to see whether there were differences in Saudi Arabian DLBCL cases in terms of diagnostic error rates, subtypes, prognostic factors and outcome. *Methods*. 117 cases of DLBCL diagnosed during the decade 1992-2002 (at the King Faisal University Hospital, Saudi Arabia) were submitted for secondary review. Paraffin embedded tissue was used from the initial diagnostic biopsy and subjected to further immunocytochemistry using tissue microarrays. The antibodies included bcl2, bcl6, PAX5, Oct2, Bob1, MUM-1, PAD44, Ki67 and a variety of antibodies to B-cell and T-cell lineage antigens. *Results*. 82 cases were fully evaluable and in 9 of these (11%) a major change of diagnosis was made from DLBCL to: T-cell rich B-cell lymphoma (3), CLL (2), Mantle cell lymphoma (1), Follicular lymphoma Grade 3A (1), Plasmablastic and acute lymphoblastic leukaemia (1 case each). The remaining 73 cases were stratified into the GC and non GC subtypes of DLBCL. Actuarial survival at 10 years was 43% for the whole cohort but non GC type and PAX5 positivity showed a trend toward a poorer prognosis. However, the strongest outcome correlation was with the intensity of staining for MUM-1 with actuarial survival at 10 years of 42% (MUM-1 weak) and 17% (MUM-1 strong). The impact was particularly notable in the non GC subtype of DLBCL (p=0.03) despite the small subgroups. *Conclusions*. The 11% diagnostic error rate for DLBCL is similar to European and US series. In 8 of these, there was a major clinical impact of treatment choice. In DLBCL cases similar prognostic effects of IPI and GC vs non GC subtypes were seen as in Europe and strong MUM-1 expression was particularly associated with a poor prognosis in this case series.

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SUPERIOR EFFICACY OF A NOVEL PLASMA SUBSTITUTE, AQIXRS-I, COMPARED TO PHYSIOLOGICAL SALINE IN A RODENT MODEL OF HEMORRHAGE

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Background. Resuscitation can exacerbate cellular injury caused by hemorrhagic shock and the choice of fluid used for resuscitation may play an important role in this injury. Traditional resuscitation has involved administration of large volumes of isotonic crystalloid or colloid solutions followed by blood products as necessary. Experimental studies have demonstrated that the use of these fluids has been associated with neutrophil activation and tissue reperfusion injury. *Aims*. This study was conducted to determine if a novel non-phosphate buffered physiological solution, designed and developed for the preservation of human organs for transplantation, can be used successfully to minimize tissue reperfusion injury and improve survival. *Methods*. The Institutional Animal Care and Use Committee approved this study. The study adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996 edition, as approved by the Institutional Animal Care and Use Committee. Nineteen Sprague-Dawley rats were anesthetized and had their abdominal aorta cannulated using a 24 gauge angiocatheter. The rats were subjected to serial withdrawal of blood in 2cc increments (simulating controlled hemorrhage)

every 30 minutes. The rats were randomly allocated to one of 3 arms: Replacement of blood volume with an equal volume of physiologic saline, replacement of blood volume with an equal volume of AQIX®RS-I (Res-Del International Ltd., London, UK) or no replacement of blood losses. The primary end point of the study was the death of the animal documented by cardio-respiratory arrest. *Results*. Rats in the no-resuscitation arm had the shortest survival and least blood volume withdrawn. Survival time and mean volume withdrawn were significantly improved by the addition of either normal saline or AQIX®RS-I resuscitation. AQIX®RS-I provided a statistically superior survival time (p<0.01) (Figure 1) and allowed larger volume of blood withdrawn compared to physiological saline (p<0.01). The mean difference in survival times between the AQIX®RS-I resuscitated rats and saline resuscitated rats was significant at 43.40±6.90 minutes (p<0.01). *Conclusions*. AQIX® RS-I appears to be a more effective plasma substitute then physiological saline with respect to survival time and volume blood loss in a rat model of controlled hemorrhagic shock. This study will serve as the basis for further detailed investigation of the potential benefits of AQIX RS-I use as a resuscitation solution in hemorrhagic shock.

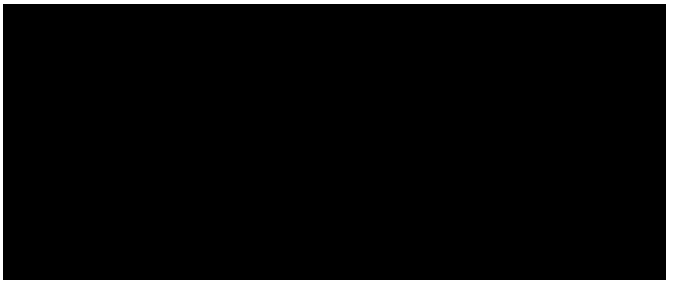

Figure 1. Survival of rats over time as a function of the resuscitation solution.

1003

QUANTITATIVE ASSESSMENT OF THE JAK2 V617F MUTATION SIGNIFICANT DIFFERENCES IN MUTANT ALLELE-BURDEN BETWEEN ESSENTIAL THROMBOCYTHEMIA, POLYCYTHEMIA VERA AND IDIOPATHIC MYELOFIBROSIS

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Background. Important insights into the molecular pathogenesis of the Philadelphia chromosome negative chronic myeloproliferative disorders (Ph-CMPD's) has been achieved since the identification of the JAK2 V617F tyrosine kinase mutation. In almost all patients with polycythemia vera (PV) the JAK2 V617F mutation can be detected, whereas about half of the patients with essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) harbors the mutation. The classification system of the Ph-CMPD's is likely to be reviewed in the near future, moving towards a molecular classification based on molecular disease identifying markers. However, several questions remain unanswered, among these is the question of how a single somatic point mutation can give rise to three different Ph-CMPD phenotypes. A *gene dosage model* describing JAK2 V617F *positive* Ph-CMPD as a biological continuum from ET, over PV to myelofibrosis has been proposed. The transformation from one phenotype to another could be related to an increasing JAK2 V617F mutation-burden. In this study we provide evidence of a significant difference in JAK2 V617F allelic burden between the three different JAK2 V617F *positive* phenotypes. *Methods*. We conducted a retrospective study on 124 patients with a JAK2 V617F *positive* Ph-CMPD (PV=90, ET=21, IMF=13). DNA was extracted by standard procedure from peripheral blood leucocytes. The proportion of JAK2 V617F mutated alleles versus JAK2 wildtype alleles was determined by two highly sensitive real-time quantitative PCR (qPCR) assays specific for the mutated and wildtype allele respectively run in parallel in duplicates. *Results*. Ninety patients with PV were investigated. Median JAK2 V617F% = 22 (95% c.i.: 17-34). ET: median JAK2 V617F% = 12 (95% c.i.: 2-15). IMF: median JAK2 V617F% = 67 (95% c.i.: 52-95). The differences in JAK2 V617F allele-burden between these three disease entities were highly significant: ET vs. PV (p=0.002) and PV vs. IMF (p< 0.00001), respectively. The same pattern was observed from samples obtained from patients at the time of diagnosis (ET= 11, PV= 42, IMF=8). ET: median JAK2 V617F% = 9 (95% c.i.: 0.9-37); PV: median JAK2 V617F% = 30 (95% c.i.: 20-38); IMF: median JAK2 V617F% = 66 (95% c.i.: 51-86). These differences were also significant: ET vs. PV (p=0.03), PV vs. IMF (p=0.0007), respectively. *Conclusions*. By qPCR we have demonstrated a significant difference in the JAK2 V617F allele burden between patients with ET, PV and IMF. This difference is significant regardless of analyzing patients at diagnosis or pooled data from both diagnostic samples and samples from patients with a longer disease duration. None of the ET patients had more than 50% mutated alleles, being accordingly interpreted as heterozygous, whereas all patients with IMF were homozygous. Interestingly, patients with PV seem to have a very wide range of proportion of mutated alleles from almost undetectable levels to levels comparable with IMF. In conclusion these data provides further evidence of a gene dosage relation between the three phenotypes with increasing allele burden from ET over PV to IMF. However, the variation of the JAK2 V617F mutated allele burden is wide in PV, which may reflect the degree of clonal expansion and myeloproliferation. Whether the proliferative potential correlates to allelic burden remains to be elucidated.

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GENE EXPRESSION PATTERN IN PARATYROID HORMONE (PTH /1-34/) TREATED ADHERENT CELL LAYERS (ACLS) OF MURINE LONG-TERM BONE MARROW CULTURE (LTBMC)

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Background. Regulation of self-renewal, quescence and differentiation of hematopoietic stem cells (HSC) is performed by the *niche* formed by elements of stromal microenvironment. PTH was shown to increase the number of long-term repopulating HSC indirectly, through regulation of spindle-shaped osteoblasts which are the main components of the niche. Earlier we have shown that PTH treatment influences on functional characteristic of ACLs of LTBMC as a model of hematopoietic microenvironment, namely it binds more hematopoietic progenitors. *Aims*. To reveal molecular mechanism of PTH influence we have studied expression level of genes regulating maintenance of HSC in ACLs. *Methods*. Rat synthetic PTH (1-34) was added in concentration 10^{-7}M, 5×10^{-8}M or 10(-8)M to LTBMC (Dexter's type) for the whole period of cultivation once a week with media change. RNA was isolated from carefully washed with PBS ACLs after 3, 5, 7, 9 and 11 weeks of cultivation. Semi-quantitative RT-PCR analysis was performed by PhosphoImager Cyclone (Packard Bell) evaluation of Southern blot hybridization. β-actin was used for normalization. *Results*. The expression of stromal differentiation marker genes such as osteopontin for osteoblasts and COMP for chondroblasts increased 2-3 fold in PTH treated ACLs during whole period of cultivation. Expression level of Angiopoietin-1 (one of genes regulating self-renewal and quiescence of HSC in osteoblastic niche) increased significantly after 3 weeks of PTH treatment and than slowly decreased up to 7 weeks of cultivation. The effect was strictly dependent on PTH concentration (increased 33 fold with 5×10^{-8}M of PTH and only 9-12 fold with 10^{-8}M or 10^{-8}M). The expression level of BMP-4 was elevated 2-3,8 fold from 5th to 11th week of cultivation independently of PTH concentration. PTH affected the expression of genes regulating self-renewing and proliferation of HSC such as Notch-1, Jagged-1 and BMI-1. Expression of Jagged-1 increased at the longevity of cultivation up to 4-fold while expression level of Notch-1 increased insignificantly. The expression level of BMI-1 increased 3,5-6 fold in ACLs after 3 weeks of PTH treatment and was still elevated after 7 weeks (2,2-3,6 fold). SDF-1 expression level in cultures to 9 week for 4 fold with subsequent elevation of β1-integrin expression to 11 week. PTH treatment also influence cell adhesion molecules VCAM-1, ICAM-1 and stromal growth factors VEGF-1, FGF-1 which expression level increased significantly (9-10 fold) and (3-9 fold) correspondingly. *Conclusions*. The PTH treatment changes expression level of genes studied. The most pronounced effect achieved at 5×10^{-8}M of PTH. Increase of expression level of osteopontin and Angiopoietin-1 was expected and reflexes the PTH influence on osteoblast maturation. Genes regulating HSC self-renewal and proliferation were always increased after PTH treatment. Increase of SDF-1 and adhesion molecules expression helps understanding of mechanism of increased hematopoietic progenitors associated with ACL in PTH treated cultures.

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INCREASED EXPRESSION OF PRO-ANGIOGENIC AND DECREASED EXPRESSION OF OSTEOGENIC GENES IN MESENCHYMAL STEM CELLS AFTER STIMULATION WITH MYELOMA - DERIVED MICROVESICLES

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Background. Despite resent advances in the treatment protocols, Multiple Myeloma (MM) is still an incurable disease with median patients'

survival of five years. This dismal prognosis is mostly due to resistance of MM to conventional therapies. Therefore, some of the attention has been redirected toward studying the role of bone marrow microenvironment in MM progression in order to find new ways to inhibit tumor growth by blocking interaction between tumor cells and their microenvironment. The important role of endothelium, osteoclasts and osteoblasts in MM progression has been documented. *Aims*. In this study we assessed the influence of myeloma-derived microvesicles (MMV) on human mesenchymal stem cells (MSC) by stimulating them with MMV. We compared also the gene expression profile in bone marrow stromal cells (MSC) from four healthy subjects (hMSC) and from six MM patients (mMSC). *Methods*. MMV were isolated from MM cell lines supernatants. Expression level of mRNA for genes involved in angiogenesis, invasion and MSC proliferation and osteoblastic differentiation were evaluated using real-time RT-PCR. *Results*. hMSC were exposed to 30 mg/mL of MMV for 8 and 24 hours and changes in gene expression were quantified. We noticed increased level of IL8 expression: 4.5 fold after 8 hours and 2 fold after 24 hours stimulation. Upregulation of MMP9 level was seen at 8 and 24 hours. HGF expression was decreased by approximately 2 folds at both 8 and 24 hours. 8 - hour exposure to MMV resulted in downregulation of RUNX2, collagen1 and osteocalcin mRNA by 1.5, 3 and 2 folds, respectively. After 24 hours, level of RUNX2 and collagen1 remained constant and level of osteocalcin decreased to 3.5 folds. When we analyzed gene expression in mMSC in comparison to hMSC we observed increased level of IL8 by 14 folds, VEGF and MMP9 by 3 folds, and decreased level of HGF by 2 folds. Difference in osteogenic genes expression in mMSC were also observed. RUNX2, collagen1 and osteocalcin were downegulated by 6, 11 and 5 folds respectively. *Conclusions*. Our study showed that MMV can induce expression of angiogenic and invasion genes and reduce expression of osteoblastic genes in MSC. This suggests presence of pro-tumorigenic activators and osteogenic inhibitors in MMV. Currently the biochemical composition of MMV is being assessed. We also noticed that MSC isolated from MM patients have skewed expression of several pro-tumorigenic and osteogenic genes in comparison to healthy controls.

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DIFFERENTIAL EXPRESSION OF CD13 IN BONE MARROW COMPARTMENTS OF CD34+ CELLS BETWEEN DIFFERENT GROUPS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. Aminopeptidase N (APN)/CD13 is a transmembrane protease present in a wide variety of human tissues and cell types (endothelial, epithelial, fibroblasts, leukocyte). This molecule is involved in extracellular proteolysis, regulation of chemokine and cytokine activity, and several other different cellular functions such us growth, secretion of angiogenic molecules, motility and apoptosis. Expression of CD13 has been shown to be deregulated in inflammatory as well as neoplastic diseases. CD13 is overexpressed in acute and chronic myeloid leukemias while information about its expression in myelodysplastic syndromes (MDS) patients is currently limited. *Objective*. In the present study we analyzed the expression of CD13 in normal myeloid CD34+ BM precursors as well as in CD34+ cells from BM of patients with MDS in order to evaluate the frequency of altered CD13 expression and its relationship with the World Health Organization (WHO) and International Prognostic Scoring System (IPSS) categories of MDS. *Patient and methods.* A total of 73 BM samples corresponding to 23 healthy individuals (control group), and 50 patients with newly diagnosed MDS were analyzed. Patients were classified according to the WHO and IPSS criteria into: refractory anemia (RA) (n=11), refractory cytopenia with multilineage dysplasia (RCMD) (n=9), RA with excess of blasts (RAEB)-1 (n=13), RAEB-2 (n=10), unclassifiable MDS (UNC) (n=2) and myelodysplastic/myeloproliferative disorder (MDS/MPD) (n=5). Low risk (LR) (n=12), intermediate risk (INT)-1 (n=14), INT-2 (n=9), and high risk (HIGH) (n=4); in 11 cases karyotypic information was not available. Among CD34+ BM cells three major cells subsets were identified: the more immature CD34+ precursors (PRin), CD34+/cyMPO+ cells already committed to neutrophil lineage (PRneu) and B-cell precursors. Expression of CD13 was analyzed by flow cytometry in the former two compartments of CD34+ precursors in terms of both percentage of CD13+ cells and the amount of expression/cell of this protein. *Results*. As compared to NBM, MDS patients showed overall increased percentages of CD13+/CD34+ cells associated with higher amounts of CD13 expression in both the PRim and PRneu CD34+ cell subsets. Such increased CD13 expression was specifically observed for those groups of cases with a poor outcome such as AREB-2 and HIGH risk MDS patients ($p<0.007$), while patients with MDS/MPD also had higher reactivity for CD13 confined to the PRneu ($p<0.03$). By contrast, MDS patients with RA showed decreased amounts of expression of CD13 associated with a lower proportion of CD13+ cells in both PRim and PRneu ($p<0.03$) CD34+ precursors; similarly, RCMD patients also showed lower CD13 expression and proportion of CD13+ cells restricted to PRneu ($p=0.03$). *Conclusions*. In summary our results show significant changes on CD13 expression among the PRim and PRneu compartments of CD34+ BM precursors in MDS patients consisting of lower reactivity in low grade MDS of the disease and increased expression in high-risk patients.

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ABSOLUTE LYMPHOCYTE COUNT AND EVENT FREE SURVIVAL IN HIGH-GRADE B-CELL NON HODGKIN LYMPHOMA TREATED WITH CHOP-LIKE CHEMOTHERAPY PLUS RITUXIMAB

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Background. Recent papers have shown that ALC recovery after autologous stem cells transplantation has a prognostic value in Lymphoma, Acute Myeloid Leukemia (AML) and Multiple Myeloma (MM). It was very recently shown in AML that ALC recovery is predictive of clinical outcome, also after Induction Chemotherapy. The role of ALC in newly diagnosed High grade B non Hodgkin Lymphoma (HG-B-NHL) treated with Immuno-Chemotherapy (IC) has not yet been described. *Aims*. We looked for a prognostic value of ALC in newly diagnosed HG-B-NHL, who were treated with CHOP-like chemotherapy plus Rituximab. *Methods*. 69 patients diagnosed with Diffuse Large B cell Lymphoma (DLBCL, n=66) and Follicular Lymphoma G3b (FL-G3, n=3), aged 21-75 (median age 58), were studied for ALC before the starting of steroids and IC (ALC-0), at day 15 (ALC-15) and thereafter every 15-20 days up to 4 months after IC. No patient of this series had leukemic dissemination. Event free survival (EFS) was analysed using the Kaplan-Meier method , the survival curves were tested for significance using the log-rank test, the Cox proportional hazard model was used to perform univariate and multivariate analysis. *Results*. The median follow-up was 18 months (range 2-46 months) Patients with ALC-0 ″800 mcL (n=12) or with ALC≥3500 (n=5) had a shorter EFS, but there was no evidence that ALC recovery at any time point after chemotherapy influenced EFS. ALC-0″800 mcL was correlated with Revised IPI (R-IPI), Bulky≥7,5 and with an activated B cell phenotype. In univariate analysis adverse prognostic factors were ALC at diagnosis 800 mcL or ≥3500mcL, R-IPI, PS, and stage. In multivariate analysis only R-IPI was statistically significant. *Conclusions*. ALC ″800 mcL or ≥3500 mcL at diagnosis were correlated with shorter EFS, R- IPI and only ALC≤800 mcL with an activated B cell phenotype. ALC recovery after first line IC did not affect EFS in our series. Further studies will better define the role of lymphocyte subpopulations in HG-B-NHL.

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CLL: A SINGLE CENTRE ANALYSIS OF THE TRADITIONAL AND NOVEL PROGNOSTIC MARKERS

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Background. Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with high variable clinical course. Genomic aberrations, IgVH genes mutational status, expression of ZAP-70 and CD38 represent novel prognostic markers increasingly used in association with the classical staging classification of Rai and Binet to predict the risk of disease progression. *Aims*. To evaluate the relationship among genomic aberrations, IgVH mutational status, CD38 and ZAP-70 expression in a cohort of 175 CLL patients referring to our centre. To study the influence of these factors on progression-free survival. *Methods*. Patient characteristics are listed on Table 1. 134 patients were followed in *wait and watch*, 26 went under treatment after progression, 15 were treated soon afterwards diagnosis. Genomic aberrations (13q, 17p-, 11q- and trisomy 12) were analyzed by FISH, IgVH mutational status by PCR and sequencing, CD38 and ZAP-70 by flow-cytometry. *Results*. High risk cytogenetic abnormalities were significantly associated with advanced stage disease ($p=0,04$) and IgVH unmutated genes ($p=0,003$). Low risk cytogenetics

abnormalities were associated with stage A disease (p=0.01), IgVH mutated genes (p=0,0001), CD38<30% (p=0.001). Patients with trisomy 12 were significantly more likely to have IgVH unmutated genes (p=0,03). Not statistically significant difference emerged comparing cytogenetics and ZAP-70 expression. A significant association was found between IgVH mutational status and both CD38 (p=0,006) and ZAP-70 expression (p=0,001). 71% of CD38- patients was also negative for ZAP-70; 53% of CD38+ patients showed ZAP-70 ≥20%. Median treatment-free survival (TFS) of patients with no cytogenetic abnormality, 17p-, +12, 11q- and multiple abnormalities was 122, 36, 34, 12 and 6 months respectively; it was not reached in 13q- patients. Median TFS in IgVH unmutated and mutated patients was 35 and 122 months, respectively. Selecting patients with low risk FISH abnormalities (no cytogenetic aberrations and isolated 13q-), median TFS remained shorter in patients with IgVH unmutated (24 months) than in patients with IgVH mutated genes (122 months). TFS was 60 months in CD38+ while 182 months in CD38- patients. TFS was not significantly different between ZAP-70+ and ZAP-70- patients. IgVH mutational status and cytogenetic abnormalities maintained their statistical predictivity for TFS in stage A patients. Clinical stage and unmutated IgVH genes resulted independent predictors of progression in multivariate analysis. *Conclusions*. Our single centre data mirror those reported in the literature. The lower prevalence of high risk cytogenetic abnormalities could be explained with the high number of stage A patients. Cytogenetic abnormalities lost prognostic significance in a multivariate analysis for disease progression because of the small sample size. Multiple abnormalities are associated with the shortest TFS. In our experience, the IgVH mutational status is the strongest progression marker in all our CLL patients regardless of cytogenetics.

Table 1.

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ASSESSMENT OF IDIOPATHIC MYELOFIBROSIS USING 18F-FDG PET

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Background. Idiopathic myelofibrosis is a clonal stem-cell disorder that leads to an ineffective haematopoiesis. This disease is accompanied with reactive bone marrow fibrosis and extramedullary haematopoiesis especially in the spleen and liver. The only curative treatment for some selected patients is allogenic haematopoietic stem cell transplantation and recognition of these patients is essential. The aim of this study was to define the role of whole body 18F-FDG PET imaging in idiopathic myelofibrosis. *Methods*. Ten patients with diagnosed idiopathic myelofibrosis were examined. They all underwent whole body 18F-FDG PET, computed multi slice tomography (MDCT) and magnetic resonance imaging (MRI). The median age of the patients was 65 (44-82) years and the disease duration was 7.3 (0.3-14) years. The median leukocyte level was 10.5 (3.2-35.8)×10^9/L, haemoglobin 110 (80-139) g/L and platelets 217 (56-957)×10^9/L. Lille scoring system revealed 0, 1, and 2 points in four, five and one patients, respectively. Visual analysis of FDG PET, MDCT and MRI were performed and the findings were correlated with clinical and laboratory parameters of good or bad prognosis. *Results*. Three patients had normal FDG accumulation, where as seven patients had diffusely high uptake of FDG in their bone marrow, which was more pronounced in femurs (six patients). High FDG uptake of femoral bone marrow correlated well with marked signal decrease in T1 weighted MR images and increase in STIR images in six patients, thought to represent bone marrow reconversion. The four patients with either normal or diffusely elevated FDG accumulation had normal MRI (one patient) or only slightly inhomogeneous signal changes in femoral bone marrow (three patients). Signal changes consistent with sclerosis of vertebrae or flat bones were found in nine of ten patients in MRI, and eight in MDCT. Spleen was enlarged in all and giant in five patients. No correlation between clinical parameters and FDG accumulation was found. *Conclusions*. High accumulation of FDG in femoral bone marrow correlates with MRI findings of bone marrow reconversion and thus possibly with activity of the disease. However, no correlation between the patients' clinical parameters and imaging findings were seen in our small series. Further studies are needed to evaluate this relationship.

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TELOMERASE ACTIVITY INCREASES IN PROGRESSION OF MYELODYSPLASTIC SYNDROMES AND IS COMMON FEATURE OF UNTREATED ACUTE MYELOID LEUKEMIA ASSOCIATED WITH A PORTION OF BLASTS IN ANALYZED TISSUE

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Background. Telomerase synthesizes repetitive sequences onto telomeres that play important role in protection of genome stability. Activity of telomerase is not detectable in somatic cells, but low levels are expressed in renewal tissues, germline and stem cells. In last few years telomerase and telomeres became one of the most studied topics in tumors due to enhancement or reactivation of telomerase activity detectable in a wide variety of tumors, including hematological malignancies. *Aims*. Telomerase activity (TA) was studied in peripheral blood or bone marrow mononuclear cells of patients with myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) before treatment and during the course of the disease with the intention to assess its relationship to clinical characteristics of patients and to determine its prognostic significance. *Methods*. Analyses were done on 93 samples of 70 patients with MDS, 13 patients with AML arising from MDS, 59 samples of 53 patients with primary AML, and 41 healthy BM/PBPC donors considered as negative controls (NC; TA=0.0162±0.0193). TA was analyzed in protein extracts using modified TRAP Assay - TeloTAGGG Telomerase PCR ELISAPLUS kit. Results were discussed together with telomere length, expression of regulatory genes of TA and clinical features (proportion of blast cells, survival analysis, individual MDS patient's risk score established according to the International Prognostic Scoring System (IPSS). *Results*. Significant increased TA, in comparison to NC (p<0.01), were found in 51% (33/65) patients with MDS. Proportion of patients with positive TA and TA levels increased towards advanced forms of MDS (RA and RARS: 49%, TA=0.0963±0.1398; RAEB, RAEB-t: 52%, TA=0.1204±0.1689). Highest values of TA were detected in AML from MDS (77%, TA=0.3408±0.5965). TA of MDS patients showed significant inverse correlation with telomere length (p=0.047). Surviving curves of patients with positive or negative TA level were not quite significantly different (p=0.071). Nevertheless, 5-years surviving probability is more than two-fold higher in patients with telomerase negativity than in patients with high TA (75% vs. 30%). Increased expression of hTERT gene (considered as main regulator of TA) was associated with positive level of TA or even forerun telomerase activation. Between TA and individual patient's IPSS risk score was found no association. In AML, increased TA was observed in 80% of untreated patients (40/50; TA=2.2357±5.6083), and average level showed more than 100-fold increase in comparison to NC. Levels of TA proposed highly significant correlation with portion of blast in analyzed tissue (p=0.004) and showed decreasing trend in relation to induction treatment. On the other hand, surviving of patients was not depended on TA level. *Summary*.Increased TA in almost half of patients with early forms of MDS and its growth towards advanced forms indicate its prognostic significance for determination of individual patients risk of conversion towards over leukemia. TA might be also non-specific molecular marker of leukemic cells, since it well characterizes majority of AML patients, moreover in highly significant association with portion of blasts and shows decrease as reaction to treatment.

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FLOW CYTOMETRIC DNA INDEX AND KARYOTYPE ARE COMPLEMENTARY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. DNA index (DI), which expresses the blast cells DNA content, is a prognostic factor in childhood acute lymphoblastic leukemia (ALL). When the leukemic clone does not show pejorative structural abnormalities, a better survival is associated with a DI > 1.16 (equivalent to a modal number higher than 53 chromosomes). On the other hand, a hypodiploid clone (≤34 chromosomes) induces a poor prognosis. Associated with the clinical and molecular characteristics, these parameters are used to include the patients in different therapeutic schedules. *Aims*. The aim of this study was to validate the accuracy of the flow cytometric (FCM) DNA index (DI) by confrontation with the karyotype, in order to provide a useful information at diagnosis in case of discrepancies between these two results. *Methods*. One hundred and twelve patients (51 girls and 61 boys) treated between 1990 and 2006 (CHU Angers and Toulouse) were included in this retrospective study. Samples were obtained at diagnosis from bone marrow (109 samples) or blood (3 samples). Depending on the sex, megabases number of each normal chromosome is a definite percentage of the total sum of the 46 chromosomes of diploid genome. Thus, X and Y chromosomes concern respectively 2.58% and 0.93% of a 46,XY cell DNA content. According to this information, we created a formula to calculate a theoretic DNA index (tDI) based on the blast cell karyotype. For every patient, the calculated tDI took into account the additional or missing chromosome material of the major clone. *Results*. In a linear regression study, DNA index correlated with modal chromosomes number (y=0.0202x+0.0685 and R=0.992) and with tDI calculated from karyotype (y=0.9709x+0.033 and R=0.995). Technical reasons may compromise the leukemic clone characterization obtained with karyotype (insufficient *in vitro* blast cells growth induced by poor cellularity or low S phase). FISH and molecular biologic methods may overcome the karyotype failure, but DNA index can alert and guide in this step (3 cases in this study). In addition, we present three cases containing 2 blast cell populations with FCM, one hypodiploid (DI=0.56, 0.73 and 0.77) and the second hyperdiploid (DI=1.11, 1.33 and 1.53 respectively). In the three cases, the karyotype detected only the hyperdiploid clone. Considering the pejorative prognosis of high hypodiploidy, DNA index was very useful to confirm the profile of triploidy/duplication of hypodiploidy 30-40 chromosomes obtained with the karyotype. *Conclusion*. The strong correlation between tDI and DNA index validates the accuracy of FCM quantification, which is technically fast and could be performed on fresh or frozen samples. If karyotype is essential to analyze chromosomal abnormalities (numerical and structural), FCM provides complementary informations when the leukemic clones express abnormal chromosomes number or heterogeneity in DNA index. We conclude that DNA index is a useful tool in the ploidy characterization of blast cells and that it should be transmitted to the cytogeneticist as soon as possible at diagnosis of childhood ALL.

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INTERLEUKIN-6 (IL-6), TUMOR NECROSIS FACTOR α (TNF-α) LEVELS AND IL-6, TNF-POLYMORPHISMS IN CHILDREN WITH THROMBOSIS

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Infection has an important role in the pathogenesis of thrombosis and it becomes more prominent in childhood cases, where the infection frequency is higher. It was suggested that patients with high TNF-α and IL-6 levels might be at increased risk of developing thrombotic complications due to the effects of these cytokines on the coagulation pathway. Functional polymorphisms in the promoter regions of the genes coding for TNF-α and IL-6 are associated with increased plasma levels of these cytokines. In this study, we planned to evaluate the serum levels of acute phase reactants such as CRP and of cytokines such as TNF-α, IL-6, and we aimed to investigate the association between the TNF-α -308 G/A and IL-6-174 G/C polymorphisms in Turkish pediatric patients with thrombosis. Fifty-eight children with thrombosis (Group 1) and 89 controls (Group 2) were included in the study. Patients who had a history of infection within the fifteen days prior to thrombosis were classified as Group 1a and those who had no infection history prior to thrombosis were classified as Group 1b. Serum TNF-α did not differ significantly between the groups. However, IL-6 level was higher in group 1a than in group 1b ($p<0.05$). The genotype distribution and allele frequencies of TNF-α G/A polymorphism were significantly higher in the thrombotic children without infection and in the control group than in the thrombotic children with an infection history ($p<0.05$). The IL-6 -174 C/C genotype was significantly higher in thrombotic children with an infection history ($p<0.05$) and there were no differences between the groups in mean of allele frequency. As a result, patients with a history of infection are seem to have a higher of CRP and IL-6 levels and IL-6 -174 C/C genotype. Venous thrombosis is also more frequent in this group than arterial thrombosis ($p<0.05$).

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INVESTIGATION OF DISEASE KINETICS IN CML PATIENTS WITH B3A2 AND B2A2 BCR-ABL FUSION TRANSCRIPTS UNDERGOING IMATINIB TREATMENT

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Imatinib mesylate induces complete cytogenetic responses in over 70% of patients with BCR-ABL positive CML. Quantitative RT-PCR (RQ-PCR) protocols for the monitoring of the BCR-ABL fusion transcript level in CML patients play an increasingly important role in assessing response to treatment. Two main variants of the BCR-ABL fusion transcript are detected in CML patients, namely b3a2 and b2a2. Using RQ-PCR, we assessed the kinetics of response to Imatimib therapy in CML in 70 patients with b3a2, (median age 48 years), and 41 patients with b2a2, (median age 47 years), in order to assess their response to treatment and the kinetics of the disease in these two subgroups of patients.Following 12, 18 and 24 months of Imatinib treatment, significantly more patients with b3a2 transcript achieved molecular response, ranging from 2-log reduction to molecular negativity, compared to patients with b2a2 transcript. 71.9% of b3a2 and 14.3% of b2a2 ($p=0.001$) patients achieved at least a 2-log reduction (minor molecular response) in transcript level. The percentage of patients with b3a2 or b2a2 transcript who showed various molecular responses to Imatinib treatment are shown in Table 1. The percentage of patients who achieved molecular responses and then went into cytogenetic relapse was 12.5% (3/24) of b3a2 and 15% (3/20) of b2a2 patients. All of these patients remained in chronic phase. RQ-PCR analysis of BCR-ABL transcripts showed different mean levels of the transcript at months 12 and 18 post treatment in the two groups of patients, as shown in Table 1 (b3a2 patients: 5.38 and 4.48, b2a2 patients: 11.25 and 11.76 respectively). ABL kinase mutations were detected in 13/70 (19%) of the b3a2 patients examined, compared to 9/41 (22%) of the b2a2 patients. These findings indicate that patients with the b3a2 fusion transcript may be more responsive to Imatinib than those with b2a2 transcript. They also show BCR-ABL transcript levels at 12 months may be of prognostic value in patients treated with Imatinib. However, it is important to validate this disease characteristic on a larger cohort of patients.

Table 1.

	B3A2					B2A2				
Months into treatment	4	8	12	18	24	4	8	12	18	24
Mean BCRL-ABL/ABL ratio	15.186± 22.509	10.127± 28.253	5.380± 13.039	4.481± 10.394	6.913± 16.572	9.943± 13.655	11.981± 18.578	11.252± 13.089	11.762± 11.762	8.724± 15.175
Minor molecular response	1/18	8/29	13/32	8/28	6/24	1/17	4/17	1/21	5/17	9/20
Major molecular response	1/18	0/29	4/32	5/28	8/24	0/17	0/17	1/21	2/17	1/20
Molecular negativity	0/18	6/29	6/32	8/28	7/24	1/17	1/17	1/21	1/17	3/20
Cytogenetic relapse	0/18	0/29	0/32	0/28	3/24	0/17	0/17	1/21	2/17	3/20

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A CASE OF DONOR DERIVED LEUKAEMIA FOLLOWING AN ALLOGENEIC CORD BLOOD TRANSPLANTATION IN A PATIENT WITH CHRONIC MYELOID LEUKAEMIA

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Background. Donor cell leukaemia (DCL) is a rare complication following allogeneic stem cell transplantation. Umbilical cord blood has been used over the past decade, as an alternative source of haematopoietic stem cells. Transplantation from an unrelated cord blood (UCB) donor has become standard practice for haematological disorders and is a safe and

feasible alternative to bone marrow (BM) transplantation in adults, when no sibling is available. *Aims*. We report a case of donor derived acute myeloid leukaemia following cord blood transplantation for chronic myeloid leukaemia in a 32 year old female. *Methods*. BM samples were analysed by G-banding and FISH. DNA was extracted from BM cells pre- and after transplantation and analysed by array comparative genomic hybridisation (aCGH) (Agilent Technologies). ACGH results were confirmed by real-time quantitative PCR (qPCR). *Results*. Cytogenetic and FISH analysis at diagnosis showed a female karyotype with the t(9;22) translocation and an inv(7) with unusual breakpoints in both arms. Seventeen months following a sex mismatch UCB transplantation cytogenetic analysis revealed an abnormal male karyotype with monosomy 7, loss of 21 and an aberrant chromosome der(17)t(17;21). Further FISH and aCGH analysis elucidated the mechanism by which the complex der(17) occurred and maps the breakpoint at 17p13.3. Moreover, we detected an amplification of the 21q11.2-q22.1 region with breaks at the flanking sites. The amplifications and deletions were confirmed by qPCR. *Conclusions*. We confirm the occurrence of donor cell leukaemia following UCB allogeneic transplantation. This study represents an extremely rare event and to our knowledge this is only the fifth case described for a donor cell leukaemia after allogeneic cord blood transplantation.

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PRAME EXPRESSION IN CHRONIC MYELOID LEUKEMIA

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Background and aims. Tumor antigens recognized by CTLs have been identified several years ago and are major targets for the creating of anticancer vaccines. PRAME is an antigen which expresses at high levels in various malignant tumors including melanomas and hematopoietic malignancies such as acute and chronic leukemias (AML, CML etc.) The aim of this study is to determine the frequency and clinical importance of the expression of this antigen in CML as well as to detect the PRAME protein in tumor cells. *Methods*. PRAME mRNA was measured by real-time RT-PCR using TaqMan technique. Periferal blood mononuclear cell (PBMC) samples from 245 cases with chronic myeloid leukemia and 25 controls were used as study material. Chi-square test for independent samples was used for statistical analysis. The monoclonal antibodies raised against truncated recombinant PRAME were used for PRAME protein analysis by Western blot and immunocytochemistry on the various tumor cells. *Results*. Seventy four of 245 cases with CML (30%) showed PRAME expression whereas all the healthy donors did not. PRAME expression was found in six of 20 newly diagnosed CML samples (30%), 55 of 209 CML-chronic phase (26%), and 13 of 16 CML with acceleration and blastic phase (80%). Highest expression was found in cases with CML-blastic phase. For some of positive PBMC samples PRAME protein expression was confirmed by Western blot assay. The control samples were negative in the same assay. Finally we have revealed the nuclear localization of PRAME protein in K562 cell line (chronic myeloid leukemia) and melanoma cell lines by immunocytochemistry and Western blot. *Summary*. PRAME is nuclear protein that expressed approximately in one third of the cases with CML. Low expression levels in remission and high levels in relapse suggest that PRAME is an important marker to detect the minimal residual disease and predict relapse.Our findings support the suggestion that this antigen may be furhter used as a target for diagnostic and therapeutic approaches.

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IMATINIB RESISTANCE POINT MUTATION LEU248VAL IN BCR-ABL GENE GIVES RISE TO MICRODELETION OF THE SAME REGION WITHOUT FRAMESHIFT

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Background. The main disadvantage of mesylate imatinib is the development of resistance to this drug in patients with chronic myeloid leukemia (CML) and with Ph-positive acute lymphoblastic leukemia (ALL). It has been found that point mutations in the ATP binding pocket of the ABL kinase domain of BCR-ABL gene confer imatinib resistance in relapsed patients. *Aims*. To study drug resistance mutations in BCR-ABL gene in CML patients that turned to be imatinib resistant according to data of quantitative RQ PCR analysis of BCR-ABL gene expression. *Methods*. PCR fragment containing BCR-ABL breakpoints and juxtaposing ABL region with ATP binding pocket of the ABL kinase domain was amplified using conventional primers. PCR fragments were purified with 6% PAAG and then directly sequenced using the same forward and reverse primers. We analyzed 2 CML patients with primary and 25 CML patients with secondary imatinib resistance. *Results*. In 2/2 CML patients with primary imatinib resistance we did not find any BCR-ABL point mutations (there was only a polymorphism not giving rise to amino acid substitution). In 19/25 (76%) CML patients with secondary imatinib resistance we found point mutations that are usual for cases of imatinib resistance. Among them there were 4 mutations known to be very significant to render imatinib resistance due to localization within ATP binding pocket of ABL P-loop (Gly250Glu, Tyr253Phe, Leu248Val, Gly250Glu). The other 2 point mutations were also common for CML patients resistant to imatinib but of mere significance as they situated outwards of P-loop region (Met244Val, Phe359Val). It was of great interest that in the same patient carrying point mutation Leu248Val we also found a microdeletion of 739-819 bp. Due to this deletion 82 bp of P-loop was removed without frameshift. The resultant BCR-ABL protein was predicted to be 23 aa shorter then usual. Point mutation Leu248Val made change of AAGC for AAGG. The same AAGG repeat was found on the other end of deletion. We suppose that point mutation Leu248Val may facilitate that deletion due to creating two perfect AAGG repeats on both ends of deletion. *Conclusions*. It is still unclear if that microdeletion may have any clinical significance. Nevertheless we consider our finding to be an interesting example of genetic instability.

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CURE OF VERY RARE PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) ACHIEVED BY BUSULFAN ALONE. A HINT FOR A ROLE OF NKT CELLS IN SUCH CASES

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Background. CML is considered to have been consistently fatal prior to development of bone marrow transplantation. An ineluctable evolution to a rapidly lethal acute transformation led to death that occurred even after an unusually prolonged chronic phase in 10 cases of the literature and in 4 personal cases, 20 to 31 years after diagnosis. *Aims*. To show that the term *cure* or *operational cure* is perhaps to be used for a patient whose CML was treated by busulfan 50 years ago and who is perfectly well in 2007 despite the persistence of very rare circulating clonogenic BCR-ABL+ cells. Preliminary results indicate that NKT cells may play a role in this situation as well as in very rare similar cases. *Case records, methods and results*. One of us has been following PEL. B since he was hospitalized in july 1956, at the age of 3 for an adult- type CML. Busulfan, given for 3 months caused a severe aplasia from which he recovered in November 1957. From 1967 to 1987, bone marrow studied 5 times showed a total of 167 metaphases, all of them normal. However in serial examinations since 1996, a small amount of b2-a2 messenger was detected in the blood leucocytes (Bcr-Abl/Abl 0.01%) and 2-5% of BCR-ABL cells+ were found by D-FISH. In cultures in presence of 4 growth factors granulocytic and erythroid colonies were obtained; 20% of which expressed b2-a2, that were suppressed by imatinib, showing their dependence on an active Bcl tyrosine-kinase. The transcript was also detected in the blood of 3 out of 6 mouse 3 months after IV inoculation of blood cells from the patient. Searching for an immune reaction we were only able to demonstrate, using flow cytometry, an unusually considerable *in vitro* expansion (294 fold) stimulated by α-galactosylceramide(α-GC) of his NKT cells as defined by the CD1d tetramer-invariant Vα24+staining, that moreover produced high levels of IFNγ, TNFα and perforine. The value of our findings is confirmed by similar molecular and immunologic results achieved in 2 other patients who have been surviving for 3 and 4 decades respectively. We are currently trying to look whether such α-GC-expanded NKT cells would inhibit the growth of colonies from our patients' blood cells. *Conclusion*. We suggest that carrying such investigations in 5 patients surviving in good health, 17 to 48 + years after diagnosis, whose previously reported observations were kindly updated for us by D. Amikam, M. Djaldetti, C. Fegan, TA. de Witte, RS. Stein and JT. Reilly, would be of great interest. At least it is thus demonstrated that an obsolete drug may allow rare patients to be considered as *operationally cured*, which remains of importance even in the era of kinase inhibitors. Our observations obviously raise a number of questions, one being whether NKT cells or α-GC might be employed as therapeutic agents in the future in some cases of CML.

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THE EFFECT OF THE SUGARBAKER PROCEDURE ON HAEMOSTASIS

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Pseudomyxoma peritonei is a progressive disease of the peritoneum, characterised by the gradual accumulation of mucinous fluid in the peritoneum. Left untreated it is ultimately fatal. Although no cure exists, the Sugarbaker procedure combining complete cytoreduction, intraoperative hyperthermic intra-peritoneal chemotherapy and post-operative intra-peritoneal chemotherapy, has shown promising results. The aim of this study was to assess haemostatic function in this group of patients pre-operatively and the effect of the Sugarbaker procedure on haemostasis. Citrated plasma was collected from 40 patients, pre and post operatively, and pre and 30 minutes post any blood components during surgery. Coagulation screening tests and whole blood global assay for haemostasis were performed. The prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fib) were performed on the MDA II (Trinity Bioscience). Thromboelastometry tests (Ex-TEM, In-TEM and Fib-TEM) were performed on the ROTEM (Biodis). Pre-operatively patients had normal PT (mean = 13.5s) and APTT (mean = 30.6s) and raised Fib (mean = 4.7g/L), suggesting the possibility of a hypercoagulable state. The PT (mean pre-o*p*=13.5s, post-o*p*= 15.9s), APTT (mean pre-o*p*= 30.7s post-o*p*= 36.6s), and fibrinogen (mean pre-o*p*= 4.7g/L post-o*p*= 1.6g/L) all displayed significant differences with p values of <0.001. Thromboelastography demonstrated deterioration in clot quality as measured by the MCF (Maximum Clot Firmness). The PT, APTT and Fib pre and post FFP (fresh frozen plasma) showed no significant difference. Results indicate that this procedure has a detrimental effect on haemostasis, identified by a reduction in fibrinogen and loss in clot quality as indicated by thromboelastography. Suggesting that cryoprecipitate, rich in fibrinogen, may be a more appropriate blood component for use in association with this surgery.

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CD95 EXPRESSION IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA AFTER TREATMENT WITH IMATINIB

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Background. Disbalance in cell lines proliferation and apoptosis leads to onset of CML. Normal granulocytes express high level of CD95 indicating apoptosis capability that is realized along with cell aging. In CML patients the granulocytes approach apoptosis much more slowly than in healthy subjects. Imatinib administration leads to selective inhibition of proliferation and to intensification of cell apoptosis. *Aims*. Assess of CD95 antigen expression and intracellular bcl-2 protein level in granulocytes and lymphocytes in CML patients. *Materials and methods*. We have examined 25 CML patients and 20 healthy controls aged 19-57. CML was diagnosed within 14-88 months before study. Ten CML patients received busulfan or hydroxyurea, otherwise interferons (1st group), other fifteen persons were treated with Imatinib (2nd group). CD95 (Fas-receptor) and bcl-2 antiapoptotic molecules were assayed in peripheral blood granulocytes and lymphocytes. *Results*. Granulocyte population in peripheral blood in CML was represented by $CD10^{+}33^{+}13^{+}Dr\text{-}71\text{-}34\text{-}95^{+}$ cells corresponding the mature granulocyte phenotype. It was found out that level of CD95-positive granulocytes was 90.9611.36% and that of lymphocytes 35.348.00*% (p<0.02) in the 1st group. Level of CD95-positive granulocytes in the 2nd group was not different from the 1st one (91.581.85%) with CD95-positive lymphocytes comprising 53.494.95*% (p<0.001) Normal reference values for CD95-positive lymphocytes are 14.92.86%. Level of positive with bcl-2 granulocytes in the 1st group was 75.845.16*% (p<0.002), being of such lymphocytes as 47.077.96% (39.72.91% and 51.74.76% in control group respectively). In the 2nd group receiving Imatinib the bcl-2-positive granulocytes comprised 63.258.40*% (p<0.05) exceeding substantially the control values. Levels of bcl-2-positive lymphocytes (61.364.67%) exceeded the reference norm with no significance. *Conclusions*. Content of CD95-positive granulocytes in both groups was similar with no change under the treatment. Number of bcl-2-positive granulocytes decreased substantially along with Imatinib treatment, whereas CD95-positive lymphocytes in both CML groups were substantially exceeding the reference norm and reflect of therapy side effect.

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DENDRITIC CELL REGENERATION IN PERIPHERAL BLOOD OF CHILDREN TREATED FOR ACUTE LYMPHOBLASTIC LEUKAEMIA BY NOPHO ALL-2000 PROTOCOL

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Background. Dendritic cells (DCs) play a pivotal role in coordinating functions of the immune system. It has been reported severe deficiency of plasmocytoid DCs (pDCs) and myeloid DCs (mDCs) at diagnosis in B-precursor acute lymphoblastic leukemia (preB-ALL) but not in T-precursor ALL (T-ALL). However, little is known on DC levels in peripheral blood (PB) in patients under treatment for acute lymphoblastic leukemia. The aim of the study was to determine the levels of pDCs and mDCs in PB in children with ALL at diagnosis and follow the kinetics of DC regeneration during chemotherapy according to NOPHO ALL-2000 protocol. *Methods*. During February 2002-October 2005, 114 children were diagnosed with *de novo* ALL at Astrid Lindgren Children Hospital in Stockholm or Academic Hospital in Uppsala, Sweden. Of these, 80 patients fulfilled the following inclusion criteria: 1) entered to NOPHO ALL-2000 MRD study; and 2) the levels of DCs in PB were measured at least twice during therapy. The median age at diagnosis was 6 years (range 1 month-17 years) and M:F ratio was 1.16 (43:37). 71 children had preB-ALL and 9 had T-ALL. Patients with preB-ALL without unfavorable features formed standard-risk (SR) group. Patients with preB-ALL with unfavorable features and T-ALL formed high-risk group (HR) group. DC frequency was evaluated using four-color flow cytometry on heparin-anticoagulated whole PB samples by Lineage cocktail 1 (lin)FITC/ CD123PE/HLA-DRPerCP/CD11cAPC antibody combination. *Results*. At diagnosis, we found severe deficiency of both DC subsets. Only four patietns (3 preB-ALL, 1-TALL) had pDC but not mDC levels in the range of control values. The relative and absolute levels of mDCs but not pDCs were significantly higher in patients with T-ALL as compared to patients with preB-ALL (p=0.015 and p= 0.014, respectively). In HR patients, the percentages pDC and mDC reached to control levels at treatment day 50. The absolute pDC values reached to control levels at day 106, but the absolute mDC levels never reached to control range. In SR patients the relative levels of mDCs reached to control range at day 50. The absolute pDC and mDC levles reached to control range at day 211. At the end of treatment the relative but not absolute levels of pDC remained within control range in all groups of patients. The relative and absolute levels of mDC decreased to significantly lower levels than controls (p=0.012 and p<0.001 for relative and absolute values, respectively). *Summary/conclusions*. Our study showed deficiency in both pDC and mDC subset in children with ALL at diagnosis, more pronounced in preB-ALL than in T-ALL. The relative levels of both DC subsets reached to control range after the first Induction block. At long-term, the mDC regeneration was more severely affected than that of pDC subset.

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EVALUATION OF THE TRANSCRIPTIVE ACTIVITY OF THE HISTONE H3 GENE IN PATIENTS WITH LOW AND HIGH GRADE NON-HODGKINS LYMPHOMAS

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Introduction. Packaging of DNA into a higher order structure known as chromatin allows an important regulatory point for the control of gene expression and other DNA-related processes. The chromatin complex is composed of DNA, histones and other nonhistone proteins. Histones are small basic proteins which bind to the chromosomal DNA and take part in the nucleosome creation. Their acetylation and deacetylation influence *respectively* the activation or stabilization of the nucleic DNA transcription ability. The level of acetylation of the histones is controlled by two opposing families of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). Therefore, a defect in the acetylation machinery appears to lead to alterations in acetylation and to development of cancer. An imbalance in histone acetylation may lead to changes in chromatin structure and transcriptional dysregulation of genes involved in the control of cell cycle progression, differentiation and apoptosis. HDAC inhibitors may block tumor cell proliferation by restoring the balance of histone acetylation resulting in the proper expression of genes. In addition, some HDAC inhibitors, like valproic acid, were suggested to inhibit angiogenesis by decreasing the production of endothe-

lial growth factors like Vascular Endothelial Growth Factor (VEGF). *Materials & methods*. To evaluate the transcriptive activity of the histone H3 gene in lymph nodes, a total of 47 patients diagnosed with Non-Hodgkin's lymphomas (NHL) participated in the study: 23 of them were diagnosed with the aggressive lymphoma (15 with Diffuse Large B-Cell Lymphoma 'DLBCL and 8 patients diagnosed with Mantle Cell Lymphoma -MCL, 24 patients diagnosed with low-grade NHL. The control group consisted of 7 persons diagnosed with a non-neoplastic lymphadenitis. The QRT-PCR method was employed to assess the activity of histone H3 gene. *Results*. A statistically significant higher activity of the histone H3 was found in high-grade NHL when compared with the indolent lymphomas ($p<0.05$). A similar situation was observed in the DLBCL sub-group when compared with low-grade lymphomas ($p< 0.01$). There was no statistically significant difference in the H3 expression between the indolent lymphoma group and the control group with lymphadenitis diagnosis. *Conclusions*. The obtained results show that the transcriptive activity of histone H3 is significantly higher in the high-grade lymphomas when compared with the indolent lymphomas. The H3 expression correlates with the clinical lymphoma grade (malignancy). Histone H3 may be found useful as a marker of the neoplastic process activity, but not as a parameter differentiating the neoplastic proliferation from the mild form. The role H3 plays in the pathogenesis of NHL suggests HDAC-inhibitors may be found useful especially in the treatment of the aggressive forms (MCL and DLBCL).

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SECOND MALIGNANCY IN PATIENTS WITH HODGKINS LYMPHOMA. THE EXPERIENCE OF A HEMATOLOGICAL CLINIC

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Hodgkin's Lymphoma is today a potentially curable hematological malignancy. In the last two decades the survival rates reached values up to 90%. The patients are often very young and therefore it is possible to observe them for decades. It has been proven through the past years that patients with Hodgkin's lymphoma in their medical history have an increased risk of developing a second malignancy in their future life. Aim of this presentation is to demonstrate data of our clinic in this field in a retrospective study. From 1974 to 2005 a total of 146 patients with Hodgkin's Lymphoma were registered. 76 (52%) of them were women and 70 (48%) were men. The median patients' age was 36 years (18 to 87 yrs). 68 (46.6%) patients were treated with chemotherapy only, 20 (13.7%) received only radiotherapy and 58 (39.7%) patients received both chemo- and radiotherapy. 10 patients developed a second malignancy. 4 were women and 6 were men. The median age of the patients was 57 years (27 to 71 yrs). The median time from the initial Hodgkin's diagnosis to the appearance of the second malignancy was 10 years (2 months to 25 yrs). 2 patients were diagnosed with Breast-Ca, 2 with Non Hodgkin's Lymphoma, 2 with Acute Myeloid Leucaemia, 1 with Colon-Ca, 1 with Prostata-Ca, 1 with Parotis-Ca and 1 patient with Multiple Myeloma. 6 out of 10 patients had received both chemo- and radiotherapy (10.35% of the total number of patients treated this way). 2 patients had received only radiotherapy, (10% of all patients treated this way) whereas 2 patients were treated with chemotherapy only (2.95% of all patients treated this way). From the total of 10 patients 7 had experienced a relapse of the Hodgkin's lymphoma and received additional therapy at a later time point. The incidence of a second malignancy among patients with Hodgkin's disease in their medical history is considerably high and has a significant influence on the patients' survival despite the good healing rates. It appears that the higher risk of a second malignancy correlates with the total load of therapy received and that the risk is not limited to the first years after treatment but extends up to decades. Therefore longer follow ups of Hodgkin's patients are required since they are a risk group for developing second malignancies. It is a fact that most patients who develop a second malignancy are those who have received the bigger load of therapy for Hodgkin's Lymphoma. Therefore it is still necessary to adapt therapy regimes in such a way that patients receive the less load of therapy possible but still effective against the disease.

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GENTUZUMAB-OZOGAMICIN, CITOSINE ARABINOSIDE, G-CSF COMBINATION (G-ARA-MY) IN THE TREATMENT OF ELDERLY POOR PROGNOSIS ACUTE MYELOID LEUKEMIA

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Backround. Gentuzumab Ozogamicin (GO) is effective as single agent in the treatment of poor risk acute myeloid leukemia (AML) patients (pts). *Aims*. To evaluate the efficacy and safety of a chemotherapy including growth factors, cytarabine and GO in the treatment of poor prognosis AML elderly patients. *Methods*. In a multicentric study involving 3 Italian Hematology Departments from September 2003 to September 2006, a total of 53 elderly AML pts [median age 69 years (range 65-77)] were enrolled in G-AraMy protocol which was divided in two consecutive trials. In the first phase from September 2003 to December 2004 27/53 pts received G-AraMy-1 treatment: rhG-CSF (5 µg/kg, on days 1-8), Aracytin as continuous perfusion (100 mg/m^2 on days 4-8), GO (6 mg/m^2 iv over 2 hours on day 9). In the second phase from January 2005 to September 2006 26 pts were treated according G-Ara-My-2 protocol: rhG-CSF (5 µg/kg, on days 1-8), Ara-C as continuous perfusion (100 mg/m^2 on days 2-8), GO (6 mg/m^2 iv over 2 hours on day 9). In pts who reached complete (CR) or partial remission (PR), consolidation therapy was performed. In G-Ara-My-1 this consisted of: rhG-CSF(5 µg/kg, on days 1-6), Ara-C as continuous perfusion (100 mg/m^2 on days 2-6), GO (6 mg/m^2 iv over 2 hours on day 7). G-Ara-My-2 group was consolidated with: rhG-CSF(5 µg/kg, on days 1-5), Ara-C (1000 mg/m^2 every 12 hours on days 2-5), GO (6 mg/m^2 iv over 2 hours on day 6). *Results*. The 53 treated pts according FAB classification were divided into: 6 M0, 10 M1, 13 M2, 7 M4, 3 M5, 3 M6, 11 AML post-MDS. Twenty three (43%) out 53 patients had a secondary AML (sAML): 11 patients had a post-MDS AML and 12 pts had received chemotherapy for a prior malignancy (3 Hodgkin's lymphoma, 5 breast, 2 thyroid, 1 bladder, 1 gut). Twenty-seven out 53 pts (51%) had previously received chemotherapy for AML being relapsed (15) or primary resistant pts (12) while 26 (49%) were untreated pts. Cytogenetic study was performed in all pts; karyotype was at *intermediate prognosis* in 30 cases, at *worse prognosis* in 12 cases, and at *favourable prognosis* in 1 patient. In 10 cases no metaphases were observed. All pts performed the CD33$^+$ evaluation on BM, the median percentage of CD33 positive blasts was 90% (range 25%-95%). After induction and consolidation therapy 30 pts (15 group 1; 15 group 2) achieved a complete remission (CR) with an overall response of 57%. Eleven patients (21%) resulted refractory to treatment and 7 (13%) patients died during the aplasia period post induction treatment. The most common adverse event was myelosuppression, as expected. Only 1 patient developed an hepatic veno-occlusive disease resolved after defibrotide treatment. Induction death occurred in 7 pts (13%): 4 due to infections and 3 due to haemorrhagic complications. No differences in term of CR and toxicity profile were observed between untreated and primary resistant/relapsed patients, *de novo* AML and sAML, and in the 2 treatment trials. Median disease free-survival (DFS) and overall survival (OS) of whole population were 8 months (range 2-23+) and 9 months (range 2-24+) respectively. Comparing DFS between G-AraMy-1 and G-AraMy-2 a difference although not statistically significant emerged (p-value 0.07) with an increase of DFS in the G-araMy-2 groups. A difference statistically significant in OS was instead observed comparing the 2 treatment groups (p-value 0.017) with an increase of median OS from 6.13 months of G-AraMy-1 to 10.7 months of G-araMy-2. *Summary/Conclusions*. These results, obtained without the inclusion in the protocol of anthracyclines, suggest that G-AraMy therapy, in particular G-AraMy-2, could be considered an useful approach for poor risk elderly AML pts, allowing CR rate comparable to literature data with low side effects also in a very selected poor prognosis population.

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KIR/HLA CLASS I MISMATCHING AND RISK OF RELAPSE IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background. Recent data suggest that, in the bone marrow transplantation setting, KIR-driven alloreactivity might be better predicted if the donor KIR genotype is considered in addition to the HLA genotype of the recipient. The prediction of NK alloreactivity relies on the missing ligand in the recipient, a scenario that can be found in HLA identical and not identical allotransplants. *Aims*. The aim of this study was to investigate the prognostic impact, at a genetic level, of the lack of recipient HLA ligand for donor KIR on the outcome of allotransplanted patients. *Methods*. We analyzed the KIR genotype of donors and the HLA genotype of 64 pediatric patients with different hematological malignancies (NHL n:8, HL n:1, LAM n:17, LLA n:30, LMC n:2, Aplastic Anemia n:1, MDS n:3, Ewing/PNET Sarcoma n:1, and neuroblastoma n:1), who received unrelated (48) and related (16) bone marrow transplantation. *Results*. Donor/recipient pairs were separated into two categories on the basis of the presence (KIR/HLA-I matched: group 1) or absence (KIR/HLA-I mismatched: group 2) of recipient HLA class I ligands for donor KIRs. Data obtained from this analysis showed that the disease free survival (DFS) was reduced in KIR/HLA-I matched patients (group 1) vs. mismatched patients (group 2), although the difference was not statistically significant. Among patients with KIR/HLA-I mismatch an increase of relapse rate was observed in patients showing both activating and inhibitory genes involved in the genetic mismatch, with respect to patients showing inhibitory genes only ($p<0.05$). *Conclusions*. These results suggest that, in a missing ligand scenario, the presence of mismatch sustained only by inhibitory KIR gene may lead to a favourable prognosis, likely supported by a Graft versus Leukemia reaction. Conversely, when the activating KIR gene is also present, a similar favourable effect can not be predicted.

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LOW INTENSITY TRANSPLANTAT REGIMENS FACILITATE RECRUITMENT OF DONOR APECIFIC REGULATORY T CELL WHICH PROMOTE HEAMATOPOIETIC ENGRAFTMENT

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Background. The vital role of the graft-versus-tumour (GvT) response in eradicating residual malignancy after allogeneic stem cell transplantation (SCT) favors allografting approaches aimed at establishing donor haematopoietic engraftment rather than tumor eradication as the initial step. Mixed haematopoietic chimerism produces host-versus-graft (HvG) tolerance allowing subsequent donor lymphocyte infusions (DLI) for the therapeutic GvT effect. Low or reduced intensity conditioning (RIC) regimens for allogeneic haemopoietic stem cell transplantation (HSCT) can be very effective at establishing donor hematopoietic engraftment and host-versus-graft (HvG) tolerance. Regulatory T cells (Treg cells) play a crucial role in the maintenance of peripheral tolerance, susceptibility to autoimmune disease, tumor immunity as well as in the induction of transplantation tolerance. *Aims*. To investigate the role of regulatory T cells on HvG tolerance induction and maintenance in an animal model in which transplantation of sublethally irradiated female recipients with bone marrow (BM) from syngeneic male donors produces mixed chimerism. *Methods*. We have used an animal model for allogeneic SCT with reduced intensity conditioning (RIC), whereby donor and recipient differ only for the minor histocompatibility antigen HY. A female mouse receives 400cGy total body irradiation and is transplanted with male bone marrow. Under this condition, male donor cell engraftment is achieved and HvG tolerance is established. FACS analysis has been used to detect the percentage of $CD4^+CD25^+FoxP3^+$ cells in the peripheral blood of mice after TBI (400cGy) with or without male or female BM transplantation. By using mixed lymphocyte reaction (MLR) assays, splenocytes or CD4/CD25 depleted cells or purified $CD4^+CD25^+$ cells from female-female or male-female chimeric mice were analyzed for their suppressive ability. *in vivo* killing inhibition assays were used by adoptively transferring the whole or CD4/CD25 depleted splenocyte from chimeric mice to test their suppressive function. The effect of chimeric splenocytes was also assessed for their ability to favour engraftment of male BM cells in female recipient mice. Donor cell engraftment was detected using CD45 polymorphisms. To determine the role of Treg cells on the generation of donor cell engraftment, anti-CD24 antibody (PC61) was used *in vivo* to deplete $CD25^+$ cells before and after BMT. *Results*. Splenocytes from chimeric mice inhibited HY-specific $CD8^+$ T cell responses both *in vitro* and *in vivo*, and their adoptive transfer facilitated donor hematopoietic engraftment. These properties were contained within the $CD4^+CD25^+$ population. The conditioning protocol alone led to a proportional expansion of regulatory T (Treg) cells, but the inhibitory activity was induced only if male BM was infused. The administration of anti-CD25 depleting antibodies to conditioned recipients at time of BMT prevented donor-recipient chimerism, but did not affect engraftment if performed after the establishment of chimerism, thus indicating that recipient Treg cells are required for the generation but not the maintenance of HvG tolerance. *Conclusions*. Donor-specific Treg cells of recipient origin are recruited when the donor antigens are present during RIC induced Treg expansion. These findings have important implications for the design of tolerogenic regimens in transplantation.

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COMPARISON OF JAK2 V617F POSITIVE AND NEGATIVE MYELOPROLIFERATIVE DISEASES

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Background. The V617F point mutation of Janus kinase 2 (JAK2) gene is believed to participate in the pathogenesis of chronic myeloproliferative disease (CMPD) characterized by clonal alteration of hematopoietic stem cell. According to current results, the frequency of V617F activating mutation is around 80% in polycythaemia vera (PV), 35% in essential thrombocythaemia (ET), and 50% in chronic idiopathic myelofibrosis (CIMF), although phenotypic variability can not be explained by this single mutation. *Aims*. The goal of the present work was to compare clinical phenotype of V617F mutation positive and negative CMPDs. *Methods*. Laboratory (hemoglobin, white blood cell and platelet count) and clinical features (sex, age at diagnosis, splenomegaly, presence of thrombotic, myelofibrotic or leukemic transformation) were recorded. We employed allele specific polymerase chain technique for detection of V617F mutation in 328 patients with myeloproliferative syndrome. *Results*. JAK2 V617F frequency was 87.4% (153/175) in PV, 61.1% (77/126) in ET, and 70.3% (19/27) in CIMF. We found significantly elevated hemoglobin level (measured at the time of diagnosis) in V617F-positive CMPD and ET patients compared to V617F-negative patients (ET: 144±21 vs. 130±14 g/L). The age of CMPD onset was higher in JAK2 V617F positive PV patients (60±12 vs. 47±16 year±SD). Female predominance was observed in V617F positive PV and CIMF patients. The incidence of vascular complications (arterial and venous thrombosis or bleeding) was higher in JAK2 V617F CMPD. The transformation rates to myelofibrosis or acute leukemia were not altered by JAK2 mutational status. *Summary*. Our data confirm earlier observations that JAK2 V617F-positive ET shares clinical features (elevated hemoglobin) with PV and suggest that JAK V617F influences the rate of vascular complications.

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IRON OVERLOAD IN PATIENTS WITH MDS: BASELINE DATA FROM STUDIES OF THE ONCE-DAILY ORAL IRON CHELATOR, DEFERASIROX

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Background. Approximately 60% of patients with myelodysplastic syndromes (MDS) require ongoing red blood cell transfusions, with resultant iron accumulation and need for iron chelation therapy to avoid the morbidity and mortality associated with transfusional hemosiderosis. Historically, many MDS patients have not received iron chelation therapy because of the difficulty of administering deferoxamine (DFO; Desferal®). The once-daily oral iron chelator, deferasirox, has demonstrated efficacy in a wide range of patients with chronic iron overload, including those with MDS. Studies continue to evaluate this patient pop-

ulation. *Aims*. To evaluate the severity of iron burden in patients with Low/Int-1-risk MDS using baseline data from two ongoing Phase II studies. *Methods*. Studies US02 and US03 are evaluating the efficacy and safety of deferasirox, and will enroll 30 and 150 patients, respectively, with Low- or Int-1-risk MDS. Informed consent has been obtained from all patients. Deferasirox dosing starts at 20 mg/kg/day, up to a maximum of 30 mg/kg/day. Iron burden is being monitored by monthly serum ferritin measurements, as well as serum iron, transferrin, transferrin saturation, labile plasma iron (LPI) and liver iron concentration (LIC). In addition, creatinine, calculated creatinine clearance and hematological status are being monitored. *Results*. To date, US02 and US03 have enrolled 14 (aged 55-81 years) and 84 (aged 47-87 years) patients, respectively. Enrolled patients had Low-risk (50% in US02; 28% in US03) or Int-1-risk (50% in US02; 72% in US03) MDS. A total of 51 patients (61%) in US03 had received DFO prior to enrolment, and two had received deferasirox. The Table 1 summarizes baseline iron parameters in patients enrolled in both trials. At baseline, concurrent therapies included 5-azacytidine (Vidaza; 6% of patients in US03), lenalidomide (Revlimid; 14% in US02 and 1% in US03) and hydroxyurea (7% in US02). In US02 and US03, baseline calculated creatinine clearance was normal (>80 mL/min) in 46% and 49% patients, respectively; mildly abnormal (51-80 mL/min) in 46% and 40% patients, respectively; and moderately abnormal (30'50 mL/min) in 8% and 12% patients, respectively. In US02 and US03, pretreatment baseline cytopenias noted, in addition to anemia, included neutropenia (<1800/µL; 8% and 17%, respectively), thrombocytopenia (<100,000/µL; 21% and 19%, respectively), and combined neutropenia and thrombocytopenia (8% and 15%, respectively). *Conclusions*. These baseline data demonstrate a high degree of iron overload among transfused patients with MDS, with serum iron, ferritin and LPI all significantly higher than the threshold values associated with clinical complications. Levels are seen to be high even in patients who had previously received DFO, indicating poor therapeutic compliance and/or efficacy. These baseline data also highlight the pretreatment levels of cytopenias in this elderly population and reduced calculated creatinine clearance, impressing the need to gain baseline measurements for subsequent patients management. The ongoing trials will assess the long-term efficacy, safety and clinical benefits of once-daily oral deferasirox therapy in patients with MDS.

Table 1.

Parameter (mean ± SD)	Study US02	Study US03	Normal Range
Total transfusions	106 ± 116	63 ± 66	0
Years of transfusions	NA	3.4 ± 1.9	0
Serum ferritin, µg/L	4645 ± 3804	3779 ± 4070	12–370
Serum iron, µg/dL	206 ± 27	205 ± 64	37–180
Transferrin, mg/dL	143 ± 19	153 ± 31	190–400
Transferrin saturation, %	114 ± 9	85 ± 15	15–50
LPI, µmol/L	0.7 ± 0.7	0.5 ± 0.6	0
LIC, mg Fe/g dw	21.8 ± 11.0	NA	<1.3

NA: Not available

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TOTAL PLASMA TFPI IN PRE-ECLAMPSIA

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Background. Tissue factor pathway inhibitor (TFPI) is a specific inhibitor of the tissue factor dependent-pathway. It is synthesized primarily by the vascular endothelium. Approximately 85% of TFPI is bound to glucosaminoglycans of the vascular endothelium while only 15% circulates in the blood. About 80% of blood-TFPI is associated with lipoproteins and 20% is physiologically active. A small amount of TFPI is stored in platelets and released upon platelet activation. Pre-eclampsia (P-Ec) is associated with a triad of symptoms including hypertension, proteinuria and edema, occurring after 20th week of pregnancy. Irrespective of intensive research, P-Ec remains one of the leading causes of maternal death worldwide. As yet, there is no reliable screening test or effective treatment to cure this disease. *Aims*. To evaluate plasma TFPI levels in non pregnant women, healthy pregnant women and women with P-Ec. *Methods*. Total plasma TFPI antigen levels were measured in a total of 57 subjects using an enzyme-linked immunosorbent assay (ELISA). These include non pregnant women (n=22), healthy pregnant women (n=15) and women with pre-eclampsia, at the third trimester (n=20). *Results*. We observed no significant difference in plasma TFPI levels between the three studied groups. The mean and standard deviation (mean±SD) for the three groups were: non pregnant women (38.50±11.05), healthy pregnant women (30.82±10.16) and pre-eclamptic women (38.89±14.49). *Conclusions*. Pre-eclampsia is a complex multisystem disorder and proteinuria is one of its common feature. TFPI has a relatively small molecular weight (38 kDa) and, under the circumstances, would easily be secreted through the kidneys. Therefore, plasma TFPI levels my not reflect the etiopathogenesis of the disease.

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CCN3: AN ADDITIONAL MARKER FOR PROFILING RESPONSE TO IMATINIB?

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Background. Chronic Myeloid Leukaemia (CML) is characterized by expression of the constitutively active Bcr-Abl tyrosine kinase. Molecular monitoring of BCR-ABL expression levels by Real-time PCR allows profiling of minimal residual disease for patients being treated with Imatinib. We have shown previously that the negative growth regulator, CCN3, is down-regulated as a result of Bcr-Abl kinase activity and that CCN3 has a reciprocal relationship of expression with BCR-ABL (McCallum *et al.*, Blood 2006; 108(5):1716-23). *Aims.* To evaluate CCN3 as a marker for response to Imatinib. *Methods*. Real-time PCR was used to determine CCN3 and BCR-ABL expression in CML patients undergoing treatment with Imatinib and in normal donors. *Results*. CCN3 expression was high in bone marrow (median 1600, range 253-7413 copies per 5 microlitres cDNA; n=6) and peripheral blood taken from normal donors (median 2900, range 120-4280 copies per 5 microlitres cDNA; n=6). BCR-ABL expression was high in all 10 CML patient samples at diagnosis (median 7250, range 275-319,000 copies per 5 microlitres cDNA). Of these, 5 patients responded well to Imatinib treatment and had less than 10 BCR-ABL transcripts per 5 microlitres cDNA, for a period of 6 months or more. These patients demonstrated low CCN3 expression at diagnosis (median 0, range 0-22.1) which then increased in response to Imatinib treatment (median 262, range 53.6-943). In contrast, the other 5 patients had high levels of CCN3 at diagnosis (median 1860, range 764-3580) and these patients did not attain less than 10 BCR-ABL transcripts per 5 microlitres cDNA for a period of 6 months. In these patients, CCN3 expression did not have a reciprocal relationship with BCR-ABL, instead CCN3 expression remained constant or mimicked that for BCR-ABL. *Conclusions*. CCN3 expression has a reciprocal relationship with BCR-ABL for patients responding to Imatinib. CML patients not achieving a molecular response have alternate CCN3 expression profiles. A larger cohort of patients is currently being investigated to determine if CCN3 may provide an additional molecular marker for CML patient response.

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JAK2 V617F MUTATION IS ASSOCIATED WITH INCREASED RISK OF THROMBOSIS IN CHINESE PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA

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Background. Recently, several groups have identified a single point mutation in the JAK2 gene in the Philadelphilia-negative myeloproliferative disorders. The presence of this mutation has been detected in high frequency in patients with polycythaemia rubra vera (PRV), essential thrombocythaemia (ET) and idiopathic myelofibrosis and suggests that it may have a fundamental role in the pathogenesis of these disorders. *Aims*. We evaluated the possible associations of the JAK2 V617F mutation with clinical characteristics, laboratory parameters and the impact on outcomes. *Methods*. Consecutive patients with ET who were followed at the Prince of Wales Hospital, a tertiary teaching hospital of the Chinese University of Hong Kong, were included in this study. Diagnosis of ET was made according to the WHO criteria and patients with PRV were excluded. Patients' clinical features, laboratory parameters, treat-

ment details and thrombotic histories were evaluated by chart review. JAK2 V617F mutation was detected qualitatively by allele-specific polymerase chain reaction method on whole blood DNA. Informed consents were obtained from patients. *Results*. Ninety-five patients were included and all were ethnic Chinese. Median age was 62 (range 21-89). There were 48 males and 47 females. The median follow-up was 68.4 months (range 5.9-227). The JAK2 V617F mutation was identified in 60 (63%) of our ET population, which is slightly higher than those reported in the literature. Univariate analysis showed that patients with the mutation were older and had significantly lower platelet counts at diagnosis. They required lower dose of hydroxyurea to control platelet count but had higher frequency of thrombotic events (32% vs 9%, p= 0.003). The effect of JAK2 mutation on rate of thrombotic events was maintained in multivariate analysis. *Conclusions*. The frequency of the JAK2 V617F mutation in our group of Chinese patients with ET was slightly higher than those reported in the literature. Our results suggested that the presence of JAK2 V617F mutation in this group of ET patients defines a subset of patients with a thrombotic tendency. Further prospective studies to evaluate the role of JAK2 V617F mutation in risk stratification for therapy are needed.

Table 1. Clinical features of ET patients.

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INVOLVEMENT OF TNF α IN LEUKAEMIA ONSET AND BONE MARROW TURNOVER

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Leukaemia is characterized by abnormal ratios of bone marrow cells from several hematopoietic lineages, meaning that normal tissue turnover is impaired in this situation. Bone marrow diseases have also been associated with an increase in bone marrow angiogenesis. It is known that changes in bone marrow microenvironment may lead to a loss of homeostasis; important players in this process may be cytokines and extracellular matrix (ECM) molecules present in bone marrow stroma. One factor possibly involved in bone marrow cell turnover, in normalcy and in disease, is TNF-α; this study focused on the functions of bone marrow TNFα, in regulating cell apoptosis within the bone marrow microenvironment and also in regulating extracellular matrix (ECM) turnover. Whilst studying bone marrow turnover following sublethal irradiation, we observed that TNF-α undergoes significant variations, acutely increasing following irradiation, and decreasing to normal values 5 days after the stimulus. To understand the possible role of TNF-α in leukaemia development, we irradiated TNF-α knock-out (KO) and wild-type (wt) mice thrice (each irradiation was separated 1 month apart), a model that has been shown to result in leukaemia induction. From the five mice we irradiated from each genotype, four wt mice died 6-7 months after the last irradiation as a result of leukaemia, while in the KO group only one mouse succumbed to a possible bone marrow deficiency (although not overt leukaemia). FACS analysis of peripheral blood (PB) and bone marrows from the different mice, revealed that WT mice had increased endothelial progenitors and increased endothelial cells, suggesting the vascular lineage was increased in these mice, following the irradiation schedules. In addition, the bone marrows of TNF-α KO mice were smaller in diameter, but after irradiation, while wt bone marrows undergo a notorious reduction, the KO bone marrows remain with more or less the same size, meaning that probably, the altered mice are more resistant to irradiation. KO bone marrow (irradiated and control) also exhibited increased megakaryocyte numbers, accompanied by a decrease in fibronectin and laminin levels, in irradiated KO mice. Another important observation is that irradiated wt mice, which developed a bone marrow disease phenotype, have increased bone marrow angiogenesis, which consisted of dilated blood vessels. Taken together, these results suggest that TNF-α plays a role in bone marrow homeostasis, which is more clearly linked with angiogenesis and ECM turnover. In KO mice, selective apoptosis may contribute to select leukaemia clones; in parallel, TNF-α is also crucial in modulating MMP activity, and as a result its absence may contribute towards a global reduction in bone marrow MMP activity. Reduced MMPs may in turn lead to a reduced availability of ECM-bound VEGF, thus regulating bone marrow angiogenesis. Globally, our data point out for a crucial role of TNF-α in modulating bone marrow turnover and angiogenesis, which may contribute to leukaemia onset.

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PHOSPHO TYROSINE KINASE PROFILLING IN PRIMARY CULTURES FROM PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Chronic myeloid leukemia (CML) is a hematopoietic disorder characterized by the malignant expansion of bone marrow stem cells. Pathogenesis of CML is associated with a single genetic defect-t(9;22) reciprocal chromosomal translocation which results in constitutive activity of BCR-ABL tyrosine kinase. Imatinib mesylate (STI571, Glivec, Gleevec) is a tyrosine kinase inhibitor specific for ABL kinase as well as for other kinases such as c-Kit, PDGF-R or ARG. Recently, Imatinib has been used successfully as the first line therapy for CML patients. Nevertheless additional alteration of BCR-ABL or other kinase can appear and lead to the treatment resistance. Investigation of active kinase pattern can disclose primary resistance prior to treatment as well as suitable targets for subsequent therapy. Expression of Wilm's tumor (WT1) transcription factor is elevated in several types of acute and chronic leukemia including CML. The positive response or upfront Imatinib resistance can be predicted on the base of WT1 expression after short-term cultivation of primary cells. *Aims*. The aim of this study was to establish a profiling pattern of tyrosine kinase activity in primary cells from CML patients and clarify changes of kinase activity after imatinib exposure. *Methods*. The effect of tyrosine kinase inhibitors (Imatinib, Herbimycin A, PP2 and JAK1) on primary culture was characterized by the expression of WT-1, proliferation marker Ki-67 and analysis of apoptosis. Gene expression was performed by quantitative real-time RT-PCR. Phosphotyrosine profiling array was used for determination of tyrosine kinase activity profile after Imatinib exposure. *Results*. Primary cells from patient in chronic phase of CML were evaluated as Imatinib sensitive by decrease of WT-1 mRNA expression after short-term exposure in culture. Response to Imatinib was accompanied by an increase of apoptosis and inhibition of proliferation. Imatinib inhibited seven (Abl1, Fyn, P85A, PTPN11, SHC1, SHC2, Src), out of forty, tyrosine kinase domains. On the other hand, four tyrosine kinase domains (EAT2, GRB14, HCK, and MATK) exhibited higher activity after short-term exposure to Imatinib. Untreated cultured cells served as a control. Effect of src kinase inhibitors (Herbimycin A and PP2) but not Jak kinases inhibitor (JAK1) correlated with the effect of Imatinib in some patients. *Conclusions*. Creating a kinase pattern disclose active signaling pathways, enables to clarify mechanism of response or resistance to distinct treatment and thus the therapy can be targeted more efficiently.

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LOSS OF HETEROZYGOSITY AND MICROSATELLITE INSTABILITY IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Chromosome 14 abnormalities -mostly translocations- are nearly seen 50 percent of multiple myeloma (MM) patients and these abnormalities are important in the pathogenesis of MM. Genomic insta-

bility is a characteristic of tumor cells. Loss of heterozygosity (LOH) is an indirect method of detecting inactivation of tumor suppressor genes and, microsatellite instability (MSI) is the other form to show DNA mismatch repair system which leads to replication rerrors. *Aims*. In this report we examined the LOH and microsatellite instability in patients with MM in order to point to genomic instability in chromosome 14 and we compared them with clinical stage and Ig type of disease. *Materials and Methods.* We selected the 5 different STR loci of cromosome 14 (14q32). 26 patients were included into the study (10 female, 16 male, mean age 63 year). 7 patients diagnosed as stage one, 7 patients stage two, 12 patients were stage 3. According to Ig heavy chain 14 patients had IgG, 5 patients IgA, 5 patients had light chain disease and one had non secretory MM. DNA was extracted from the bone marrow plasma cells after the separation procedure with magnetic beads from the residuel bone marrow cells and hair DNA was used as control. After the PCR with fluorescein congugated primers, the MSI and LOH was detected by capillary electrophoresis (ABI 310). *Results*. MSI was detected %35 of patients in D14S65 locus. LOH was detected %23 of patients in D14S985 loci. The other MSI and LOH findings were rare. Fifthy per cent of IgG MM had MSI in D14S65 locus. Four patients with IgG MM (%28) and one patient with IgA MM had either MSI or LOH in all 5 locus (%20). No significant association of any molecular defect to Ig type and clinical stages of disease was found. *Conclusions*. In present study we showed that MSI and LOH are comman findings in MM especially chromosome 14q32 region which we know that Ig Heavy chain is being encoded.

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MONITORING LEUKEMIA PATIENTS' RESPONSE TO CHEMOTHERAPY BY A NOVEL IR LIGHT SPECTROSCOPIC METHOD

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Background. Diagnosis and monitoring leukemia progression have great importance in determining prognosis and choosing the appropriate protocol for treatment. Present diagnostic methods such as FACS and cytogenetics of bone marrow are expensive and involve invasive procedures and thus cannot be used for daily monitoring of individual patients' response to chemotherapy. Fourier transform infrared (FTIR) spectroscopy is an inexpensive, rapid and simple optical method for daily monitoring biochemical changes of nucleic acids, lipids and proteins in mononuclear cells. In preliminary studies, we were able to identify leukemia patients and follow their reaction to chemotherapy treatment daily by analyzing FTIR spectra of peripheral blood mononuclear cells. *Aims*. We aim to use FTIR for diagnosis and follow-up of leukemic children undergoing chemotherapy treatment. Furthermore, we will search for parameters which provide information on the biochemical changes in mononuclear cells during chemotherapy treatment. To investigate the nature of FTIR spectral changes following drug treatment, we will use leukemic cell lines as a model system. *Methods*. Mononuclear cells from blood samples were obtained from 30 acute lymphoblastic leukaemia (ALL) patients and 27 healthy controls by the standard Ficoll-Hypaque technique. FTIR measurements of mononuclear cells were carried out using a FTIR microscope IRscope II. CCRF-CEM cells were treated with doxorubicin in different concentrations. Apoptotic and necrotic morphology was evaluated by staining CCRF-CEM cells with acridine orange and ethidiume bromide, and examining them using a fluorescent microscope. *Results*. By calculating the ratio of the functional groups CH3/CH2, which are related to biochemical changes of proteins and lipids respectively, we were able to distinguish between leukemic and healthy groups (Figure 1a). This ratio also served as a parameter to monitor the effect of chemotherapy on mononuclear cells of the leukemic patients. Figure 1b depicts the response of two leukemic children to chemotherapy monitored by FTIR. According to the analysis, child #1 responded faster than child #2 (owing to the development of a blood infection in child #2) to the treatment as reflected by the return of the CH2/CH3 ratio to normal values. In order to understand the effects of chemotherapy on an FTIR spectrum of mononuclear cells, we conducted *in vitro* experiments on CCRF-CEM cells treated with doxorubicin. As seen in Figures 1c and 1d, several spectral changes appeared due to modifications in quantity/conformation of membrane lipids, nucleic acids and proteins following doxorubicin treatment. These modifications correlated with the rate of apoptotic and necrotic cells monitored using a fluorescent microscope. Additional experiments with other known cell death inducers such as Ara-C, H2O2 and KCN were carried out using FTIR spectroscopy and were compared with biochemical standard methods (*data not shown*). *Conclusions*. We conclude that FTIR is a powerful biochemical tool that can be used for leukemia diagnosis as well as for monitoring the effects of chemotherapy on mononuclear cells.

Figure 1.

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PRE-TRANSPLANT LOW DOSE ATG AS PROPHLYLAXIS FOR CHRONIC GVHD AFTER PERIPHERAL BLOOD STEM CELL MYELOABLATIVE SIBLING TRANSPLANTS IN AML: A SINGLE CENTER EXPERIENCE

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Background. Chronic graft versus host disease (cGvHD) incidence and severity are generally found to be greater after peripheral blood stem cell transplantation (PBSCT) than after bone marrow transplantation, and some studies increased mortality from cGvHD, particularly the extensive type. *In vivo*, T-cell depletion with antithymocyte globulin (ATG) is an effective strategy for decreasing the incidence of cGvHD but may include side effects such as high risk of infection and relapse. The main utilization of ATG has been as prophylaxis of GvHD in unrelated bone marrow transplantation but few data are available on myeloablative HLA-identical sibling PBSCT. *Aims*. In our study we evaluated the incidence of infections, relapse and cGvHD in 30 patients with acute myeloid leukemia (AML) in first complete remission after a peripheral stem cell myeloablative HLA-identical sibling transplant. *Patients and Methods*. Conditioning regimen was oral busulfan (16 mg/Kg), cyclophosphamide (120 mg/Kg) and ATG-Thymoglobuline (4 mg/Kg total dose given on days -2 and -1); the median dose of $CD34^+$ cells and $CD3^+$ cells infused were 4.6×10^6/Kg (range 3.1-9) and 250×10^6/Kg (range 84-442), respectively. GvHD prophylaxis was Cyclosporin A (2 mg/Kg/die) and short-term Methotrexate (15 mg/m^2 on day +1, 10 mg/m^2 on day +3,+6,+11). *Results*. The median time to neutrophil (>0.5×10^9/L) and platelet (>20×10^9/L) engraftment was 15 (range 13-17) and 18 (range 15-20) days, respectively. Acute GvHD grade I-II was observed in 6/30 (20%); no patients experienced aGvHD grade III-IV. CMV antigenemia was monitored twice a week for the first 100 days; 18/30 (60%) patients developed CMV reactivation but no CMV disease occurred. As to immunological recovery, the median number of $CD3^+$ and $CD4^+$ after 1, 3 and 6 months after transplantation was 690, 850, 930 and 125, 214, 256×10^9/L, respectively; the median number of CD8 and NK cells after 1, 3 and 6 months was 430, 538, 653 and 216, 156, 230×10^9/L, respectively. At a median observation time of 34 months (range 8-60) the overall cGvHD was 8/30 (26%) (limited 5/8 or 17% and extensive 3/8 or 9%); the relapse rate was 8/30 (26%). *Summary*. Our data suggest that low dose of ATG is effective in preventing acute and chronic GvHD without increase in relapse; prospective randomized trials are needed to evaluate the role of ATG in AML undergoing allogeneic stem cell transplantation from HLA-identical sibling.

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INCIDENCE AND SPECTRUM OF INFECTION IN PATIENTS TREATED WITH ALEMTUZUMAB: A MULTICENTER EXPERIENCE

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Introduction. Alemtuzumab (Campath 1-H) is a monoclonal antibody reactive with the CD52 antigen used for therapy in lymphoproliferative disorders and T-cell lymphomas. As a result of this therapy, patients often experience profound T-cell immunodeficiency. Consequently, the spectrum of infections organisms is changing, and they can cause serious morbidity and mortality. We designed a retrospective study in our hospitals, with the aim of evaluating the incidence and spectrum of infections in these patients. *Patients and Methods*. From June 2001 through December 2006, 24 patients who received alemtuzumab were evaluated. The median age was 66 (range, 23-70). Median of prior regimens was 3 (range, 0-6) and the median weeks of alemtuzumab therapy was 7 (range, 3-20). All patients received antiviral infection prophylaxis, Pneumocystis prophylaxis and 11 patients (45,8%) oral fungal prophylaxis. *Results*. 35 episodes of infection were noted in 17 patients at a median of 27 days (range, 10-203) after the first dose of alemtuzumab. 9 patients had a single episode, 3 had two, 2 had three, 1 had four and 2 patients had five episodes of infection. Infectious side effects included CMV reactivation (31,4%), pneumonia (31,4%), fever of unknown origin (11,4%), urinary infection (11,4%), gastro-intestinal infection (8,5%) and cutaneous infection (5,7%). 12 episodes occurred during neutropenia (neutrophil count less than 1.0×10^9/L). 2 patients showed clinical evidence of CMV disease, a patient with pneumonia and other with gastrointestinal disease. Fungal isolation occurred in 7 patients: 2 Candida albicans, 1 Aspergillus fumigatus, 1 Aspergillus niger, 1 Saccharomyces cerevisiae, 1 Penicillium and 1 mould infection detected in a cutaneous biopsy without culture. Other virus infection distinct than CMV were 1 adenovirus and 1 varicella zoster. Gram-negative bacteria were isolated in three cases and Mycobacterium tuberculosis in one. 30 episodes of infection improved or resolved with therapy and the overall mortality related to infection was 14,28%. By univariate regression analysis risk factors for infection includes: neutropenia and non-responders to alemtuzumab. Age, number of prior regimens and previous use of prednisone were not risk factors for infection with a trend towards significance for prior fludarabine therapy. *Conclusions*. Despite use of herpesvirus prophylaxis, Pneumocystis pneumonia prophylaxis and, in some patients, fungal prophylaxis, serious infections complications occur in our patients receiving alemtuzumab. Infections are more varied and extended than previous reports, particularly in those who have advanced disease and poor responses to alemtuzumab.

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ASSOCIATIONS BETWEEN GENDER AND AGE AT PRESENTATION WITH CLINICAL STAGE, IGVH MUTATIONAL STATUS, GENE USAGE, AND CYTOGENETIC ABERRATIONS IN B-CLL

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Background. B-CLL is a heterogenous disorder with a highly variable clinical course and median survival of approximately 10 years. It affects mainly older individuals and shows a male preponderance. The prognostic relevance of patient characteristics such as age and gender has been studied extensively. However, the relationship between such characteristics and the molecular features defining poor outcome in B-CLL, such as 17p chromosomal deletion, high CD38 expression and lack of IgVH hyper-mutation, remains to be determined. *Aims*. The aims of this study were to determine if age at presentation, or gender, showed an association with IgVH mutational status and/or gene usage, clinical stage or cytogenetic aberrations. *Methods*. Three hundred and three B-CLL patients were recruited for this study. Binet clinical staging, immunophenotype, lymphocyte doubling time and time to treatment were available on most patients. IgVH mutational status, gene usage and CDR3 sequences were determined using multiplex BIOMED-2 primers (InVivoScribe Technologies) and protocol and by sequence analysis. Interpahse FISH analysis was performed on all patients to screen for common cytogenetic aberrations. *Results*. Eighteen patients were ≤ 50 years of age and 285 were >50 years upon presentation. There were no significant differences in the male:female ratio, IgVH mutational status, or poor prognosis cytogenetic aberrations between the 2 age groups. However, there was a significant increase in the number of Binet stage B and C patients in the older age category. In addition, IgVH4-34 gene usage was more prevalent in the younger age group (23.5% compared to 11.0% in the older group). Overall, in all patients the CLL cohort consisted of 61.4% males. Binet stage B and C disease, and unmutated IgVH gene status, were significantly more frequent in males than females. Interestingly, IgVH3-30 and IgVH4-34 gene rearrangements were significantly more common in females than males. Females were also more likely to have no detectable poor prognosis cytogenetic aberrations, whilst trisomy 12 was significantly more prevalent in the male sub-group. *Summary/Conclusions*. In conclusion, our findings confirm that male CLL patients have biomarkers associated with poorer prognosis, a factor accentuated with advancing age at diagnosis. The associated molecular basis for this phenomenon remains to be elucidated. The biased gene usage and proportion of somatic hyper-mutation in the female sub-group detected in this study suggests the possibility of gender specific infective aetiology. Ongoing studies are being carried out to investigate this possibility.

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PALIFERMIN REDUCES THE INCIDENCE OF ORO-PHARYNGEAL MUCOSITIS IN MULTIPLE MYELOMA PATIENTS RECEIVING HIGH-DOSE MELPHALAN WITH STEM CELL RESCUE

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Background. Mucositis is a frequent and debilitating complication experienced by patients receiving high-dose chemotherapy and/or radiotherapy, which significantly affects treatment outcomes and health care resources used for its management. *Aims*. To assess possible differences in incidence, duration and severity of oro-pharyngeal (OP) mucositis and its related clinical sequelae between 2 groups of multiple myeloma (MM) patients undergoing myeloablative chemotherapy and autologous haematopoietic stem cell transplantation (HSCT), one receiving OP mucositis prophylaxis with a standardized regimen of Palifermin (PAL) and one receiving no prophylaxis. *Methods*. This observational, retrospective and comparative study involved 23 patients with MM treated with high-dose melphalan (HDMEL, 200 mg/m^2) conditioning regimen followed by autologous stem cell rescue. Seven patients who received PAL (PAL Group) were compared to 16 patients who received no prophylaxis for chemotherapy-induced OP mucositis. OP mucositis was assessed according to the WHO Oral Toxicity Scale. *Results*. In PAL Group, the median age was 58.0 years (range 45-62), 5 patients (71%) were male, 5 (71%) had Salmon-Durie (SD) stage IIIA at diagnosis, and the most frequent type of monoclonal paraprotein was IgG/kappa (3 patients, 43%). In Control Group, the median age was 56.5 years (range 42-66), 11 patients (69%) were male, 11 (69%) had Salmon-Durie (SD) stage IIIA at diagnosis, and the most frequent type of monoclonal paraprotein was IgG/kappa (7 patients, 44%). Groups were homogeneous with regard to previous radiotherapy, chemotherapy (median two treatment lines for the PAL Group vs one treatment line for the Control Group, previous autologous HSCT, number of CD34$^+$ cells infused, and therapeutical aplasia. Four patients (57%) developed OP mucositis in PAL group, compared to 15 (94%) in Control Group (p=0.03). Time to development of OP mucositis was longer in PAL Group (median 4.5 vs 3.0 days after HSCT, p=0.062). OP mucositis lasted longer in Control Group (median 11.0 vs 8.5 days, p=0.192). No statistically significant difference concerning WHO score was found between the two groups. All 23 patients developed febrile neutropenia, with longer median duration in Control Group (5.0 vs 2.5 days, p=0.231). *Summary/Conclusions*. Prophylaxis with PAL significantly reduced the incidence of chemotherapy-related OP mucositis in patients treated with HDMEL and autologous HSCT. A larger patient sample is needed to study other relevant variables, such as analgesia and need for total parenteral nutrition. Nevertheless, this positive response warrants further investigation.

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LONG-TERM OUTCOME AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC TRANSPLANTATION IN THERAPY-RELATED MYELODISPLASTC SYNDROMES AND ACUTE MYELOID LEUKEMIAS

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Therapy-related MDS (tMDS) and AML (tAML) generally have a very poor outcome. Curative treatment strategies are not yet standardized

and the role of hematopoietic stem cell transplantation is still not fully elucidated. We performed an observational analysis on 26 patients (pts) with tAML (n=15, FAB M0, M1, M2, M4) and tMDS (n=7 RAEB-2. n=4 RAEB-1) developed after chemo/radiotherapy for lymphoma or breast cancer. Conventional karyotype analysis was available in 14 pts (n=8 complex, n=1 normal, n=2 isolated del(7q) or monosomy 7, n=1 isolated del(5q), n=1 trisomy 21, n=1 trisomy 8). All patients were considered as candidates to allogeneic stem cell transplantation (SCT), and 21 of them already received the planned SCT. The purpose of this study was to determine the long-term outcome. Median age was 53 years (range:23 - 68); all pts, because of comorbidities, received a reduced-intensity conditioning (RIC) followed by allogeneic peripheral blood SCT. One pt died for disease progression before transplant and 4 pts are still completing consolidation therapy before allo-SCT. Disease status at transplant was categorized as low risk (n=7 CR1 or CR2), high risk (n=3 PR, n=5 PD, n=2 refractory) and 4 pts were treated up-front. The median time from diagnosis to allografting was 6 months (range: 1-80 months). Pts received allogeneic stem cells from HLA-matched/1ag mismatched siblings (n=15), or HLA-matched unrelated doors (n=4), or haploidentical related donors (n=2). All pts engrafted. Acute GVHD grade II-IV occurred in 6 pts (n=1 post-DLIs), chronic GVHD developed in 6 pts (n=1 post-DLIs). OS at 5.5 years was 5.3%, the 4-year EFS was 0%, TRM at 100 days and at 1 year were 29.8%. No statistical differences were observed in TRM, EFS, and OS according to disease status at transplant and diagnosis of tMDS or tAML. In conclusion, our data show that the outcome of patients with therapy-related leukemias was very poor even after allogeneic SCT

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EFFECT OF BUSULFAN AND CYCLOPHOSPHAMIDE ON ENDOTHELIAL AND HEPATIC CELLS IN CULTURE: TOWARDS UNDERSTANDING PATHOGENESIS OF HEPATIC VENO-OCCLUSIVE DISEASE

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Background. Hepatic veno-occlusive disease (HVOD) is the major complication of Busulfan (Bu)/Cyclophosphamide (CPA)-based conditioning regimen prior to hematopoietic stem cell transplantation (HSCT). Although clinical efficacy of Bu/CPA combination is well established, interindividual differences in susceptibility to drug-induced toxicities are significant. The pathogenesis of HVOD is complex. Even though age, sex, preexisting liver damage and type of tranplant have been associated with this complication, high bioexposure to these drugs is considered to be the major determinant. Analysis of biological markers suggests the potential involvement of cytokines and haemostasis in mediating the drug-induced damage of sinusoidal endothelial cells (SEC) and adjacent centrilobular hepatocytes in HVOD. *In vivo*, Bu is metabolised by conjugation with glutathion (GSH), catalysed by a family of Glutathion S-transferase (GST) enzymes. Of the 4 main subfamilies of GST (GST A1, GST M1, GST P1 and GST T1), GST M1 and GST T1 are highly polymorphic, with homozygous deletion of either one or both genes found in significant frequencies in different ethnic groups. Although these isoforms are considered to be less efficient than GST A1 in the metabolism of Bu, we reported earlier that both Bu clearance and GST M1-null genotype were significantly associated with the incidence of HVOD. *Aims*. We hypothetize that Bu and CPA could modulate GST expression and so act on their own metabolism and that these drugs and/or their metabolites affect molecules implies in hemostasis (TF), vasomotricity (ET-1), endothelial adhesion (ICAM-1) and GvHD (PECAM-1) leading to HVOD. *Methods*. We have studied the effect of Bu and CPA on an endothelial cell line (TrHBMEC), as well on primary endothelial cells (HUVEC) from several donors and an hepatic cell line (HepG2). These cells had previously been genotyped for GST A1, GST M1 and GST T1. We analysed the mRNA expression of GSTs, TF, ET-1, PECAM-1 and ICAM-1 by real-time quantitative PCR (Q-PCR) and the protein expression by ELISA for ET-1 and ICAM-1. *Results*. Effect of CPA on mRNA expression of these genes in these cell types was not significant but we show that Bu: i) moderately up-regulates GST A1, a major Bu metabolising enzyme, in the hepatic cell line, ii) does not alter GST M1 and ICAM-1 expression but down-regulates GST T1 (2 fold), iii) down-regulates the expression of ET-1 (2 fold), PECAM-1 (2 fold) and TF (2 fold) *Summary/Conclusions*. These data are not in agreement with the previously proposed involvement of TF and ET-1 up-regulation in the pathogenesis of HVOD. Our study also demonstrated that the expression status of ET-1, ICAM-1 and TF is not related to the GST genotype in endothelial cells. Ongoing studies of Bu/CPA-induced functionnal interactions between EC and hepatic cells must provide further insights into the pathogenesis of HVOD. The striking observation is the absence of expression of GST A1 in all endothelial cell types, regardless of GST genotypes and may provide an explanation for the Bu-induced endothelial desquamation that ensues conditioning in HSCT. A better understanding of molecular mechanisms of HVOD pathogenesis must allow designing of novel therapeutic strategy for this lethal complication in HSCT.

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ASSESSMENT OF THE RISK FOR THROMBO-EMBOLIC COMPLICATIONS BY QUANTITATIVE ALLELE-SPECIFIC PCR FOR THE JAK2 V617F MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Thrombo-embolic events are considered the most relevant complications in patients with essential thrombocythemia (ET). Thus, cytoreductive therapy is widely recommended for patients with the major riskfactors for thrombosis, i.e. age over 60, platelet counts over 1,5 M/l or a history of prior thrombo-embolic events. Recently, the JAK2 V617F mutation has been associated with an enhanced risk for thrombosis in patients with ET. The objective of this study was to test for a correlation of peripheral blood JAK2 burden with thrombo-embolic events. Nucleated peripheral blood cells from 21 patients (7 male, 14 female; median age 69 yrs, range 27-87) with newly diagnosed or hitherto untreated ET were tested for occurrence of the JAK2 V617F mutation by quantitative allele-specific PCR. The proportion of JAK2 V617F was calculated by correlation with GAPDH. The sensitivity of this assay was below 1%. To establish the risk of thrombo-embolic events in untreated patients, JAK2 mutational status and JAK2 V617F burden in peripheral blood was correlated to the patients history of thrombo-embolic complications. With this highly sensitive test the V617F mutation was found in 17 out of 21 patients (81%) with a quantitative range from 1,1 to 52,7% (median 8,0%). 10 of these 21 patients had a history of thrombo-embolic complications. 3 patients had suffered apoplectic strokes, 4 patients had a history of acute coronary syndrome, including 2 myocardial infarctions, 2 patients had peripheral arterial occlusive symptoms and one patient suffered from sinus vein thromboses. The mutated allele was found in 9 of 10 patients with trombo-embolic events. Statistical analysis with the Mann-Whitney U-Test showed a significant association of thrombo-embolic events with with age (p 0,015) as well as with the burden of mutated JAK2 in peripheral blood (p 0,036). Due to the low number of patients with extremely high platelet counts, the role of platelet numbers could not be addressed. The JAK2 V617F mutation is a risk factor for thrombo-embolic complications in patients with ET. The question, whether occurrence of this mutation is an indication for cytoreductive therapy, should be addressed in larger studies. However, if mutated JAK2 is considered a clinically relevant prognostic factor, the detection methods should be highly sensitive, since thrombo-embolic events are also prevalent in patients with lower copy numbers of mutated JAK2.

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USE OF CD34+ CELL COLLECTION EFFICIENCY CALCULATIONS FOR DOSE PREDICTION, PROCESS QUALIFICATION AND PRODUCT SPECIFICATION DURING PBSC COLLECTION USING THE MNC PROGRAMME ON THE COBE SPECTRA CELL SEPARATOR

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Background. The clinical heterogeneity of PBSC collection, which is carried out in widely different patient groups, using widely different mobilising regimes and with a very wide range of peripheral CD34 cell counts, makes validation, process qualification and definition of a *product specification* potentially difficult. We describe the use of calculated CD34 cell collection efficiency values to address these issues. *Methods*. 330 consecutive peripheral blood stem cell collections were audited, 32 allogeneic collections (donors mobilised with G-CSF only) and 298 autologous collections (mobilised using a wide variety of chemotherapy regimes plus G-CSF). The MNC collection programme on the Cobe Spectra cell separator was used for all procedures. Absolute number of $CD34^+$ cells processed by the machine was calculated as the product of peripheral $CD34^+$ count and the blood volume processed by the machine (final *inlet flow* minus final *AC volume* from the machine's end-run data). Absolute $CD34^+$ cell count in the PBSC collection bag is measured rou-

tinely by our stem cell lab prior to cell storage. Collection efficiency was calculated as the ratio cells collected / cells processed. Effect of a variety of parameters on collection efficiency was assessed, including: individual Cobe Spectra machine used for collection; gender; length of procedure; paediatric versus adult collection; average inlet flow speed (both absolute and relative to total blood volume); allogeneic versus autologous collection; and day of collection (for donors collected on several days running). *Results*. There was a very strong linear correlation between *cells processed* and *cells collected* (r=0.979), corresponding to a relatively constant and predictable collection efficiency averaging out at 55%. Factors appearing to influence collection efficiency positively included paediatric procedures, and very slow absolute runspeeds (less than 25 mls/min), presumably in both cases due to longer PBSC *dwell time* in the collection channel. Factors influencing collection efficiency negatively included very short procedures (less than 150 minutes) and *third or subsequent day of collection* (presumably due to collection on rapidly falling peripheral CD34 counts). Factors which did not influence collection efficiency significantly included: very long procedures (interestingly, there was no evidence of stem cell *depletion* in procedures lasting 240 minutes or more); gender; very fast runspeeds; runspeed relative to total blood volume; and allogeneic versus autologous collection. *Conclusions*. Comparison of average collection efficiencies between the five individual Cobe Spectra machines was possible, yielding valuable information for validation and process qualification of PBSC collection using the MNC programme on these machines. Although there was some slight machine-to-machine variability, this did not reach statistical significance. The data were useful for two other specific quality purposes. Firstly, the relative constancy of collection efficiency allows the predicted $CD34^+$ dose in the PBSC product to be calculated at the start of each collection procedure, using the predicted end-run data provided by the machine and the formula: Predicted dose = (5 x Peripheral $CD34^+$ count/mL x Final Inlet Flow in mls) / (Weight in kg x 10,000). (This formula includes a correction for anticoagulant volume.) Secondly, the actual dose achieved can be compared retrospectively against the predicted dose, and (rare) collections where the actual dose achieved is less than half of the predicted dose can then be investigated for quality purposes: in other words, this provides a *product specification* for PBSC collections. Calculation of predicted cell dose with a good degree of accuracy at the start of a collection procedure is also useful to apheresis staff and to the patient for planning purposes.

Figure 1. Correlation between Cells processed & Cells collected (all patients).

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EXPRESSION OF APOPTOSIS-REGULATING GENES BCL-2 AND BAX EVALUATED BY QRT-PCR IN LYMPH NODES OF PATIENTS WITH HIGH GRADE NON-HODGKIN'S LYMPHOMA

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Background. Impairment of apoptosis is central to cancer development (including lymphoma) and renders tumors refractory to cytotoxic therapy. The balance between Bcl-2 and Bax is important for the induction of programmed cell death; when Bcl-2 predominates, apoptosis is inhibited. BCL-2 expression has been studied extensively in aggressive Non-Hodgkin's lymphomas, principally Diffuse Large B-cell Lymphoma /DLBCL/, and has been correlated with worse prognosis. In contrast, Bax expression and their clinical significance are not so well studied, the results often being discordant. Moreover, most of the research conducted so far has analyzed the activity of pro- and antiapoptotic genes in peripheral blond mononuclears and little research has assessed this activity in lymph nodes by means of mRNA analysis. *Aims*. The aim of study was to evaluate the expression of apoptosis-regulating proteins Bcl-2 and Bax in the lymph nodes of 23 never-treated high grade Non-Hodgkin's lymphoma patients in different stages of the disease (15 of these patients were diagnosed with DLBCL, 8 with the Mantle Cell Lymphoma-MCL; the control group consisted of 7 patients with persistent chronic lymphadenitis. *Methods*. The QRT-PCR method was employed to assess the activity of Bcl-2 and Bax. *Results*. No difference in the expression of Bcl-2 and Bax was found between MCL and the DLBCL groups /p>0,05 U Mann-Whitney test/. The T-Student test, however, did show a significantly higher expression level of Bcl-2 in the MCL group when compared with the DLBCL group / p<0,05/. A difference in the Bcl-2/Bax ratio was found between the mantle cell lymphoma and the DLBCL group (U Mann-Whitney test, p<0,01) and between the mantle cell lymphoma and the control group (U Mann-Whitney p<0,001). Strong positive correlations were found for Bcl-2 and Bax for the mantle cell lymphoma group (p<0,001), for the DLBCL group (p<0,001) and also for the control group (p<0,05). No statistically significant correlation was found between the survival time and the expression of the studied genes; however, in the case of Cox's proportional hazards regression model we did obtain a result showing a tendency to a shorter survival time with a higher expression of Bcl-2 in the DLBCL group (p=0.0771). *Summary/Conclusions*. The obtained results indicate a significantly more important role of apoptosis impairment in the pathogenesis of mantle cell lymphoma when compared with DLBCL group. Moreover, the obtained results show that the analysis of Bcl-2 activity alone does not provide precise information in the assessment of apoptosis in high-grade NHL patients and should be accompanied by an assessment of the structure of apoptosis-regulating genes of Bcl-2 family, including the assessment of interdependence of Bcl-2 and Bax. Further studies are required which will analyze the influence of the expression of studied genes on the survival time as well as will compare the expression of genes related to apoptosis in lymph nodes and peripheral blood mononuclears in high grade lymphoma patients.

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CHRONIC MYELOPROLIFERATIVE DISORDERS WITH THROMBOCYTHEMIA: THROMBOSIS ASSOCIATED WITH INHERITED THROMBOPHILIA OR JAK2 V617F GENE MUTATION

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Introduction. Patients with Ph- chronic myeloproliferative disorders with thrombocythemia (MPD-T) may be at increased risk of thrombosis in case that an additional thrombophilic state (such as inherited thrombophilia) or the JAK2 gene V617F mutation is present. *Aims*. To evaluate the impact of additional thrombophilic states and of JAK2 V617F mutation on the incidence of major thrombosis in our patients. *Patients*. A series of 66 consecutive institutional patients (26 males / 40 females) with MPD-T has been studied. According to the Polycythemia Vera Study Group (PVSG) criteria, the cohort comprised 53 patients with ET, 5 with PV, 4 with an unclassifiable MPD, and 4 with IMF. According to the European Clinical and Pathological (ECP) criteria, 16 patients with ET previously diagnosed according to PVSG criteria had to be reclassified as IMF. The median age at diagnosis was 46.1 years (17-76), the platelet count was 969 (430-1972) and the peak follow-up platelet count was 1192 (430-4376). *Methods*. Additional thrombophilia as defined according to the recommendations of the Czech Hematological Society comprised either inherited thrombophilia (in this series, protein C and S or antithrombin III deficiency, and factor V Leiden or prothrombin gene G ->A 20210 mutations), or an acquired additional thrombophilic state at the time of the thrombotic event (in this cohort, 1 patient had widespread colon cancer and 1 had DIC due to missed abortion). JAK2 gene mutation V617F was evaluated as a separate risk factor, using real-time RT-PCR with 2 locked nucleic acid (LNA)-modified TaqMan probes hybridizing to the mutated and the unmutated alleles, respectively. The assay allowed discrimination between hetero- and homozygots without the need of sequencing. *Results*. Of the 21 patients with serious thrombosis (9 arterial, 14 venous-2 patients suffered both types of events), 9 (43%) had an additional thrombophilic state, whereas of 45 patients without thrombosis, thrombophilia was demonstrated only in 6 (13%; p=0.01). In addition, a higher proportion (13 of 20 examined;

65%) of patients with a major thrombotic event had a mutated JAK2 gene, in comparison to patients without thrombosis (12 of 45; 27%; p=0.005). All of the JAK2 mutations were heterozygous in this series (1 IMF patient only acquired homozygosicity during follow-up). Of the 13 patients with JAK2 mutation in whom thrombosis occurred, only 3 had inherited thrombophilia (F.V Leiden-1, heterozygous protein C deficiency-1, combination of the two-1) in parallel. Interestingly, the vast majority of the patients with thrombosis (19 out of 21; 90%) had either additional thrombophilia or JAK2 mutation. In contrast, in patients not experiencing thrombosis, either additional thrombophilia or JAK2 gene mutation was demonstrated only in 18 out of 45 (40%; p=0.0001). *Conclusions*. Thrombosis in MPD-T may result from coincidence of major risk factors: elevated platelet counts, an additional thrombophilic state (mostly inherited thrombophilia) or JAK2 gene V617F mutation, the latter yielding increased adhesiveness to leukocytes and platelets. Larger scale prospective studies of these risk factors are warranted.

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EXPLORING THE ROLE OF HEPCIDIN IN ERYTHROPOIESIS OF PATIENTS WITH MULTIPLE MYELOMA

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Background. Anemia is a frequent clinical manifestation in patients with multiple myeloma (MM). Although its origin is multifactorial, an imbalance of inflammatory cytokines (IL-1, TNF, IL-6), resulting in anemia of chronic disease (ACD), appears to be its main cause. Recently, it has been demonstrated that hepcidin, a peptidic hormone produced in the liver under the stimulus of IL-6, has a key role in ACD by inhibiting iron supply to the erythron. *Aims*. To explore if prohepcidin levels have any impact on iron metabolism and erythropoiesis in untreated MM patients. *Patients and Methods*. Blood samples from 31 MM patients [M/F: 13/18, median age: 66(40-83) years] and 16 healthy volunteers [M/F: 7/9, median age: 60(56-75) years] were analyzed. Hemoglobin, serum pro-hepcidin (pro-HPC), erythropoietin (EPO) and interleukin-6 (IL-6) levels, CRP, iron, ferritin, total iron binding capacity (TIBC), transferrin, transferrin saturation and soluble transferrin receptors (sTFRs) were measured in both groups. β-2 microglobulin and tumor necrosis factor-A (TNF-A) levels were studied in the patients' group, only. None of the patients had smouldering myeloma, infection or second malignancy. Mann Whitney U test, independent samples T test and Spearman's rho test were used for statistical analysis. *Results*. Compared to healthy volunteers, MM patients had lower Hb (median: 10.6 g/dL vs 14.75 g/dL, p<0.001), TIBC (median: 237 micromols/L vs 313 micromols/L, p<0.001) and transferrin (median: 196 mg/dL vs 275 mg/dL, p<0.001). Patients' group was characterised by significantly higher pro-HPC (median: 209 ng/dL vs 181 ng/dL, p: 0.046), EPO (median: 28 IU/L vs 8 IU/L, p:0.001), ferritin (median: 201 ng/dL vs 60 ng/dL, p:0.016), transferrin saturation (median: 28.5% vs 25%, p:0.05) and CRP levels (median: 0.48 mg/dL vs 0.2 mg/dL, p<0.001). Hemoglobin level in the patients' group was negatively correlated to EPO (rho:-0.672, p<0.001), and β-2 microglobulin (rho:-0.709, p<0.001) while no correlation was found for pro-HPC, IL-6 and TNF-A. Prohepcidin was correlated positively to IL-6 (rho: 0.548, p: 0.002) and negatively to transferrin (rho:-0.428, p: 0.042). *Conclusions*. In the current study, pro-HPC levels were found high in patients with MM. The absence of expected correlations between pro-HPC and erythropoietic indices, indicates the complex pathophysiology of MM anemia. Further studies are needed in order to deeply clarify the aspect of iron metabolism in MM.

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INCIDENCE AND EVOLUTION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) IN ISANITARY AREA

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Background. The risk of MGUS progression to Myeloma (MM) or other plasma cell related disorders (PCD) occurs at an annual rate of 1-3%. To identify the variables associated with a high risk for transformation will allow development of evaluation strategies *Aims and methods*. In order to evaluate the incidence of Monoclonal Gammopathies (MG) in our area (300.000 inhabitants), to determine associated diseases, to analyse their clinical course and to identify factors related to progression we studied all MG detected from January 1992 to February 2004. A complete data base was created with several demographic and hematological variables recorded at diagnosis. *Results*. Incidence and distribution: 836 patients with MG were studied. According to the SOWG diagnostic criteria 610 (72.9%) were considered MGUS, 179 (21.4%) met criteria for MM; 36 (4.3%) for Waldenstrom Macroglobulinemia (WM) and 11 (1.3%) other lymphoproliferative diseases. The annual incidence of MG was 30-40. Since 1994 the rate increased two fold because a change in the electrophoresis method which implied and increment of discrete bands identified as MGUS. Age and sex: The mean age was 71.4 years (32-100), only 3.2% of patients were younger than 45, the male/female ratio was 1.34. Associated diseases: Infections 328; cardiac diseases 249; rheumatoid pathology 211; liver diseases 108, Neoplasia 80; Neuropathies 43. Immunoglobulin heavy-chain: IgG was involved in 493 cases (MM 86;MGUS 407); IgA in 143 (MM50;MGUS 93); IgM in 114 (WM36; MGUS 78); IgD in 2 (MGUS 2); 16 were biclonal (1.9%), 1 triclonal (0.1%) and 64 (7.6%) no exhibited any heavy-chain. Light-chain: Kappa 515 (61.6%) M-protein level in serum: the mean was 15 gr/l (14 gr/l MGUS; 25 gr/l MM; 24 gr/dL WM) (p<0.05) Erythocyte sedimentation rate: the mean was 47.4 mm (MGUS 32.4; MM 43.6; WM 66.7) (p<0.001) Bone marrow (BM) plasma cells: the mean was 5.9%; more than 20% was found in 85% MM and 7% MGUS (p<0.001) β2 Microglobulin: the mean was 3.43 mgr/L (2.59 MGUS; 4.18 MM; 3.52 WM).Albumin : The mean was 30 gr/L (30.1 MGUS; 20.9 MM; 30.2 WM). CRP: The mean was 101 mgr/dL (152.6 MGUS; 133.5 MM; 18.4 WM) Metastatic bone survey: 40% of patients had osteoporosis and 16% lytic lesions. Flow cytometric in 2% of the patients: atypical 57.1% (CD 138+, CD 38+, CD19-) Transient MG: 20 MG disappeared (Ig G 16; Ig M 3; ig A 1) within a mean period of 2.6 months (1.4-4.6); 70% associated fever at diagnosis. Transformation rate: 24 cases of MGUS have developed MM and 15 other lymphoid neoplasia. The median time to progression was 3 years (IC 1.82-4.3). Survival and mortality: The total median survival (ms) was 77.3 months (90.66 MGUS; 40.76 MM; 73.26 WM). During follow-up 362 patients died (136 MM; 19 WM; 207 MGUS). *Conclusions*. In the present study factors associated with transformation were: 1. Ig A heavy chain (p<0.002) 2. Serum monoclonal protein level 3. Increased ESR rate (p<0.001) 4. Age < 70 years (p<0.05) 5.BM plasma cell percentage (p<0.002) 6. Osteoporosis (p<0.005). We propose a risk-stratification model and a follow-up scheme.

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PRESENCE OF A MINOR PHILADELPHIA-POSITIVE CLONE IN YOUNG ADULTS WITH DE NOVO T-CELL ALL

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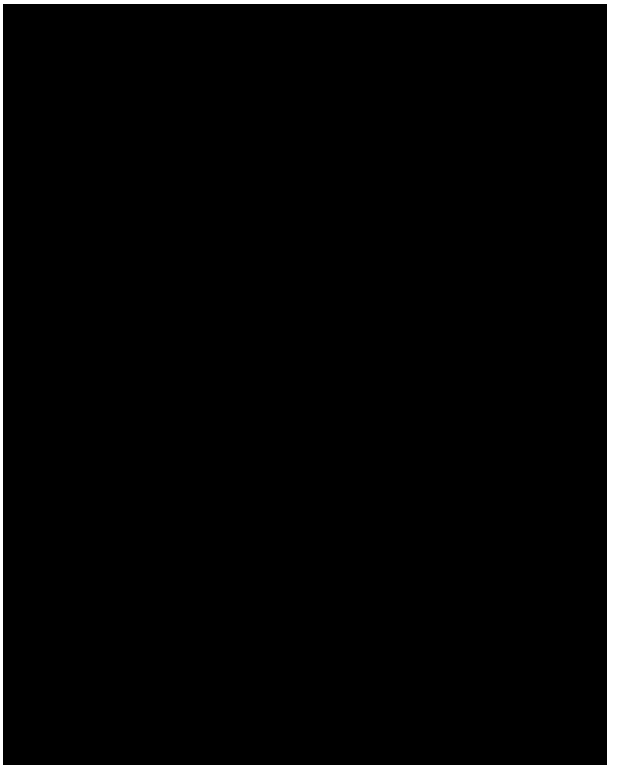
Background. BCR-ABL and its chromosomal counterpart Philadelphia chromosome (Ph) are considered as the hallmark of chronic myeloid leukemia but are also associated with B cell acute lymphoblastic leukemia (B-ALL). Philadelphia positive T-cell ALL have been occasionally reported in the setting of CML blast crisis, of *de novo* childhood ALL (Graux *et al.* Leukemia 2006) but remains very rare in *de novo* adult ALL patients. After the diagnosis of a first case of clonal Ph positive T-ALL, we decided to investigate the incidence of this presentation and its potential significance. *Methods*. A retrospective analysis was performed on 32 bone marrow samples from patients with *de novo* adult T-ALL treated in our institution. Analysis of BCR-ABL was performed by conventional cytogenetics, FISH (Bcr-Abl VYSIS dual color ES probe, Abbott, France) and RQ-PCR (TAQMAN). Multiplex PCR was performed to eliminate other transcripts (e6a2 e6a3, microBCR-ABL). *Results*.Two patients over 32 presented a minor Ph^+ clone at diagnosis (Table 1 presents the treatment strategies and outcome): Case #1: a 17 year-old female was admitted for anemia and cervical lymph node enlargement. Blood counts revealed a deep anemia and hyperleucocytosis with 92% lymphoblasts. Immunophenotyping showed a $CD34^+$ $CD33^-$ $CD38^+$ $CD3^+$ $CD4^+$ $CD8^+$ pattern with TCR γ/δ expression consistent with a mature T-ALL phenotype (TIII subtype of EGIL classification). Conventional cytogenetics showed no abnormalities. Nevertheless, RQPCR showed a mBCR-ABL transcript detected at a low expression level (0.27% Abl control gene). Multiplex PCR showed no other BCR-ABL transcript. Interphase and metaphase FISH analysis found 2% of Ph^+ nuclei in addi-

tion to a translocation t(5;14)(q35;q32) confirmed using Chromosome painting (WCP14 metasystems probe). The patient achieved a complete remission following induction chemotherapy. Peripheral blood RQ PCR showed the persistence of a very weak positive signal not quantifiable. RQ-PCR turned negative after consolidation chemotherapy. Case #2: a 21 year-old male was admitted with diffuse lymph node enlargement and pancytopenia. Marrow aspirate showed ALL with 87% blasts. Immunophenotyping showed $CD34^+$ $CD33^-$ $CD38^+$ $CD3^+$ $CD4^-$ $CD8^-$ and no expression of TCR, consistent with an immature T-ALL (T II EGIL). Conventional cytogenetics found a t(11;12) (p11;p13). This translocation has not yet described in ALL. M BCR-ABL transcript was detected by RQ-PCR at a low expression level (0.36% control Abl). Interphase FISH confirmed the presence of a minor Phi^+ clone (10%, 50 of 500 nuclei). No other transcript was detected by multiplex RT-PCR. No response to initial steroid treatment was seen and medullary blasts persisted by day 8 following induction chemotherapy. Complete response was achieved after addition of Imatinib Mesylate treatment (800 mg/day) to chemotherapy. Complete molecular response was obtained after 3 months of treatment. *Conclusions*. Those two cases illustrated the interest of a systematic detection of BCR-ABL by RQ-PCR in T-ALL. The prognosis significance of this minor clone remains unclear even if chemoresistance and proliferative advantages associated with BCR-ABL expression should be taken into account. The usefulness of the addition of Tyrosine-Kinase Inhibitors should be suggested as illustrated in case #2.

Table 1. Treatment strategies and outcome.



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GRANULOCYTE COLONY-STIMULATING FACTOR MODULATES ARHGAP21 GENE EXPRESSION AND CELL VIABILITY IN MONONUCLEAR CELLS FROM PATIENTS WITH MYELODYSPLATIC SYNDROMES

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Background. Granulocyte colony-stimulating factor (G-GCF) in combination with erythropoietin has a significant therapeutic value for the treatment of patients with low-risk MDS, being capable of decreasing the number of apoptotic bone marrow precursors. We have recently described ARHGAP21, a Rho-GTPase activating protein (Rho-GAP) that was shown to be upregulated during erythroid differentiation of normal mononuclear hematopoietic cells and associates with α-catenin. RhoGTPases mediate many aspects of cell biology, including proliferation, apoptosis, survival and adhesion. *Aims*. Since other genes involved in erythroid differentiation are deregulated in MDS cells, we aimed to evaluate the expression of ARHGAP21 in bone marrow cells of low risk MDS patients and normal donors. We also attempted to verify the effect of G-CSF treatment on MDS cells regarding apoptosis and ARHGAP21 mRNA expression. *Methods*. Bone marrow aspirates were obtained from 5 normal donors and 8 patients with MDS: 6 RA, 1 RARS and 1 RA with excess of blasts (FAB classification). The National Ethical Committee Board approved the study; informed-written consent was obtained from all patients and donors. Total cells were submitted to RNA extraction and the expression level of mRNA was detected by real time RT-PCR, normalized by β-actin control. For the evaluation of the effect of G-CSF, mononuclear cells (MNCs) from bone marrow samples of two patients (1 RA and 1 RAEB) were cultured in the presence or not of stromal cells, and then subjected to treatment with G-CSF (100ng/mL). After 4 hours, MNCs were harvested to detect apoptotic cell death (AnnexinV/Propidium Iodide) by FACS analysis and expression of ARHGAP21 by Real Time PCR. *Results*. ARHGAP21 mRNA expression was similar in cells from low-risk MDS (median=0.235) and normal donors (median=0.22). In MNCs from the low risk MDS patient (RA), G-CSF was able to increase cell viability (up to 3.3 fold) and to downregulate the expression of ARHGAP21 (down to 20 fold), compared with MNCs cultured without GCSF. Moreover, when the MNCs were co-cultured with stromal cells, in the presence or not of G-CSF, we also observed an increased cell viability (up to 8 percent) and a more evident downregulation of ARHGAP21 (down to 56 fold) mRNA expression when compared to stroma-free cultures. The same results were observed in the culture of MNCs from a patient with high risk MDS (refractory anemia with excess of blast). *Conclusions*. The downregulation of ARHGAP21, in parallel to the increased viability of bone marrow cells by G-CSF and stroma contact may suggest that ARHGAP21 is involved in the physiopathology of this disease. It is well known that G-CSF decreases the number of apoptotic bone marrow precursors and the stroma is able to maintain the cell in an undifferenciated status. Thus, ARHGAP21 may participate in cell signaling involving apoptosis and cell adhesion in MDS cells.

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PRIMARY PROPHYLAXIS WITH PEGFILGRASTIM WAS MORE COST-EFFECTIVE THAN FILGRASTIM IN PATIENTS WITH NON-HODGKINS LYMPHOMA RECEIVING CHOP-21 IN GERMANY

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Background. Febrile neutropenia (FN) is a major toxic effect of chemotherapy, predisposing patients with cancer to serious and often life-threatening infections, and often also resulting in lengthy treatment delays and dose reductions, which in turn have been shown to compromise treatment. Primary prophylaxis (ie, starting in the first cycle of chemotherapy and continuing for all subsequent cycles) with granulocyte-colony stimulating factors (G-CSF) is recommended by the 2006 ASCO and EORTC guidelines when the risk of FN is equal to or greater than 20%. Both filgrastim (daily injections) and pegfilgrastim (once per cycle) have commonly been used. However, in clinical practice filgrastim has often been used with shorter-than-recommended courses of administration, which are associated with less clinical benefit. *Aims*. We evaluated the cost-effectiveness of pegfilgrastim vs. filgrastim used for 11 days (as used in the randomised trials demonstrating efficacy) and 6 days (as often used in clinical practice) in patients with aggressive NHL receiving CHOP-21 in Germany. *Methods*. A decision-analytic model was constructed from a health care payer's perspective, with a study time horizon set at life-time. Costs for model inputs were acquired from official price lists or literature; they included drugs, drug administration, FN-related hospitalisations, and subsequent medical costs. Other model inputs, including FN risk (varied by days of filgrastim use), FN case-fatality, relative dose intensity (RDI), and the impact of RDI on survival were based on data from a comprehensive literature review and expert panel validation. NHL mortality data and all-cause mortality data were obtained from official statistics. Using data from a meta-analysis (pegfilgrastim vs. 11 days of filgrastim) and from observational studies (pegfilgrastim vs. 6 days of filgrastim), we estimated that the absolute risk of FN in patients receiving pegfilgrastim decreased by 6.5 percentage points (19.6% vs. 13.1%) vs. 11-day filgrastim, and by 12 percentage points (25.1% vs. 13.1%) vs. 6-day filgrastim. Next, we estimated the impact of a difference in FN risk on FN-related mortality, RDI, and long-

term survival. Model robustness was tested using sensitivity analyses. Outcomes were measured as incremental cost-effectiveness ratio (ICER) including € per percentage (absolute) FN risk decreased, € per FN event avoided, and € per life-year gained (LYG). *Results*. Pegfilgrastim was cost saving compared with 11-day filgrastim (€9,011 vs. €12,211 for pegfilgrastim vs. filgrastim). Compared with 6-day filgrastim, the ICER was €15,817 per FN avoided or €158 per 1% decrease in absolute risk of FN. Pegfilgrastim achieved 0.095 more LY at a moderate cost increase of €1,898 (€9,011 vs. €7,113) per person, yielding an ICER of €19,979/LYG. Results were sensitive to the assumption of RDI impact, relative risk of FN for 6-day filgrastim vs. pegfilgrastim and study time horizon. *Summary/Conclusions*. In Germany, pegfilgrastim was cost saving compared with 11-day filgrastim, and was shown to be cost-effective compared with filgrastim used for 6 days per cycle of CHOP-21 chemotherapy. To substantiate our results, more studies are needed on the impact of RDI supported by G-CSF and its impact on long-term survival.

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ANALYSIS OF IN-HOSPITAL CAUSES OF DEATHS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. The survival rates for adult acute lymphoblastic leukemia (AALL) in developing countries are significantly lower than the developed countries. Some of the reasons for poor results are due to early deaths due to infection and bleeding due to constraints on resources. *Aims*. We choose to analyse the causes of deaths in admitted patients with AALL at a large tertiary care multispecialty hospital of north-west India. *Methods*. We collected death summaries of all adult (age >12 years) patients suffering from various hematological malignancies and who died in the hospital from January 2002 till December 2005. The death summaries were then reviewed including presenting complaints of the patients, duration of illness before presenting to hospital, duration of hospitalisation, microbiological cultures and possible causes of deaths. Patients were divided into three groups depending upon their diagnosis and disease status. Group I was newly diagnosed patients in whom the diagnosis of ALL was made in this admission. Group II were those patients who were previously diagnosed with ALL and who were in remission and died during this hospitalisation. Group III were those patients who were previously diagnosed with ALL but were in relapse. *Results*. Out of 236 deaths that occurred during this 4 years period, AALL constituted 13.1% (31/236). The median age of the patients was 24 years (range 13-55 years) and male female ration was 24:7. There were 16 patients in group I (median age 16.5 years), 6 patients in group II (median age 27.5 years) and 9 patients were in group III (median age 31 years). The median symptom duration was significantly higher in group I (30 days) as compared to group III (6 days) and II (2.5 days ;($p<0.05$). The median white cell count was 23500/µl in group I, 1000/µl in group II and 8600/µl in group III ($p<0.05$). The median platelet count was also significantly lower in group I (21000/µL) than group III (73000/µL) and group II (100000/µL). Bleeding and infections were the causes of deaths in 37.5% & 68.8% in group I, 16.7% & 100% in group II and 22.2% & 88.9% in group III. Proven bacterial infections were cause of deaths in 11 patients, proven fungal infections in 5 and viral infection in one patient. The median duration of hospitalisation was 14 days in group I, 7 days in group II and 15 days in group III. *Conclusions*. Infections are still the most common causes of deaths in AALL while in a proportion of patients leukostasis and bleeding are contributing factors. Early referral of patients and early administration of antibiotics may help in preventing deaths in some patients with AALL.

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CHANGING CHARACTERISTICS OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL DURING EX VIVO CULTURE: IMPORTANT CONSIDERATION FOR RESEARCH AND CLINICAL APPLICATION

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Background. Therapeutic application of *ex vivo* expanded mesenchymal stem cells (MSCs) are increasing rapidly in the recent years. However, the characteristics of MSCs may probably be changed during *ex vivo* culture because of the changing microenvironment. We have previously reported that the result of induction of CD45 expression on MSCs by using demethylating agent 5-aza-2-deoxycytidine is different from passage to passage (S-P Yeh, *et al*. Leukemia 2006,20:894-896). F-B Zhang *et al*. had also been report that the differentiation potential of MSCs into cardiomyocyte-like cells was*passage-restrictive*. These data suggested that MSCs can become different after *ex vivo* culture, even though they derived from a same origin. *Aims*. To examine the effect of *ex vivo* culture on the gene expression profile (GEP) of bone marrow-derived MSCs. *Methods*. We used cDNA microarray to evaluate the difference of GEP between passage 2 (P2) and passage 5 (P5) MSCs of the same origin. Bone marrow-derived MSCs were isolated from 2 patients of AML, myeloma, and 1 normal adult respectively. They were all *ex vivo* cultured at the same condition. RNA were extracted from P2 and P5 cells (of the same origin) and then subjected to cDNA microarray (Agilent Human 1A(V2) OligoMicroarray) to compare the difference of GEP between P2 and P5 MSCs of the same origin. Western blot and immunocytochemical staining were further used to confirm the difference at protein level. *Results*. We found the GEP of MSCs were markedly different between P2 and P5 in each pair, especially in case of AML. The highest differently expressed genes and their normalized expression ratio in each pair are showed in the Table of Figure 1. Of these, increased expression of vimentin and proenkephalin in P2 cells were found in all the 3 specimens, which were further confirmed by western blot at protein level. Since more hematopoietic cells may be contaminated in P2 MSCs and may subsequently mislead the interpretation of cDNA microarray and Western blot, we further using immunocytochemical staining to document the strikingly different intensity of staining of vimentin on the *fibroblast-like* MSCs (Figure 1). *Conclusions*. Our study showed that bone marrow-derived MSCs will be changed during *ex vivo* culture and P2 MSCs are definitely different to P5 MSCs, even though they came from the same origin. The difference may reflect the fact of how MSCs were significantly influenced by the microenvironment, specifically in case of AML. This factor should also be considered seriously when using MSCs in laboratory experiments or clinical studies because MSCs of different passage might probably lead to different result.

Table. Gene Expression Profile Between P2 and P5 MSCs.

P5<P2	Specimen Origin	Normalized Expression Ratio
Proenkephalin	MM/AML/Normal	0.33/0.38/0.24
Vimentin	MM/AML/Normal	0.43/0.41/0.40
Ch.20 ORF 129	MM/AML	0.26/0.02
Cyclin B2	AML/Normal	0.15/0.31
Selenoprotein P	MM	0.33
P5>P2		
Angioprotein-like 4	MM/AML	2.62/13.83
TGF-α	AML	27.425
TFPI	AML	8.49

Figure 1. GEP and vimentin staining of P2 and P5 MSCs

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DETERMINATION OF IMATINIB PLASMA LEVELS: CONSEQUENCES IN PATIENTS MANAGEMENT AND PREDICTION OF RESPONSE TO TREATMENT OF CHRONIC MYELOID LEUKEMIA

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Background. Imatinib mesylate (IM) exerts a potent and selective inhibition of tyrosine kinase bcr-abl. IM is currently used for front line treat-

ment of CML and induces a high rate of complete cytogenetic response and molecular response. However, variable responses to IM are incompletely understood. Pharmacokinetic data indicate large variability especially in plasma exposure with many factors which could be involved like absorption, metabolism, drug-drug interactions. Furthermore, other groups pointed out that response to IM could be correlated to IM plasma concentration. *Methods*. Analytic procedures : plasma concentrations of IM were determined by use a liquid chromatography-UV visible methods. In brief, plasma samples (1 mL) were collected 20 to 24 hours after IM intakes, and were mixed in a microcentrifuge tube with internal standard (clozapin) before plasma proteins were precipitated with sulfosalicylic acid. The dried residue was dissolved in 150 µL borate buffer/acetonitril and transferred to autosampler vial, and 10 µL was injected into HPLC system. The HPLC system consisted of an Alliance 2690 separation module (Waters, Milford, MA) piloted by the Millenium software. Standart curves were linear over the range of 100 to 2,000 ng/mL, and the intraday and interday coefficients of variation were less then 1%. We assessed through plasma IM concentration in CML patients who responded to standart-dose IM, after at least one year's treatment. Complete cytogenetic response (CCR) was defined as 0% Ph-positive celles in bone marrow. Major molecular response (MMR) was defined as a ratio of transcripts bcr-abl/abl <0.1% from peripheral blood using real-time quantitative reverse-transcriptase polymerase chain reaction. *Results*. 47 patients with chronic phase of CML were included. Mean through plasma IM concentrations (±SD) were: 1269,33±659.75; 1502.34±741.68; and 1300±331 for 300 (n=6), 400 (n=34) and 600 (n=15) mg/day of IM respectively.The ratio metabolits/imatinib slighly stable for the different schedule of IM: 6.65 (300 mg), 7.57 (400 mg) and 6.04 (600 mg). No correlation was found with toxicity profil. No correlation was found between weight and IM concentration in the 34 patients receiving 400 mg of IM per day. Eleven patients did not experience a CCR. The through plasma IM concentration were significantly lesser than CCR patients (850.56±600 vs 1550±550). Furthermore, 36 patients showed a CCR and mean through plasma imatinib concentration were significantly higher in the group with MMR (19 pts) than in the group without (2135±713 ng/mL vs 1341±761 ng/mL). *Conclusions*. Monitoring of plasma imatinib concentration should be a part of standard management of CML patients, especially in case of poor compliance, risk of drug interaction or case of resistance. Studies correlation with larger cohort of patients is necessary to clarify the role of imatinib drug monitoring.

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SPANISH REGISTRY OF ESSENTIAL THROMBOCYTHEMIA. RETROSPECTIVE ANALYSIS OF MYELOID TRANSFORMATION EVENTS

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Background. Essential thrombocythemia (ET) is considered a chronic myeloproliferative disorder (CMPD) having little or no influence on life expectancy. However there are not too many data about myeloid transformation events (MTE) of this disease, such as transformation into myelofibrosis (MF), polycytemia vera (PV) or acute myeloid leukemia / myelodysplastic syndrome (AML/MDS). Moreover, there is controversy about if myeloid transformation represents a natural evolution of ET or if it is drug related. *Aims*. Using our data base of Spanish patients with ET, we performed a retrospective analysis in order to assess the role of treatment in the evolution to MF, PV or AML/MDS. *Patients and Methods*. 411 ET patients from 54 sites from February 2005 to August 2006 were included in a retrospective chart review. ET was diagnosed according to PVSG criteria (1997). All patients had started treatment with anagrelide before December 2004, either as a first line or as a second line therapy. Data were sent to a CRO for blind analysis by two external data managers. According to previous platelet-lowering treatment received, patients were classified into 3 groups: A) anagrelide as first line treatment, (n=110), B) previous hydroxyurea + anagrelide (n= 280), C) previous treatment with other cytoreductive drugs not including hydroxyurea + anagrelide (n=21). There were 13 patients in group B who had received another cytoreductive agent in addition to hydroxyurea. *Results*. There were 31 patients who presented 33 MTE, as follows: MF= 20, PV= 4, AML/MDS= 9. According to the 3 therapeutic groups MTE distribution was: 3/110 in group A (2.7%), 28/280 in group B (10%), 0/21 in group C (0%), Table 1. A significant statistic difference (p= 0.02) concerning the appearance of MTE was observed comparing rates of group A and B. Comparing group A and B, the odds ratio to suffer a MTE was 4.7 (95% CI=1.4-15.9; p=0.006). All AML/MDS events (n= 9) were described in patients previously treated with hydroxyurea, although 3 patients were treated with another cytoreductive agent additionally to hydroxyurea. 18 MF events were reported in group B. 4 patients were treated with additional cytoreductive drug. There were 2 MF, 1 PV and 0 AML/MDS events in the group of patients only treated with anagrelide. *Conclusions*. These results are rather coherent with other previously reported. MF seems to be the most frequent haematological event in ET. No AML/MDS event has been reported in patients only treated with anagrelide. On the other hand, all of the AML/MDS events were described in patients previously treated with hydroxyurea. Due to the methodologic limitations of any retrospective study, a cause-effect relation with hydroxyurea cannot be confirmed. New prospective studies are still needed to clarify the long term effects of these two drugs. Nevertheless, anagrelide should be considered as an attractive treatment option in ET because of the absence of leukemogenic effect.

Table 1.

Myeloid transformation events	Group A (N=110)		Group B (N=280)		Group C (N=21)	
	N	%	N	%	N	%
MF (N=20)	2	1.8	18	6.4	0	0.4
PV (N=4)	1	0.9	3	1.1	0	0.0
AML/MDS (N=9)	0	0.0	9	3.2	0	0.0

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GLUCOCORTICOID-INDUCED MODULATION OF MIR-203 EXPRESSION IN LEUKEMIA CELLS

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Background. Glucocorticoids (GCs) are the principal therapeutic agents in acute lymphoblastic leukemia, but molecular targets of GCs are still under discussion. MicroRNAs (miRNAs) are a recently discovered class of small noncoding RNAs that can play important regulatory roles by targeting the messages of multiple protein-coding genes. *Aims*. Identification of miRNAs potentially involved in GC specific cytotoxicity and cell cycle regulation in leukemia cells. *Methods*. The study has been performed using human B-lineage leukemia cell lines with different sensitivity to GC treatment (TOM-1 and 697 cells: G1-arrest and apoptotic cell death; NALM-6: G1-arrest only; Reh: no response). miRNA profiling was performed using TaqMan MicroRNA Assays Human Panel (Applied Biosystems) including 157 miRNA assays. Expression of individual miRNAs was measured by real-time RT-PCR normalized by U6 RNA expression. *Results*. miRNA profiling was performed with TOM-1 cells, either untreated or treated with dexamethasone for 24 hrs. The majority of miRNAs did not change their expression significantly (fold change <3). Expression changes of the candidate miRNAs have been validated using individual miRNA assays. As the result, we identified miR-203 which revealed high and reproducible expression increase in GC treated TOM-1 cells (mean change by 13-fold). The GC-induced upregulation of miR-203 could be further confirmed in the GC sensitive 697 cell line. By contrast, in the apoptosis-resistant NALM-6 cells and in the totally GC-resistant REH cells expression of miR-203 did not change through dexamethasone treatment. *Conclusions*. These data for the first time demonstrate GC-induced modulation of miRNA expression and point to the potential involvement of miR-203 in GC-specific signaling.

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COMPARISON OF PLATELETPHERESIS ON THE FENWAL AMICUS AND FRESENIUS COM.TEC CELL SEPARATORS

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Background. Apheresis platelets (PLT) are obtained from single donors. Single donor PLT transfusions are more advantageous than random donor PLT transfusions. A variety of apheresis devices are now available on the market for plateletapheresis. However, there is no published study comparing the Fenwal Amicus and the Fresenius COM.TEC cell separators

used for plateletapheresis. The aim of this study was to compare two apheresis devices (Fenwal Amicus and Fresenius COM.TEC) in terms of parameters such as processing times, platelet (PLT) yields, and efficiencies, and white blood cell content. Donors were randomly assigned to undergo plateletapheresis with either Amicus or COM.TEC cell separators. There were no significant differences in the sex, age, weight, height and TBV between the Amicus and the COM.TEC device in pre-apheresis setting. However, pre-apheresis PLT count was higher in the COM.TEC than in the Amicus device (198×10^3/μL vs. 223×10^3/μL; p=0.035). The blood volume processed to get a target PLT yield of ~3.3×10^{11} was higher in the COM.TEC compared to the Amicus instrument (3481 mL vs. 2850 mL; p<0.001). The median separation time was also significantly longer in the COM.TEC than in the Amicus device (61 min vs. 44 min; p<0.001). Ninety-one% and 88% of the PLT products collected with Amicus and COM.TEC had a PLT count of ~33.3×10^{11} (p=0.325). There was no statistical difference in terms of the collection efficiency between the Amicus and the COM.TEC machines (55±15 vs. 57±15; p=0.477). However, collection rate was significantly higher with the Amicus compared the COM.TEC device (0.077±0.012 vs. 0.057±0.008; p<0.001). In conclusion, platelets could be efficiently collected with both instruments. Additionally, consistent leukoreduction could be obtained with both machines, however; the advantage of Amicus machine was faster to get the target yield of PLTs compared to COM.TEC instrument.

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DEFINITION AND VALIDATION OF NOVEL PROGNOSTIC MARKERS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. There is no doubt about prognostic importance of IgVH mutational status in B-cell chronic lymphocytic leukemia (B-CLL). Unfortunately, detection of this parameter is technically highly sophisticated and time-consuming process. There is an effort to determine easily detectable marker or group of markers that could supplement or even substitute the detection of IgVH mutational status. *Aims*. In our study we performed microarray analysis of the gene expression in order to define new prognostic markers in B-CLL and compare their predictive value with recently published prognostic markers (e.g. LPL and ZAP70). *Methods*. B-CLL cells from 20 patients (10 with mutated and 10 with unmutated IgVH) were isolated and microarray analysis using Agilent Human 1A Arrays was performed. Obtained data were statistically analysed using SAM (Significance Analysis of Microarrays) followed with PTM (Pavlidis Template Matching). Expression ratios were clustered according to IgVH mutational status of B-CLL cells. mRNA levels of selected genes were tested on cohort of 100 B-CLL patients using quantitative RT-PCR and protein levels were determined by Western blotting. *Results*. Using microarray analysis we detected ~50 genes with significantly changed expression levels that differentiated patients with mutated and unmutated IgVH. Our results confirmed data previously published in similar studies, e.g. LPL, ZAP-70, ADAM29 and SEP10. In addition, we detected LAG3 (lymphocyte-activation gene 3) that has not been previously reported in connection with IgVH mutational status in B-CLL and its high expression very closely correlates with unmutated IgVH and worse prognosis. We have detected expression of LAG3, LPL, ZAP70, CLLU1, ADAM29, BCL2 and p21WAF1 with quantitative RT-PCR. We proved significantly higher expression of LAG3 and LPL in patients with unmutated IgVH in comparison with very low or no expression of these genes in patients with mutated IgVH. mRNA level of ADAM29 followed opposite trend to LAG3 and LPL and its higher expression correlated with mutated IgVH. On the other hand we have detected discrepancies in correlation between ZAP70 expression and IgVH mutational status. Western blot analysis of LAG3, LPL and ZAP 70 confirmed our findings on protein level. *Summary/Conclusions*. From subset of ~ 50 differentially expressed genes that resulted from microarray analysis of B-CLL cells of patients with different IgVH mutational status we further validated the prognostic relevance of LAG3, LPL, ADAM29 and ZAP70. We found discrepancies between ZAP70 expression and IgVH mutational status, while differences in expression of LAG3, LPL and ADAM29 were in agreement with IgVH mutational status in 82%, 91% and 78% cases, respectively. Therefore LAG3, LPL and ADAM29 appear to be promising potential prognostic markers in B-CLL. Simple detection of this gene set could reliably complete and possibly substitute the detection of IgVH mutational status.

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PHASE II TRIAL OF CLADRIBINE (2-CDA) AND RITUXIMAB IN PATIENTS WITH CLL AND SLL: PRELIMINARY REPORT OF A SINGLE INSTITUTION

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Background. Byrd *et al.* (Blood 2005) confirmed the synergistic effect of Rituximab and Fludarabine in the treatment of CLL patients (pts). The choice of purine analogue still remains controversial. *Aims*. We evaluated feasibility, efficacy, and toxicity of 2-CdA-Rituximab combination in the treatment of CLL and SLL pts requiring treatment. *Methods*. The combination therapy consisted of intravenous Rituximab at a dose of 375 mg/m^2 on Day 1 and 2-CdA at a dose of 0.1 mg/kg (sc injection) per day on Days 2 to 6. The treatment was repeated once every 4 weeks for 4 cycles in total. 32 pts (22 CLL and 10 SLL) were enrolled in the study and the median age was 59 years (31-73); 44% of pts were pre-treated. A CT scan or ultrasound of the abdomen was abnormal in all pts with CLL. Immunophenotypic evaluation by ZAP-70 was positive in 68% of pts while no evaluable pts showed adverse prognostic cytogenetic features by FISH. Minimal residual disease (MRD) assessment was performed by flow-cytometry and PCR methods. *Results.* 2 pts had to discontinue therapy after 2 cycles: one due to herpes zoster reactivation and the other one to progression of disease (PD). We observed grade 3 and 4 neutropenia in 4 pts (12%), major infections in 4 pts (12%) and no episodes of grade 3-4 thrombocytopenia. 27 pts were evaluable for response with an ORR of 92%. At the end of the therapy 12 pts (44%), 7 CLL and 5 SLL, achieved a CR, with negative MRD (by PCR) in 5 pts (4 untreated); 13 pts (48%) obtained a PR; 2 pts had no responce. With a median follow-up of 17 months (range 6-39) 6 pts (2 pts with MRD$^+$ CR and 5 pts with PR after treatment) experienced PD; 5 of these pts were pre-treated and 2 died due to the disease. *Conclusions*. The combination of 2-CDA and Rituximab seems to be tolerable and active principally for untreated pts and able to induce a molecular clearance also in pre-treated pts. The achievement of a CR with negative MRD seems to be the most important issue in improving the outcome.

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IS THERE ANY EFFECT OF INVASIVE FUNGAL INFECTION ON THE OUTCOME OF HEMATOPOETIC STEM CELL TRANSPLANTATION

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Introduction and aims. Invasive fungal infection (IFI) in patients underwent hematopoetic stem cell transplantation (HSCT) is one of the significant infection-related complications. Despite of the improvement of the therapy IFI has proved high morbidity and mortality. Since the reactivation of the infection during the transplantation period was 60% - 90% for patients who had a history of IFI prior to transplantation. In this study we aimed to evaluate outcome of patients receiving antifungal therapy for IFI during HSCT retrospectively. *Patients*. In our center, between June 2004-August 2006 233 patients underwent an allogeneic HSCT (n=127) or an autologous HSCT (n=106). Only 44 patients (18.8%) received antifungal therapy for IFI during the transplantation period. Of these patients 39 was performed allogeneic-HSCT in which graft source was peripheral blood (n=32) or bone marrow (n=7). The rest of them (n=5) was undergone auotologous peripheral HSCT. Median age was 37.5 years (16-63 ys). In allogeneic group, most of the patients (n=36) were conditioned a myeloablative regimen. Only 3 patients were given a reduced intensity regimen. In our cohort, 21 patients had received long-term antifungal therapy prior to the transplantation because of proven (n=1), probable (n=8) or possible IFI (n=12) (Group 1). Therefore, all of the patients in this group were initiated amphotericin-B at the prophylactic dose (n=17) or therapeutical dose (n=3) along with conditioning regimen and continued at posttransplantation period. In this group, transplantation were also performed on 4 patients with active IFI because of the underlying malignant disease progression (relapsed/progressive AML, n=3; refractory/progressive NHL, n=1). Twenty-three patients received antifungal therapy firstly for either probable IFI (n=8) or empiric therapy (n=15) according to febrile neutropenia guideline (Group 2) as well. *Results*. The median time of antifungal use at the peritransplant period was 41 days (4-153 days) in group 1 versus 14 days (2-56 days) in group 2. In the first group, the doses of antifungal therapy were changed into therapeutical doses in 6 patients (29%)

due to reactivation and/or progression of IFI. In 2 patients, IFI was treated two antifungal drugs and also granulocyte infusion (n=1). There was no difference for engraftment kinetics, the frequencies of transplantation-related mortality and acute graft-versus host disease and three-month the probability of survival between group 1 and group 2. Only one patient died of progressive IFI after the transplantation in each group. No significant differences of transplant outcomes in patients with high probability or active IFI in each group were also found. *Conclusions*. Our data showed that the history of IFI and also active infection was not absolute contraindication for a transplantation program. So the decision of transplantation should be given according to the biological behavior of the underlying disease individually, not the characteristic of IFI alone.

Table 1.

	Group 1	Group 2	*p*
Median age	21(19-51)	23(16-53)	
Diagnosis			
Acute leukemia	16	8	
Chronic leukemia	3	3	
Aplastic anemia	-	4	
MDS/Lymphoma/MM/PNH	1/1/0/0	2/1/4/1	
Conditioning regimen	20	21	
Myeloablative	1	2	
Non-Myeloablative			
Dead due IFI	1/21	1/23	
Engraftment	17/21	21/23	0.403
Neutrophil <0.5×10^9/L	16(11-25) gün	16(10-69)	0.561
TRM	7/21	6/23	0.599
3 months survival	%70.33±10,26	%78,26±7.90	
2 year survival	%42.20±18.83	%62.17±10.75	0.565

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UNDERUTILIZATION OF VENOUS THROMBOEMBOLISM PROPHYLAXIS IN PATIENTS IN A TERTIARY CARE CENTER

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Background. Venous thromboembolism (VTE) remains a serious disease requiring hospitalization, treatment and proper prophylaxis. Many consensus groups recommend identifying at risk patients and giving them appropriate thromboprophylaxis. However, there is still no consensus on which patients should receive thromboprophylaxis. *Aims*. In Lebanon, physicians' practice with respect to VTE prophylaxis was not previously investigated. We designed a retrospective observational study at the American University of Beirut Medical Center (AUBMC) to evaluate VTE prophylaxis. *Methods*. All patients admitted under the care of the Department of Internal Medicine at AUBMC during 2005 were listed. Out of patients staying more than 2 days, 250 were randomly selected, and their medical records were reviewed. Patients who were receiving anti-coagulation at admission time or those who were started on anti-coagulation in hospital for their associated diseases were excluded from the study. The remaining patients had their risk factor profile reviewed, and contraindications to anticoagulation were recorded if present. We also checked whether those patients received the appropriate VTE pharmacologic prophylaxis during their stay. Patients were considered as eligible for prophylaxis if they had two or more risk factors, one or more absolute contraindication, or two or more relative contraindications. Patient with two to three risk factors were considered moderate-risk for VTE, whereas patients with four or more risk factors were considered high-risk. *Results*. Forty-six patients of the 250 were excluded because of receiving anti-coagulation for non-prophylaxis purposes. The remaining patients were 54.4% males with a mean age of 60.7 years. The mean stay in hospital was 8.8 days. 144 patients were found to have two or more risk factors, with no contraindications. Only 40 patients (27.8%) received VTE prophylaxis, 37 of whom received low-molecular weight heparin (92.5%). Upon reviewing the risk factors profile, the majority of patients (71.3%) were found to have 2-4 risk factors. Among this group, 24% received prophylaxis. Table 1 shows the distribution of risk factors among the studied patients and those who were anticoagulated. *Conclusions*. Risk assessment models can help physicians identify at-risk patients, give prophylaxis in timely manner, and increase physicians' awareness of risk factors. Underutilization of VTE prophylaxis was observed among inpatients with medical illnesses spending more than 3 days in hospital. This rate is similar to the rates reported around the world. Most of the deficiency was observed in the moderate risk group which constituted the majority of medical patients. A high rate of prophylaxis was observed among critical patients staying in ICU, having central lines, or with respiratory failure. Patients with heart failure, chronic lung disease, or infections had lower rates of prophylaxis. The lowest rate of prophylaxis, among significant risk factors, was observed in cancer patients. The data obtained from this study will raise the awareness of the institution physicians about the current practice and areas of deficiency. Re-auditing will be conducted to evaluate the implementation of thromboprophylaxis guidelines.

Table 1. Risk factor distribution among the studied patients and those anticoagulated.

Risk Factor	Number (%)	Anticoagulated patients
Age > 40	170 (83.3%)	30.3%
Intensive Care Unit	34 (16.7%)	62.5%
Prior VTE7	7 (3.4%)	83.3%
Body mass index > 30	63 (30.9%)	26.9%
Ischemic stroke	16 (7.8%)	33.3%
LVEF < 50	32 (15.7%)	22.6%
Chronic lung disease	42 (20.6%)	35.9%
Infection	77 (37.7%)	32.4%
Malignancy	65 (31.9%)	17.7%
Respiratory failure	14 (6.9%)	72.7%
Thrombophilia	0 (0%)	0%
Inflammatory bowel disease	3 (1.5%)	0%
Central venous catheter	9 (4.4%)	66.7%
Varicose veins	1 (0.5%)	0%
Collagen vascular disease	6 (2.9%)	0%
Family history of VTE	0 (0%)	0%

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STRONG CORRELATION BETWEEN SYSTEMIC MASTOCYTOSIS AND HIGH SERUM TRYPTASE LEVELS IN PATIENTS WITH SEVERE ANAPHYLAXIS AFTER HYMENOPTERA BITES: ROLE OF FLOW CYTOMETRY

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Background. Patients with systemic mastocytosis (SM) may suffer from severe anaphylactic reactions after wasp or bee stings. Serum tryptase level is used as a surrogate marker of mastocytosis and correlates with mast cell (MC) burden. *Aims*. Aim of our study was to evaluate the incidence of SM in patients with severe anaphylactic reactions after hymenoptera bites and high serum tryptase basal levels. *Methods*. 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 high serum tryptase levels (>11.4 ng/mL). In addition, multiparametric flow cytometric analysis (5- or 6-color combinations of monoclonal antibodies) was applied to BM samples to identify MCs (CD45$^+$/CD117$^+$/CD34$^-$) and to evaluate abnormal expression of CD25 and CD2 on MCs (total cell number analyzed: 1,000.000). Finally, we assessed the presence of D816V KIT mutation in BM mononuclear cells by restriction fragment length polymorphism analysis, using Hinf I on RT-PCR product corresponding to the second tyrosine kinase domain of c-Kit; the same PCR product was then sequenced to confirm the presence of the D816V mutation. *Results*. Basal serum tryptase levels were increased (median 16.9 ng/mL; range 11.8-103 ng/mL) in 34/276 patients (12.3%). Ten of these patients were studied with BM analysis (Table 1). Nine/10 patients had previous severe anaphylactic reactions (type III: 3 cases, type IV: 6 cases) and one had reaction of type I. BM immunohistochemistry showed typical infiltrates of tryptase+ spindle-shaped cells in 5/10 patients (50%). The KIT point mutation D816V was found in 5 (50%) cases. Six/10 (60%) patients showed atypical MCs in BM smear. In all cases, BM CD117^{++} MCs, eval-

uated by flow-cytometry, expressed CD2 and/or CD25 (median 0.041% of CD45+ BM cells, range 0.004-0.28%). By contrast, MCs from normal BM (n=2), other hematological malignancies (n=4), and cutaneous mastocytosis (n=4) did not express CD2 and/or CD25. Cytogenetic analysis was normal in all cases. One patient revealed typical cutaneous lesions of CM (confirmed by histology). In summary, the final diagnosis was indolent SM (ISM) according to WHO criteria in 8/10 patients (80%). Five patients had both major and minor criteria of SM, whereas only three minor criteria were found in 3 patients. In one patient only two minor criteria were found (expression of CD25/CD2 on MC and tryptase serum levels >20 ng/mL), and one 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). *Summary / Conclusions*. Our results show strong association among anaphylaxis due to hymenoptera bites, abnormal basal serum tryptase levels and SM. In addition, the multiparametric flow cytometry analysis seems to be the most sensitive and reliable method to identify occult BM involvement, especially in patients without skin lesions and indolent disease.

Table 1.

Sex	Age	Anaphylactic Reaction#	Serum Tryptase (ng/ml)	BM Biopsy	BM Smear	BM MCs CD2/CD25+ (%)	D816V Kit Mutation	Diagnosis
F	63	III	22,7	pos	pos	0,009	neg	ISM
M	33	IV	56,2	pos	pos	0,2	pos	ISM
F	58	IV	24,8	neg	pos	0,05	pos	ISM
F	58	IV	42	pos	pos	0,035	pos	ISM
M	62	III	31,6	neg	pos	0,027	pos	ISM
M	46	I	27	neg	neg	0,008	neg	MMAS
M	19	III	20,1	neg	neg	0,004	pos	ISM
M	45	IV	13,7	pos	pos	0,28	neg	ISM
M	55	IV	12,7	neg	neg	0,047	neg	MMAS
M	74	IV	21,2	pos	pos	0,079	neg	ISM
# severity of the anaphylactic reactions was graded according to Mueller								

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HIGH EXPRESSION OF APAF-1XL IN BONE MARROW CELLS OF LOW RISK MYELODYSPLASTIC SYNDROME

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Background. Apoptosis has a crucial role in myelodysplastic syndromes (MDS). Early disease is associated with excessive apoptosis and the apoptotic rate diminishes during disease progression. Cytochrome c/ APAF-1/ CASP-9 pathway is the main pathway involved in apoptosis initiation by several stimuli. Original APAF-1 comprises three functional domains; APAF-1XL and APAF-1LN isoforms have an insertion between the CARD and ATPase domains and APAF-1XL also has an additional WDR. It has been reported that only the isoforms with the extra WDR activate pro-caspase 9. We hypothesized that APAF-1XL expression could be related to the higher rates of apoptosis found in early-stage disease. *Aims*. To analyse the expression of APAF-1 transcripts in bone marrow cells from MDS patients and correlate these findings with IPSS. We also attempt to verify the modulation of APAF-1 mRNA expression during erythroid differentiation of CD34+ normal and MDS cells. *Methods*. Marrow aspirates were obtained from 7 normal donors, 14 patients with low risk MDS (IPSS: 9 low and 5 intermediate-1), and 8 patients with high risk disease (IPSS: 5 intermediate-2 and 3 high), out of treatment (11 males, 11 females; 69 [40-91] yo). The National Ethical Committee Board approved the study; informed-written consent was obtained from all patients and donors. Total cells were submitted to RNA extraction and the expression level of mRNA was detected through real time RT-PCR. The relative quantification value of gene expression was calculated using 2-DDct. For erythroid differentiation, bone marrow samples from one MDS patient with refractory anemia (IPSS low) and one normal donor were collected and CD34+ cells were separated from mononuclear cells using the MIDI-MACS immunoaffinity columns. CD34+cells were plated on plastic culture dishes in methylcellulose medium with appropriate growth factors for 6 days. BFU-E, CFU-E and proerythroblasts were then cultured in α MEM for an additional 8 days. At days 6 and 14, cells were collected and submitted to real time RT-PCR and apoptosis analysis. Apoptosis was quantified with anexin V and propidium iodide; erythroblast differentiation was observed with transferrin receptor and glycophorin A by flow cytometry. *Results*. APAF-1XL expression was significantly higher in low risk (15.327[3.095-121.1]) when compared to high risk MDS (4.379[2.412-44.632]; p=0.0103, Wilcoxon rank-sum test). APAF-1XL/APAF-1LN ratio was also significantly different comparing low and high risk groups (1.614[0.6598-6.964] versus 0.8904[0.155-2.99], p=0.0197). Erythroid differentiation of CD34+ cells from low risk MDS was characterized by increased apoptosis (20% at day 14) and increased expression of APAF-1XL mRNA (six fold at day 14 compared to day 6). In contrast, erythroid differentiation of CD34+ normal hematopoietic cells was characterized by a low rate of apoptosis (8% at day 14) and low expression of APAF-1XL mRNA (half fold on day 14 compared to day 6). *Conclusions*. High levels of APAF-1XL mRNA expression in low risk disease and its positive correlation with the apoptotic rate, observed during erythroblast differentiation of low risk MDS cells, suggest that APAF-1 participates in the augmented susceptibility of myelodysplastic cells to intramedullary death, since APAF-1XL, instead of APAF-1LN, is the isoform constitutionally capable of activating pro-caspase 9.

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MYELOID SARCOMA: A CLINICO-PATHOLOGICAL STUDY INCLUDING IMAGING OF STROMAL COMPONENTS

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Background.The term myeloid sarcoma (MS) is currently used to designate extramedullary tumors composed of immature myeloid cells. *Aims*. Our purpose was to analyse clinico-pathological aspects of a large series of MS. Since the homing to extramedullary tissues resulting in MS may be promoted by the local microenvironment, we further characterized stromal cell populations of MS occurring in different anatomic sites. *Methods*. Paraffin-embedded specimens containing representative formalin-fixed MS biopsy material were retrieved from our files. Tissue sections stained for a panel of antigens including myeloperoxidase, lysozyme, CD3, CD4, CD20, CD34, CD41, CD61,CD68, CD117, CD99, CD 56, TdT , hemoglobin were reviewed. Clinical and laboratory informations including CXCR4 expression and survival data were obtained. Additional imaging studies focused on the spatial localization of microvessels and of stromal cells expressing stromal cell-derived factor-1 (SDF-1), cellular retinol-binding protein-1 (CRBP-1) and α-smooth muscle actin (SMA). *Results*. We obtained representative samples and clinical informations from 61 patients. MS developed simultaneously with bone marrow infiltration as initial manifestation of acute myeloid leukaemia (AML, n=11), as relapse after chemotherapy or allogeneic bone marrow transplantation of a previously diagnosed AML (n=12), as extramedullary transformation of a myelodysplastic syndrome (n=11) or as blast phase disease in typical or atypical myeloproliferative disorders (n=16). Moreover, 11 patients presented with an isolated MS without any bone marrow involvement. The myeloid infiltrates showed phenotypic features of an acute myelomonocytic or monoblastic leukaemia in 48% while the remaining cases corresponded to various AML categories including megakaryoblastic leukaemia or were classified as blastic type, not further specified. In contrast to the large variety of phenotypic profiles and clinical settings, a common feature of all samples was an increased density of SDF-1, CRBP-1 and SMA-expressing stromal cells.The spatial organization of the stroma was highlighted by confocal imaging. *Summary/Conclusions*. According to our observations, the functional interaction of local stromal cells with myeloid population via chemokines and chemokine-receptors may be crucial for blast cell infiltration of extramedullary sites in MS.

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ABNORMAL METHYLATION STATUS OF THE PROMOTER OF APAF-1 IN *DE NOVO* AML PATIENTS

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Background. Apaf-1, the apoptotic protease activating factor 1, is an important signaling protein involved in the activation of the Caspase 9. During DNA damaging stimuli-induced apoptosis, Apaf-1 is released in the citosol from its interaction with Bcl-2. Apaf-1 forms the so called apoptosome complex with Pro-Caspase 9 in the presence of cytosolic cytochrome c and dATP. This association ultimately leads the Caspase 9 activation and subsequent activation of Caspase 3, one of the key executioners of apoptosis. It has been reported that the inactivated Apaf-1 gene in malignant melanoma could be switch off by DNA methylation and can be turned back by inhibitors of DNA methylation. Similar findings found in other malignances point to the possibility that abnormalities of Apaf-1 play a role in the pathogenesis of a wide variety of cancer. Besides, recently it has been shown that methylation silencing is a mechanism of inactivation of Apaf-1 in several human leukemia-lymphoma cell lines (Furukawa *et al*. Mol Cancer Res 2005;3(6):325). *Objective*. Studying the abnormal methylation status of the promoter of Apaf-1 in patients with *de novo* AML by means of the methylation specific PCR method (MSP) (Herman *et al*. Proc Natl Acad Sci USA,1996; 93:9821). *Patients and Methods*. We have studied bone marrow samples from 88 patients at the time of diagnosis [(48 M / 40 F, median age: 61 yr (range: 18-88), FAB subtype: 0 M0, 18 M1, 23 M2, 10 M4, 15 M5, 14 M6, 1 M7, 2 unknown)]. Genomic DNA was extracted using standard protocols (Quiamp DNA Mini kit). After bisulfite technic prodedure (Epitect Bisulfide, Quiagen) the DNA was PCR amplified with primers specific for the methylated and unmethylated alleles of the gene. The PCR products were separated on 2% agarosa gel. Bone marrow DNA from healthy donors was used as negative control. CpGenome™ Universal Methylated DNA (Chemicon, Millipore) was used as a positive control. *Results*. Aberrant methylation of Apaf-1 promoter region was found in 19 out of 88 samples (22%). Hypermethylation of Apaf-1 did not correlated significantly with any clinical, biological, morphological, immunophenoypic or molecular characteristics between the methylated and unmethylated group. Moreover, no significant differences in overall survival and relapse risk were observed between both groups. *Conclusions*. In agreement with previous studies carried out in AML cell lines our data show that hypermethylation of the promoter of Apaf-1 is a frequent event in *de novo* AML patients. However, no correlation with relevant clinical or biological data was found. Relevance of this finding for the treatment with hypomethylating agents should be explored in the clinical setting.

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ABT-737 ACTIVITY ON PRIMARY MULTIPLE MYELOMA SAMPLES

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Background. The bcl-2 family proteins are key regulators of cell survival and are frequently found aberrantly expressed in lymphoid malignancies and in multiple myeloma (MM) conferring resistance to chemotherapy. *Aims*. In the present study we investigated the cell cycle and apoptotic effects of ABT-737 (kindly provided by Abbott Laboratories), a Bcl-2 (BH3) inhibitor, on MM cell lines and on primary CD138+ malignant plasma cells. *Results*. The KMS18 MM cell line was exposed to increasing concentrations of ABT-737 (from 0.1 to 1 μM) up to 72 hours. A dose- and time-dependent cell growth inhibition was documented (IC50s 0.286 microM at 72 hours) due to a significant increase of apoptotic cells as demonstrated by the increment of Annexin V+ cells, at 24 hours, from 17.3%±4.4 in DMSO to 31.7%±12.9, 45.8%±9.1 (p=0.032), 49.8%±7.8 (p=0.017) and 60.8%±5.8 (p=0.0026) in the presence of ABT-737 at 0.1, 0.25, 0.5 and 1 microM, respectively. These data were confirmed by measuring the subG0/1 peak (Acridine-Orange). Cell cycle analysis demonstrated at 72 hours a significant G1-phase depletion: from 54.6%±4.5 (DMSO) to 26.9%±1.4 (1 μM ABT-737) (p=0.021). The effects of ABT-737 were then examined on primary cells from untreated patients: 6 MM and 1 plasma cell leukemia (PCL). Bone marrow aspirate cells, following CD138 enrichment (>80% of purity), were cultured *in vitro* with ABT-737 (at scalar concentrations from 0.1 to 1 μM) up to 72 hours. Effective apoptosis was observed in all 6 MM samples. Importantly, low concentrations (0.1 microM) of ABT-737 significantly (p=0.01) increased CD138+ apoptotic cells from 19.8%±9.0 to 52.9%±17.2 (24 hours) in MM samples. ABT-737 triggered similar effects in the PCL sample (a 3- and 5-fold increase of apoptotic cells at 0.1 microM and 0.5 microM). *Summary*. In conclusion, ABT-737 shows potent *in vitro* growth-inhibitory and pro-apoptotic activity at nanomolar concentrations in MM cells, indicating that it has great potential for the treatment of this disease. A further pre-clinical/clinical development of this compound is warranted.

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JAK2 V617F MUTATION SCREENING IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS AND CORE BINDING FACTOR ACUTE MYELOID LEUKEMIAS (CBF-AML)

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Background. An acquired V617F mutation of the JAK2 gene is of diagnostic and prognostic value in myeloproliferative disorders (MPD). Low incidence of JAK2 mutation has been also described in patients with CBF-AML, negatively affecting their prognosis. *Aims*. We have updated our results of JAK2 V617F mutation screening in a cohort of 604 patients with suspected Ph- MPD and 50 patients with CBF-AML. *Methods.* For detection of the JAK2 V617F mutation, allelic discrimination real-time RT-PCR assay with specific locked nucleic acid (LNA)-modified TaqMan probes was used. The expression of AML1/ETO and CBFβ/MYH11 in CBF-AML was assessed by quantitative real-time PCR. C-KIT mutations in AML patients were determined by gel electrophoresis and direct sequencing. *Results*. 604 samples of patients with suspected Ph- MPD were analyzed. In 267 patients (44.2%), JAK2 mutation was detected. 29 of 267 JAK2 mutations (10.9%) were homozygous. Out of 113 patients suffering from polycythemia vera (PV), 112 had mutations (99.1%), which were homozygous in 21 cases (18.8%). In WHO criteria-defined essential thrombocythemia (ET), JAK2 mutations were demonstrated in 33/63 (52.4%) patients, none of them was homozygous. 34 of 65 (52.3%) individuals with idiopathic myelofibrosis (IMF) had JAK2 mutations (5 were homozygous). Of another 96 MPD patients with thrombocythemia not discriminated according to WHO criteria, 77 (80.2%) had detectable JAK2 mutations. Within patients with thrombocythemia of unknown reason (i.e., patients that might have MPD or secondary thrombocythemia), 5/90 (5.6%) had mutated JAK2 genes, whereas none of 14 patients with well defined secondary thrombocythemia had their JAK2 genes mutated. Of 73 patients with polyglobulia of unknown reason (i.e. patients that might have PV, secondary polyglobulia, or idiopathic erythrocytosis), none had mutated JAK2 genes. Likewise, 11 patients with well defined secondary polyglobulia had their JAK2 genes unmutated. Among the remaining 79 patients with miscellaneous diagnoses, 6 cases with JAK2 mutation were found. In the cohort of 50 CBF-AML patients, having either AML1/ETO (n=29) or CBFβ/MYH11 (n=21) fusion genes, 2 (4.0%) had JAK2 mutation. The first of them was 12-year-old female with AML1/ETO positivity, diagnosed in July 2004. She carried a heterozygous JAK2 mutation. After one month of induction chemotherapy, she became JAK2 negative but remained AML1/ETO positive, with a decreasing trend until December 2004 when she reached molecular remission. In May 2005, she relapsed clinically with comparatively high AML1/ETO expression as at diagnosis, but with undetectable JAK2 mutation. She underwent allogeneic stem cell transplantation in September 2005 and since November 2005 she remains in molecular remission. The second patient was a 22-year-old male presenting with extramedullar disease, AML1/ETO positivity, heterozygous JAK2 mutation and D820G exon 17 C-KIT gene mutation. Following induction chemotherapy, he became AML1/ETO and JAK2 negative. *Conclusions*. Our results confirm that incidence of JAK2 mutation is extremely high in PV (99%) and moderate (about 50%) in either ET or IMF, in case allele-specific PCR is employed. The rather low percentage of CBF-AML patients with detectable JAK2 mutation may share a course with clinical problems (extramedullar disease, relapses).

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EVOLUTION OF PATIENTS WITH HODGKINS DISEASE STAGE I AND II AFTER RADIOTHERAPY

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Background. Patients with stage I and II Hodgkin's disease and favorable prognostic are treated with both chemotherapy and radiotherapy, regimens with modified doses. Our study is retrospective and the purpose is presenting the evolution of patients, from our center, with stage I and II Hodgkin's disease treated with radiotherapy alone. *Material and methods*. The study contains 74 patients (median age 28±2,5 years) with stage I and II Hodgkin's disease treated with radiotherapy alone. The study period was from 1984 to 1999. *Results*. 26 (35,1%) patients with stage I and II Hodgkin's disease treated with radiotherapy alone are relapsed. The median age of this patients was 31 ±3 years. Relapse rate varied according to the stage of the disease, stage IA and IIA representing 23,07% of the relapses (6 patients), stage IB and IIB representing 73,07% (19 patients) and stage IIIA 3,84% (1 patient). 21 patients (80,7%) present early relapse. Disease free survival was 18±3 months. The rest of 5 patients (19,2%) represents late relapses (over 3 years). 19 of the patients (73%) present relapse limited to nodal sites, and 7 patients (27%) present extranodal relapse. Most of extranodal relapses are pulmonary and are due to bulky mediastinal adenopathy. 61,5% (16 patients) of the total of relapses present tumors over 7 cm in diameter. From 26 patients who relapsed, 14 patients (53,84%) present in-field relapse, and in 12 patients (46,16%) the relapse occur outside the radiation field. 17 cases (65,3%) occurred after localized radiotherapy, and 9 patients (34,6%) are relapsed after wide-field radiotherapy. Indifferent to the type of relapse, the specific chemotherapy has re-induced the complete remission in 92,3% of the patients and the durable responses was in 86,3% of cases. *Conclusions*. Relapse rate in localized Hodgkin's disease treated with radiotherapy alone is 31,5%. Most of relapses are early (80,7%) and they involved nodal sites. The relapse rate seems influenced by the stage B of the disease, by the maximum tumor volume and by the type of applied radiotherapy (local/extended). The sensitivity to chemotherapy is for this patients is similar at the time of initial therapy and salvage chemotherapy can achieve durable responses and remissions in approximately 86,3 percent of these patients This explains why, although the relapse rate for favorable prognosis stage I-II Hodgkin's disease is higher with radiotherapy alone compared to combined with chemotherapy, survival is similar

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NO RELATIONSHIP BETWEEN THE JAK2V617F LOAD AT DIAGNOSIS AND THROMBOSIS IN 57 PATIENTS WITH ESSENTIAL THROMBOCYTAEMIA

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Background. Essential Thrombocytaemia (ET) is a myeloproliferative disorder (MPD) frequently complicated by thrombotic events. The JAK2V617F mutation has been recently described as a risk factor to develop thromboses along the clinical course of MPD. However, in most of the studies, the JAK2V617F mutation was only qualitatively determined, but the JAK2V617F load was not analysed. *Aims*. To evaluate whether the JAK2V617F load at the time of diagnosis is associated with thrombotic risk along the clinical course. *Methods*. Fifty-seven ET patients (26 males and 31 females), with a median age at diagnosis of 54 years (range, 16-94) diagnosed according to the PVSG classification from a population-based registry, were studied. Bone marrow smears at diagnosis were scraped and DNA was isolated using the QIAamp DNA Blood Mini kit (Qiagen, Courtaboeuf, France). The JAK2V617F was determined using a sensitive, allele-specific, quantitative PCRs in genomic DNA previously described.[1] Fifty patients were treated using cytoreductive therapy and 39 received anti-platelet drugs. The median follow-up duration was 69 months (2-203). *Results*. The JAK2V617F mutation was observed in 43 patients (75%), with a median value of JAK2V617F / JAK2WT ratio of 20.2% (range, 2 to 85%). Patients were divided in 3 classes depending on the percentage of JAK2V617F:0% (n=15), 1-20.2% (n=21), >20.2% (n=21). The percentage of patients with cardiovascular risk factors was respectively 47%, 57% and 71% in these three classes. Six (10%) patients had a prior history of thrombosis, mainly venous thrombosis. There were 6 thrombotic events at the time of diagnosis in 6 different patients, and 22 thrombotic complications (12 arterial and 10 venous) in 14 different subjects during the follow-up. No difference in thrombotic vascular events was noticed between the 3 classes, either for arterial or venous thromboses. However, among the 4 patients with a JAK2V617F load above 50%, three had thrombotic events during the clinical course, without myelofibrosis evolution. *Conclusions*. No clear association between the JAK2V617F / JAK2WT ratio and the risk of thrombosis was observed in our patients. These results have to be confirmed in a larger cohort, especially regarding very high JAK2V617F / JAK2WT ratio.

Reference

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GEMTUZUMAB OZOGAMICIN WITH CYTARABINE AND MITOXANTRONE AS A THIRD LINE TREATMENT IN A POOR PROGNOSIS GROUP OF ADULT ACUTE MYELOID LEUKEMIA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background. Patients with refractory acute myeloid leukemia (AML) after 2 courses of chemotherapy or in second relapse have very poor prognosis. Most patients have already been exposed to intensive multiagent chemotherapy and most reinduction regimens in current use cause substantial toxicity; such patients are not always eligible for intensive chemotherapy. Antibody-targeted chemotherapy is presumed to be less toxic than conventional chemotherapy and has been developed for treatment of CD33-positive AML. *Aims*. We conducted a study of GO combined with mitoxantrone and cytarabine as third-line treatment in a homogeneous *poor prognosis* group of 23 patients with refractory/relapsed AML to evaluated the safety and toxicity of the drug in this group of patients. *Patients and Methods.* Patients median age was 52 years (range 36-70); twelve patients were refractory after DCE (daunorubicin, etoposide, and cytarabine) as first-line therapy and FLAG-IDA (fludarabine, cytarabine, and idarubicin) as second line; eleven patients were in second relapse and were treated with DCE at onset and with FLAG-IDA in first relapse; this *poor prognosis* group was therefore homogeneous as regards previous chemotherapy regimens. GO at a dosage of 3 mg/m^2 was administered as a 2-h intravenous infusion on days 1 and 14, cytarabine at 100 mg/m^2 on days 1-7 and mitoxantrone at 12 mg/m^2 on days 1-3. *Results*. The overall complete remission rate was 5/23 (21.7%): 4 of 11 (36%) in second relapse and 1 of 12 (8.3%) in refractory patients; CR and CRp were achieved in 2/23 (8.6%) and 3/23 (13%), respectively; of these 5 responder patients one was in the favourable cytogenetic group [t(8;21)] and 4 were in the intermediate (3 with normal karyotypes and 1 with +8); no response was achieved in the poor cytogenetic risk group. Four patients died during therapy (overall treatment-related mortality 17.3%): two due to sepsis (P. aeruginosa), one to cerebral haemorrhage and one to acute respiratory distress syndrome. Baseline characteristics including age, type of AML, WBC count and marrow blasts were not predictive of treatment in this study. Furthermore, the CR/CRp rate after GO, cytarabine and mitoxantrone was not influenced by CD33 positivity. All patients experienced profound neutropenia (<0.1×10^9/L); in patients achieving CR/CRp the median time to reach absolute neutrophil count (ANC) >0.5×10^{9}L and 1×10^9/L was 28 days (range 21-33) and 34 days (range 29-39), respectively. Grade III-IV anemia and thrombocytopenia were observed in all cases; a grade I/II bilirubin increase occurred in 5/23 cases (21.7%); grade I/II ALT/AST elevation was documented in 3/23 cases (13%); grade I/II alkaline phosphatase elevation was observed in 4/23 (17.3%) cases; no veno-occlusive disease (VOD) occurred. The most common non-hematologic adverse events were infusion-related allergic reactions (17/23 or 73.9%), infection (5/23 or 21.7%) and febrile neutropenia (20/23 or 86.9%). *Summary*. In our experience, the addition of GO to mitoxantrone and cytarabine is feasible in refractory or second relapse acute myeloid leukemia patients but yields a low response rate (21.7%) when used as a third line treatment.

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CLINICAL RELEVANCE OF REGULATORY NETWORK IN THE BONE MARROW COMPARTMENT OF PATIENTS WITH MULTIPLE MYELOMA

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Regulatory network in the bone marrow (BM) compartment of patients with multiple myeloma (MM) consists of functional interplay between the myeloma cells and microenvironment resulting with the interaction of various cytokines, their receptors and adhesion molecules. The aim of study was to analyze prognostic significance of different tumor-host interactions in the BM of patients (pts) with MM by immunohistochemical markers of sensitivity to the IL-6; adhesion molecules; osteoclastogenesis; and angiogenesis. *Patients and Methods*. Sixty newly diagnosed MM pts (33 male/27 female pts, mean age 60 years, range 35-75) were distributed according to the clinical stage (CS, Salmon&Durie) as: I 8pts, II 22pts, III 30pts. IgG myeloma was diagnosed in 35pts; IgA in 12pts; light chains in 12pts. Regarding ISS score, the group included: ISS1 18pts, ISS2 13pts, ISS3 29pts. All patients were treated with conventional chemotherapy. All samples of BM biopsies were analyzed for the immunohistochemical expression of gp 130, VCAM, OPG and RANKL. The intensity of these staining was graded as weak (0-30% cells), moderate (31-60% cells), and strong (>60% cells). Analyzing the microvessel density (MVD), BM vessels were visualized by immunohistochemical staining for CD34. The number of vessels per 400x high power field (HPF) was counted in the area of the most dense vascularization. *Results*. The expression of gp130 was higher in III vs. I CS (32 vs.15%, p<0,05). High level of the expression of the VCAM indicated a significantly shorter overall survival (36 vs. 18m, log rank, p<0,001). Significantly stronger expression of RANKL was detected in III vs. I CS (67,5 vs. 38,5%, p<0,05), and in pts with ISS3 vs. ISS1 (55 vs. 38,5%, p<0,05). This correlated with low expression of OPG in III CS (Me 27,5%, range 10-40%), and ISS3 (Me 20%, range 5-30%). MVD was significantly higher in III vs. I CS (15 vs.7,5/ x400 field, p<0,001); and in ISS3 vs. ISS1 (17,5 vs. 9,7/ x400 field, p<0,05). Intensified expression of IL-6 receptors accompanied with a strong activity of adhesion molecules, high levels of angiogenesis and osteoclastogenesis in III CS pointed out aggressive course of disease followed by a significantly shorter overall survival (26 vs. 43,5 m, log rank, p<0,05). In conclusion, markers of sensitivity to the IL-6, stromal adhesion activity, osteoclastogenesis, and angiogenesis as a key points in regulatory network of myeloma represents important prognostic factors of significant predictive value on course of disease and possible therapeutic targets.

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PERSISTENT MONOCLONALITY AFTER HISTOLOGICAL REMISSION IN GASTRIC MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA TREATED WITH CHEMOTHERAPY AND/OR SURGERY: INFLUENCE OF T(11;18)(Q21;Q21)

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Background. The molecular response in patients with gastric MALT lymphoma achieving histological response after chemotherapy or surgery is not well established. *Aims*.To study the molecular response and outcome of 19 patients with gastric MALT lymphoma achieving histological remission after treatment with chemotherapy and/or surgery. *Methods*. IgVH gene rearrangements were studied by PCR in gastric biopsies obtained at diagnosis and during follow-up. Presence of t(11;18)(q21;q21) was studied by FISH or PCR. Sequencing analysis of PCR products of 3 t(11;18)(q21;q21) positive and 2 t(11;18)(q21;q21) negative lymphomas with persistent monoclonality was performed. *Results*. At diagnosis, t(11;18)(q21;q21) was found in 3 patients (15.7%). After achieving histological response, monoclonality was demonstrated in 11/19 patients (58%) and maintained monoclonality in 8 of the 10 patients (80%) who had sequential studies. Median follow-up was 73 months (13-240). All 3 t(11;18)(q21;q21) positive patients had maintained monoclonality and sequencing analyses revealed the presence of the same mutated IgVH alleles in diagnostic and in follow-up samples. In 2 t(11;18)(q21;q21) negative lymphomas the monoclonal rearrangements corresponded to unrelated alleles. Persistent monoclonality was detected after a median of 49 months (6-134) and did not condition histological relapses, either in negative or positive t(11;18)(q21;q21) patients. *Conclusions*. More than half of patients with gastric MALT lymphoma achieving histological response after chemotherapy and/or surgery have long term persistent monoclonality. t(11;18)(q21;q21) determines long term persistence of the initial lymphoma population. However, the presence of maintained monoclonality did not condition histological relapse.

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CD38 AS A PROGNOSTIC FACTOR IN CHINESE PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. B-cell chronic lymphocytic leukaemia (B-CLL) is the most common type of adult leukaemia in western countries, however, infrequent in eastern. Although the median survival is around 10 years, B-CLL patients have a highly variable clinical course and prognosis. Many studies were unanimous in showing that a high proportion of CD38 expressing cells among the leukemic cells was associated with a significantly poorer prognosis, defined either as overall survival (OS) or as event-free survival (EFS) or as treatment-free interval (TFI). However, a number of questions remain unclear, including the optimal cut-off for CD38 positivity. The characteristics of Chinese patients with B-CLL compared with western countries have not yet been clarified. *Aims*. To estimate the clinical value of CD38 expression as B-CLL prognostic factors in Chinese patients, we present study from our a single centre of 89 patients with B-CLL. *Methods*. CD38 expression was analysed on $CD19^+/CD5^+$ leukaemic cells using flow cytometry and correlated the results with the clinical outcome and risk factors like the stage of the disease, the lymphocyte count, the lactate dehydrogenenase (LDH) and β2-microglobulin level, ZAP-70 expression, and molecular cytogenetic aberrations. The prognostic significance of CD38 expression (<7% vs.≥7%; <20% vs. ≥20%; <30% vs. ≥30%) and of age (<60 vs. ≥60 years), gender, Rai stage (0 vs. I-II vs. III-IV), lymphocyte count ($<50\times10^9$/L vs. $\geq50\times10^9$/L), peripheral blood morphology (typical vs. atypical), immunophenotypic score (3 + 4 vs. 5), LDH value (normal vs. elevated), β2-microglobulin level was (normal vs. elevated), ZAP-70 expression (<20% vs. ≥20%), molecular cytogenetic aberrations (normal vs. abnormal) analysed on survival duration. *Results*. According to the different cut-off points of 7%, 20% and 30%, the percentage of $CD38^+$ cases was 39.3%, 31.5% and 27.0%, respectively. We did not find any significant differences in estimated parameters among these three cut-offs. The level of CD38 were correlated with Rai stage, the expression of ZAP-70, β2-microglobulin level, and chromosomal aberrations like del(13q14), del(17p13), and del(11q23). At univariate analysis, Rai stage III-IV, del(17p13), del(11q23), ZAP-70 expression, and CD38 expression according to all three cut-offs shown significant impact on OS. At multivariate analysis, Rai stage and CD38 expression according to all three cut-offs remained significantly related to OS. *Conclusions*. Our data demonstrates that CD38 was a predictor of clinical outcome in Chinese patients with B-CLL.

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PROGNOSTIC SIGNIFICANCE OF IPS, BULKY DISEASE AND ESR IN CLASSICAL HODGKIN LYMPHOMA -SERBIAN LYMPHOMA STUDY GROUP EXPIRIENCE

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The International Prognostic Score (IPS), as well as the other clinical and laboratory parameters (bulky disease, red blood cell sedimentation rate-ESR>50) are considered to have prognostic relevance in Hodgkin lymphoma (HL). The aim of the study was to determine the prognostic value of clinical and laboratory parameters including IPS, bulky disease and ESR>50 in cohort of patients with classical HL. Their significance was evaluated regarding response to treatment and survival period. Optimal initial prognostic model was determined according to these findings. A retrospective study was performed on cohort of 90 patients randomly selected from large number of treated patients with classical HL, nodular sclerosis subtype. In all pts, initial IPS, presence of bulky disease and ESR>50 were determined. The median follow-up was five years. All patients were treated according to standard clinical approach, ABVD regimen. The mean age was 34.5±10.3 yrs, range 15-74 (77% of pts were <45 yrs). Gender distribution was 45 male / 45 female. The overall survival rate was 73.3% after 5 years of follow up. The IPS distribution of HL pts was as follows: 0,1-27%, 2-30%, 3,4,5-43%. According to the IPS of HL, positive correlation was found between bulky disease and ESR. The inci-

dence of bulky disease was higher in pts with IPS 3-5 than in pts with IPS 0,1. (23.3% vs 6.6% $p<0.01$). Similarly, higher expression of ESR>50 was found in pts with IPS 3-5 comparing to the pts with IPS 0.1 (33.3% vs 10% $p<0.01$). These findings of increased incidence of bulky disease in correlation with ESR>50 found in pts with high IPS pointed out shorter survival of those pts (40.86 m vs 64.4 m $p<0.01$). The patients with IPS>3, bulky disease and ESR>50 are in risk of relapse and treatment failure, and are eligible for the initial aggressive therapeutic approach.

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FLAG-IDA IN THE TREATMENT OF REFRACTORY/RELAPSED ACUTE LEUKEMIA: A SINGLE INSTITUTIONAL STUDY

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Background. Relapsed or refractory adult acute myeloid leukaemia (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 leukemic blasts and the response to chemotherapy may be improved by addition of idarubicin, an anthracycline that is less susceptible to multidrug resistance *Aims*. Based on these consideration, we evaluated the efficacy and the toxicity of FLAG-IDA in a series of 78 refractory/relapsed AML. *Patients and Methods*. Fifty-two patients (66%) were in first relapse and 26 (34%) were refractory to conventional chemotherapy. The patient group included 44 men and 34 female with a median age of 43 years (range 15-63). All patients were treated with fludarabine (30 mg/m^2 iv for 5 days), cytarabine (2 gr/m^2 iv for 5 days), idarubicin (10 mg/m^2 iv for 3 days) and G-CSF (5 mcg/Kg/day subcutaneous 24 h after completing chemotherapy and until neutrophil regeneration). *Results*. The overall CR rate was 55% (43 of 78): 31 of 52 (59%) in relapsed and 12 of 26 (46%) in refractory patients; there were 5 of 78 (6%) deaths during therapy: 2 due to cerebral haemorrhage and 3 due to infection. In patients achieving remission, the median time to reach absolute neutrophil count (ANC) more than 0.5×10^9/L and 1×10^9/L was 21 (range 16-26) and 24 days (range 20-28) from the start of chemotherapy, respectively. Platelets level of more than 20×10^9/L and 100×10^9/L were achieved in a median time of 24 (range 19-26) and 32 days (range 28-39) days, respectively. Fever more than 38.5°C was observed in 63 of 78 patients (80%): 45 (71%) had fever of unknown origin and 18 (29%) documented infections. Nonhematological side effects, consisting mainly of mucositis (60/78 or 76%) and transient liver toxicity increase (30/78 or 38%). All 43 patients who achieved CR received a second course with FLAG-IDA, and 18 received allogeneic stem cell transplantation, 8 patients received autologous stem cell transplantation, 9 were junged unable to receive any further therapy, and 8 refused other therapy. The median overall survival (OS) for all 78 patients was 6 months (range 3-62); for the 43 responders patients, the disease free survival (DFS) and OS were 9 (range 4-62) and 11 (range 7-62) months, respectively; the 18 patients who received allogeneic transplantation had a DFS of 13 (range 7-62) months. *Summary*. In our experience, FLAG-IDA is a well-tolerated regimen in refractory/relapsed AML with a CR rate of 55%; the toxicity of this treatment is acceptable, enabling the patients who achieved CR to receive further treatment, including transplantation procedures.

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POLYMORPHISMS OF DNA REPAIR AND DETOXIFICATION GENES IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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B-cell chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults in the Western World. As its clinical course is highly variable, prognostic factors for disease progression such as particular cytogenetic abnormalities, the mutational status of the immunoglobulin heavy chain (IgH) genes, LPL, or CD38 expression, have been identified. However, a convincing prediction of disease progression can not be made in early stages of the disease. Further prognostic factors would be helpful for meaningful prediction of disease progression. There is evidence that multiple low-penetrance genetic factors, including genetic polymorphisms, predispose to CLL or modify its clinical course. The aim of this study was to identify such allelic variants of genes, primarily concentrating on DNA repair and detoxification genes. We studied the distribution of 50 polymorphisms in 500 CLL-patients and 500 control persons using PCR amplification followed by restriction enzyme digestion. Whereas for most polymorphisms an association with CLL could be excluded, the risk of the development of CLL increased with the number of high-risk alleles in the DNA base excision repair gene XRCC1 and in a combined analysis on alleles in XRCC1 and the detoxification gene CYP1B1. Further investigations are now under way to proof the relevance of these polymorphisms in the clinical course of CLL and its putative significance for CLL in combination with other prognostic factors. The results of this study will lead to new insights into the etiology of CLL and may help to predict disease progression.

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THRESHOLDS OF IRON TOXICITY IN PATIENTS OF MYELODYSPLASTIC SYNDROMES

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Background. Patients with myelodysplasia (MDS), osteomyelofibrosis (OMF), or severe aplastic anemia (SAA) suffer from ineffective erythropoiesis due to pancytopenia, which is often treated with red blood cell transfusion. Especially in low-risk patients with mean survival times of > 5 years (MDS-RA, -RARS, -5q-), potentially toxic levels of liver iron concentration (LIC) can be reached. *Aims*. Although, the 2005 consensus of Nagasaki gave recommendations for chelation treatment based on a wide range of ferritin levels, there is still no evidence based agreement about the initiation of chelation treatment in these patients. Moreover, ferritin levels can vary independently from the true iron stores as assessed by LIC. Therefore, we aimed to establish a relationship between LIC and ferritin in the patients of the myelodysplasia syndrome. *Methods*. In the present study, a total of 67 transfused patients with MDS (n=20, age: 17-75 y), OMF (n=4, age: 48-68 y), SAA (n=43, age: 5-64 y) were measured by SQUID biomagnetic liver susceptometry (BLS) and their liver and spleen volumes were scanned by sonography at the Hamburg biosusceptometer. Less than 50% were treated with DFO. LIC (µg/g-liver wet weight, conversion factor of about 6 for µg/g-dry weight) and volume data were retrospectively analyzed in comparison to ferritin values. *Results*. LIC values ranged from 149 to 8404 with a median value of 2705 µg/g-liver, while serum ferritin (SF) concentrations were between 500 and 10396 µg/l with a median ratio of SF/LIC = 0.9 [(µg/L)/(µg/g-liver)] (range: 0.4 to 5.2). The Spearman rank correlation between SF and LIC was found to be highly significant (RS=0.80, $p<0.001$), however, prediction by the linear regression LIC = (0.83±0.08)αSF was poor (R2=0.5) as found also in other iron overload diseases (Fischer *et al.*, Am J Hematol 1999; 60:289-99). Progression of hepatic fibrosis has been observed for LIC > 2700 µg/g-liver (Angelucci *et al*. Blood 2002; 100:17-21) within 60 months and significant cardiac iron levels have been observed for LIC > 3300 µg/g-liver (Jensen *et al.* Blood 2003; 101:4632-9). These thresholds were exceeded by 51% and 39% of our patients for hepatic fibrosis progression and for cardiac iron toxicity, respectively. The total body iron burden is even higher as more than 50% of the patients had hepatomegaly (median hepatomegaly factor: 1.2 of normal). *Summary/Conclusions*. A liver iron concentration of about 3000 µg/g-liver or 18 mg/g-dry weight has to be seen as the latest intervention threshold for chelation treatment as MDS patients are affected by more than one risk factor. A more secure intervention threshold would be a LIC of 1000 µg/g-liver or 4 - 6 mg/g-dry weight, corresponding with a ferritin level of 900 µg/L for transfused MDS patients. Such a LIC value is not exceeded by most of the heterozygous hereditary hemochromatosis subjects and is normally well tolerated without treatment during their life-time.

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SERUM LEVELS OF SOLUBLE HLA CLASS-I MOLECULES, ICTP, AND RANKL AS PROGNOSTIC PARAMETERS IN MULTIPLE MYELOMA PATIENTS

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Given the highly variable clinical course of multiple myeloma, the analysis of prognostic parameters bears considerable clinical interest. We here have analyzed the clinical significance of serum levels of soluble HLA class I molecules (sHLA-I), carboxy-terminal telopeptide of type-I collagen (ICTP), and receptor activator of nuclear factor kappa B ligand (RANKL). sHLA-I (median, range in µg/L) were significantly (p<0.001) elevated in myeloma patients (667 (203-2930)) as compared to healthy controls (61 (44-1010)), and 50% and 43% patients showed increased levels of ICTP and RANKL, respectively. sHLA-I correlated significantly with the International Staging System (ISS), and serial determination of sHLA-I in 11 patients revealed significant higher sHLA-I levels at time points of active versus remittent disease (700 (250-2090) versus 380 (130-920)). ICTP correlated to ISS and the presence of osteolytic lesions, however, no differences were found in patients with active disease as compared to patients in remission. With regard to RANKL, no significant correlation to ISS stage, active versus remittent disease, and osteolytic lesions were observed. Importantly, patients with increased serum levels of sHLA-I (≥1000 µg/L versus <1000 µg/L, 23 months versus > 48 months, p=0.01) or ICTP (≥5 µg/L versus <5 µg/L, 10 months versus > 48 months, p=0.0008) had a significantly decreased median overall survival, while RANKL were of no prognostic value. In conclusion, sHLA-I and ICTP serum levels seem to be of prognostic significance in myeloma patients and might be helpful to identify patients with a poor prognosis.

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ALTERATIONS OF CD44 METHYLATION IN MYELOID NEOPLASMS

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Background. CD44 comprises a family of class I transmembrane glycoproteins, which exists in several isoforms. This surface molecule has been involved in various processes such as tumorigenesis, haematopoiesis and inflammation. The standard CD44 isoform is expressed on all types of mature blood cells, on the majority of mononuclear bone marrow precursors and on all $CD34^+$ haematopoietic progenitor cells. Changes in expression of CD44 have been shown during the early differentiation of primitive haematopoietic cells as well as in certain aggressive lymphomas. Elevated expression of CD44 has been reported from patients with acute and chronic myeloid leukaemia (AML, CML) or a late form of myelodysplastic syndrome (MDS). Methylation of CpG sites in the promoter region has been often described as a potent way of restricting gene expression, called *gene silencing*. A small part of the total DNA comprises CpG islands, in which CpG dinucleotides occur at a higher frequency than in the rest of the genome. These regions are normally unmethylated. When methylated, CpG islands in gene-regulatory (promoter) sequences may repress transcription. *Aims*. The aim of this study was to gain information about the methylation pattern of CD44 and furthermore to evaluate if alterations in methylation represent a regulatory mechanism of CD44 expression. Methylated regulatory sequences are discussed in diverse malignancies like colorectal or gastric carcinoma but often without any information about the percentage of methylated versus unmethylated CpGs. This implicates the question after a consistent relationship between methylating/demethylating events and expression and needs to be further elucidated. *Methods*. Genomic DNA was isolated from three cell lines, one AML and one MDS patient, converted by bisulfite modification and analysed by methylation-specific polymerase chain reaction. Cloning and subsequent sequencing the amplificates allowed an analysis of the methylation pattern within and beside exon 1 of CD44, an approximately 700 bp region. *Results*. All three cell lines showed a diverse methylation pattern of CD44. The lowest amount of methylation was observed in the CML cell line K-562 (2%). An increased percentage of methylated CpGs was displayed in the AML cell line NB-4 (19%), whereas the highest CD44 methylation was registered in the cell line U-937. *Summary/Conclusion*. These analyses reveal a differential pattern of CD44 methylation in haematological malignancies, displaying in general a lower percentage of methylation sites as compared to neuroblastoma (100%) or prostate cancer (30-100%). The analysis of the patients' methylation pattern and the corresponding CD44 expression are currently under investigation.

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ABSENCE OF JAK2 EXON 12 MUTATIONS IN ESSENTIAL THROMBOCYTHEMIA WITH CLONAL V617F-NEGATIVE HEMOPOIESIS

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Background and objective. The JAK2 V617F mutation has been described in a subset of patients with Essential Thrombocythemia (ET). The aim of the study was to assess the incidence of this mutation according to clonality status in ET, to evaluate possible implications on thrombotic risk and finally to search for new mutations of JAK2 exon 12 in those patients with monoclonal X-chromosome inactivation pattern (X-CIP) and without JAK2 V617F mutation. *Design and methods*. We analyzed clonality status by X-CIP of 59 ET female patients younger than 60 years old. All patients were studied for JAK2 V617F mutation using allele-specific PCR according to Baxter *et al.* Seventeen major venous thrombotic events were recorded, mostly at diagnosis (29%). We also performed allele-specific PCR screening for JAK2 exon 12 mutations recently reported in a subset of these patients(K539L, N542-E543del, F537-K539delinsL, H538QK539L). *Results*. JAK2 V617F mutation was present in 69% of the overall patients; 42% of mutated patients had thrombosis, while all JAK2 wild-type patients were asymptomatic (p=0.001). Among patients with thrombosis and JAK2 V617F mutation, monoclonal hemopoiesis was predominant (65%). We did not observe a significant association between clonality status and JAK2 V617F mutation. Five out of 25 patients (20%) with monoclonal X-CIP resulted JAK2 V617F negative and thus were investigated for the presence of other JAK2 exon 12 mutations. None of such new mutations was detected in this small subgroup of patients. *Interpretation and conclusions*. JAK2 V617F mutation in our patients was higher than previously reported and it was significantly associated with development of thrombosis. JAK2 V617F mutation and monoclonal X-CIP define a group of female patients with ET at high risk for life-threatening thrombosis. JAK2 V617F mutation is present in the vast majority of our patients independently from their X-CIP and it is not surprising due to the low resolution of X-CIP analysis in the background of polyclonal hemopoiesis compared to the resolution of allele-specific PCR. The mechanisms underlying monoclonal hemopoiesis in the absence of JAK2 V617F mutation are still unclear despite the recent identification of exon 12 new mutations and need further investigations.

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OSCILLATIONS OF PLATELETS AND LEUKOCYTES IN PATIENTS WITH POLYCYTHEMIA VERA UNDER TREATMENT WITH HYDROXYUREA: ANALYSIS OF 5 INDIVIDUALS FROM GERMANY

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Background. Hydroxyurea (HU) is commonly used in polycythemia vera (PV) for cytoreductive treatment. While efficacy is usually high, in some patients difficult blood cell control can be observed since marked oscillations in platelet counts of up to several hundred thousands as well as in leukocytes can occur. It has been speculated that these oscillations may cause an increased risk for thrombosis in these patients. *Aims*. Our goal was to identify in our PV cohort HU treated patients with oscillations and to determine the frequency and amplitude of the oscillations in platelet counts and possibly other cells. Whenever possible we measured changes in periodicity after switch to other drugs. *Methods*. Blood cell counts were collected from our patient files in a retrospect analysis. Spectral analysis of periodicity was made using the Lomb periodogram, which is a special version of a Fourier spectral analysis that can be used for unevenly sampled data. *Results*. We identified five patients (4 of them JAKV617F homozygous positive, one not yet analysed) with marked oscillations in platelet counts and checked them also for red (RBC) and white blood cell counts (WBC). All 5 patients were initially treated with phlebotomies alone but therapy had to be changed to HU due to increasing platelet counts. Patient 1 is a female (age 22 at diagnosis). Under HU she showed marked oscillations with a minimum platelet count of 21 000 /µL and a maximum of 1 417 000/µL. Fourier analysis yielded a peri-

od of approximately 27 days, WBC also showed oscillations with a period of about 27 days. After change to anagrelide, oscillations promptly ceased. Patient 2 is also a female (53 years at diagnosis). During therapy with HU platelets (nadir 68 000 /μL, zenith 1 690 000 /μL) and WBCs oscillated with a period of approximately 27 days. She still remains under therapy with HU. Patient 3 is a 73-year-old woman who was treated with HU for 4 months during which she showed marked oscillations (nadir 71 000 /μL, zenith 1 145 000 μL) with an approximate period of 29 days which ceased under change to anagrelide. Patient 4 (female, 52 years at diagnosis) exhibited oscillations of platelet count (nadir 104 000 /μL, zenith 1 420 000 μL) with a period of approximately 30 days. Patient 5 (male, 62 years) showed under with HU for 10 months marked oscillations (nadir 46 000 /μL, zenith 1 122 000 /μL) with a period of approximately 30 days. The oscillations disappeared with switching therapy to busulphane. None of the five patients experienced thromboembolic events under HU therapy. *Summary*. We have identified 5 patients with marked oscillations under HU therapy. The mean period was 28.2 days. No thromboembolic complications over an accumulated observation period of 55 patient months have been observed. Since it is unclear in which percentage of HU treated patients such oscillations do occur and whether unrecognized oscillations may account for dosing difficulties, systematic analyses of larger patient cohorts are required.

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PRENATAL GENOTYPING OF FETAL RHD IN MATERNAL PLASMA FROM RHD NEGATIVE WOMEN

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The Rhesus (Rh) system is one of the most important and complex blood group systems in humans. The Rh antigens are encoded by two highly homologous, closely linked, genes on the short arm of chromosome Anti-D antibody was once the most common cause of haemolytic disease of the fetus (HDN). In most cases, the anti-D of an RhD negative mother results from immunization by transplacental haemorrhage during a previous birth of an RhD positive baby. With the introduction of the Rh immunoglobulin prophylaxis program, there has been a dramatic drop in mortality due to Rh HDN. The purpose of this study was to confirm the accuracy of a non- invasive prenatal determination of the RHD typing and determination of the fetal DNA in the maternal plasma from RhD negative women with the use of real time PCR. We studied 27 RhD negative pregnant women while 6 positive subjects were used as controls and the results were compared with serologic RhD typing of the newborns. DNA was extracted from maternal plasma using QIAamp DNA Blood Mini Kit (Qiagen) and then analyzed for the RhD gene with a Real-Time PCR and Taqman method. Amplification was carried out in a Light-Cycler instrument (Roche Biochemicals). Among 27 RhD negative women, 7 were in their second trimester of pregnancy and 20 were in their third trimester. Eighteen fetuses were RhD positive, and 9 were RhD negative. Fetal D status was predicted with 100 percent accuracy from maternal plasma. The discovery of fetal DNA in maternal plasma has opened up an approach for noninvasive prenatal diagnosis. Molecular genetics of Rh blood group system has become a reality in practical transfusion medicine the last years. Pregnant women carrying RhD negative fetuses that can be specifically detected by RhD genotyping may be excluded from receiving unnecessary Rh Ig prophylaxis.

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METHYLATION STATUS OF P57KIP2 AND P16INK4 IN PATIENTS WITH PLASMA CELL NEOPLASMS

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Background. Growing evidence has implicated silencing of tumor suppressor genes (TSG) by aberrant methylation in the molecular pathogenesis of several human cancers. The p57KIP2 and the p16INK4 are cyclin-dependent kinases (CDKIs) that have been implicated in tumorigenesis as TSG. Although the cytogenetic and molecular abnormalities underlying plasma cell (PC) neoplasms are becoming better understood the role of CDKIs methylation, if any, is largely unknown. *Aims*. In this study we wished to estimate the frequency of p57KIP2 and p16INK4a methylation in patients with PC neoplasms, to see whether there is any implication in the pathogenesis of the disease and to find any possible correlation with clinical and laboraratory variables. *Methods*. The methylation-specific polymerase chain reaction (MSP) with primers for methylated and unmethylated alleles of the p57KIP2 and p16INK4a gene was employed to study prospectively bone marrow samples from 22 patients with multiple myeloma (MM) and 2 patients with Waldenström's macroglobulinemia (WM). Age range was 47-84 years, median 68 years. All samples were taken at diagnosis, except for one patient that sample was taken when progression to plasma cell leukemia (PCL) occurred. Genomic DNA was extracted using the QIAmp DNA mini kit of Qiagen. Bone marrow DNA from 11 individuals with leukopenia or thrombocytopenia that were proved to have no haematological malignancy served as negative controls. Human male genomic DNA universally methylated for all genes (Intergen Company, Purchase, NY) was used in all experiments as positive control for methylated alleles. *Results*. MM patients were classified using the Durie and Salmon criteria; 3 patients had smoldering myeloma, 1 patient stage IA disease, 9 patients IIA, 5 patients IIIA and 4 patients IIIB. Classical cytogenetic analysis was available in 9/23 patients and was normal in all but one patient that had a 45,X,-Y karyotype. Patients younger than 60 years requiring treatment received VAD chemotherapy and high dose melphalan with PBSC rescue, whereas patients older than 60 years received oral melphalan and methylprednisolone. Four patients (3 MM and 1 PCL) died due to refractory disease and the remaining 20 patients are on regular follow up. In all experiments there was a strong visual band for the positive control. All patient samples were found to be completely negative for methylation of the p57KIP2 gene whereas 4 patients with MM (14%; one patient with smoldering myeloma, 2 patients with stage III disease and 1 patient with PCL) and 1 patient with WM were found to be methylated for the p16INK4 gene. 2/3 MM patients and 1/1 PCL patient with p16INK4 methylation died due to disease progression or refractory disease. *Conclusions*. P57KIP2 methylation has not been studied before and it seems that it is not a frequent event in this group of patients. The prevalence of p16INK4 methylation found falls within the range of 10% to 51% previously reported in the literature. P16INK4 methylation might be associated with advanced disease. Further studies are needed to confirm the above mentioned results.

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EBV RELATED TRANSFORMATION EVENTS IN CLL

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Less than 5% of patients with CLL undergo histological transformation to diffuse large B cell lymphoma (DLBL) while transformation to Classical Hodgkins lymphoma (CHL) is also recognised. It has been assumed that such events reflect genetic changes in the CLL clone but there is now emerging evidence to suggest that at least some transformation events may be Epstein Barr virus (EBV) related neoplasms. In this analysis we have reviewed the clinical and laboratory features of 15 CLL patients with biopsy proven histological transformation to DLBL and 2 patients who developed CHL. Histological sections were assessed for the expression of a wide range of immunophenotypic markers as well as EBV latent membrane protein 1 (LMP-1). Of the 15 patients developing DLBL 5 appeared on phenotypic grounds to be related to the underlying CLL clone. In the remaining patients the tumour cells appeared phenotypically distinct as they lacked CD5 and CD23 and demonstrated expression of germinal centre markers in some instances. LMP1 positivity was demonstrable in 5 patients with DLBL (1 apparently related and 4 unrelated to the underlying CLL). Of the 2 patients who developed CHL 1 was associated with EBV and lacked CD20 expression. All the patients with EBV+ tumours were heavily pretreated (median prior therapies 4, range 1-5) while the median time from original diagnosis to histological transformation was 74.5 months (range 17-114). Previous therapies included chlorambucil (n= 4), fludarabine monotherapy (n=3), FC (n=2), FCR (n=1), high dose methyl prednisolone (n= 1) and alemtuzumab (n=2). 2 patients received only one line of therapy for CLL and 1 of theses was heavily immunosuppressed with steroids and azathioprine for autoimmune neutropenia. 4 patients presented with nodal disease and 2 patients presented with extranodal disease (bone marrow and liver). The outcome of the EBV associated tumors in these patients was death in 2 patients (opportunistic infection and complications of intensive chemotherapy), remission with intensive combination chemotherapy in 2 patients 1 of whom died 10 months later of progressive CLL, and spontaneous remission of nodal disease in 1 patient. 1

patient is still undergoing intensive chemotherapy and the outcome is not known yet. We would conclude that not all transformation events in CLL occur within the original clone. The majority appear to be clonally distinct and a significant proportion of these appear to be EBV associated. This presumably occurs as a result of both the underlying immune-deficiency seen in CLL as well the potent immunosuppressive agents given as therapy. This phenomenon may also be a feature of other B-cell disorders as we have recently seen a phenotypically and genotypically distinct EBV associated DLBL in a patient treated with chlorambucil, CHOP and FCR for mantle cell lymphoma. The natural history of these tumours remains uncertain at this stage but it is clear that at least a minority may resolve spontaneously. All biopsies demonstrating histological transformation in CLL patients should be assessed for the presence of EBV.

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RH-INCOMPATIBLE PLATELET TRANSFUSIONS AND EVALUATION OF ANTI-D ALLOIMMUNIZATION

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Background. Transfusions of platelet concentrates (PC) are indicated both for the prophylaxis of bleeding and for thrombocytopenic patients. PC can sometimes contain enough red blood cells to cause immunization against antigens of the Rh system, thus Rh D+ PC should not be transfused to Rh D- female patients in child-bearing age. Moreover, the platelet membrane carries numerous structures with varying degrees of antigenic potential, including substances A and B absorbed from the plasma. For these reasons, platelets are transfused according to the following criteria: a) ABO-matched to the recipient; b) according to compatibility of plasma; c) platelets suspended in a crystalloid solution. *Aims*. The aim of our study was to evaluate anti-D alloimmunization caused by transfusions of Rh incompatible platelets, produced from platelet-rich plasma, in both immunosuppressed and immunocompetent subjects. *Methods*. 115 Rh D- patients, with not detectable irregular antibodies prior to transfusion, were studied in Caserta's Hospital and in University. 58 of them were affected with onco-hematological disorders (12 with acute leukemia, 21 with Non-Hodgkin's lymphoma, 15 with myelodysplastic syndrome and 10 with chronic myeloid leukemia in blast crisis); these had undergone chemotherapy for their malignancy and some had received conditioning therapy for a subsequent bone marrow transplant; the supportive therapy was based on transfusion of red blood cell (RBC) concentrates selected for both ABO and Rh compatibility and PC administered regardless of Rh compatibility. The others were 45 surgical and 12 neonatal patients, which received Rh-matched RBC transfusions but unmatched PC. Rh incompatible platelet administration was carried out because transfusion therapy could not be delayed and compatible blood components were not available. However, the blood components for all the hematological patients and for the variably immunosuppressed patients were filtered at the bed-side. The indirect antiglobulin test, by gel-test, was carried out, in all patients, before the first transfusion and repeated 1 month after the last one. *Results*. 562 PC, obtained from single units of whole blood, were transfused. ABO compatibility was respected in only 43% of the cases. On average every transfusion episode consisted of 5/6 units of platelets. No one of the Rh-negative hematological or pediatric patients developed alloimmunization. On the contrary, 3 on 45 surgical patients (6.6%) showed alloimmune antibodies: two anti-D (4.4%) and one anti-E (2.2%) were identified. Alloimmunizations became detectable by laboratory tests 37±4 days after the first transfusion. *Conclusions*. The incidence of anti-D alloimmunization has been reported to range between 0-19% in immunosuppressed patients and to be greater than 80% in immunocompetent subjects. Our data differed from that in literature, in fact we found no cases of alloimmunization among immunosuppressed patients. This is because hematological patients received strongly immunosuppressive chemotherapy and newborns were not immunocompetent yet. Despite the limited sample size, we can conclude that: the risk of alloimmunization from incompatible platelet is low, but does exist; this risk is related with the volume (>0.03 mL) of RBCs contaminating the PC and it might be inversely related to immunomodulation due to massive or prolonged transfusion therapy.

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CHARACTERIZATION OF IG VH4-34 EXPRESSED BY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS IDENTIFIES A SUBSET WITH A VIRTUALLY IDENTICAL HEAVY AND LIGHT CHAIN THIRD COMPLEMENTARY DETERMINING REGION (HCDR3 AND LCDR3)

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Previous studies suggest that the Ig heavy and light chain variable region (VH and VL) repertoire expressed by patients affected by CLL is restricted. Evidence of biased Ig heavy and light chain variable region genes indicates a possible response to a common antigen epitope. To further explore the antigen-driven selection in CLL, we focused on CLL patients expressing the VH4-34 gene. DNA was extracted from peripheral blood mononuclear cells isolated from 126 CLL patients studied at diagnosis at a single institution (patients selected by age less than 65 years, median age: 50±9). PCR amplification was performed using VH family specific and consensus JH primers, followed by automated sequencing. VH4-34 light chains were sequenced using VL and JL primers. The sequences were aligned to Ig sequences from the IMGT, GeneBank and V-BASE databases. The VH family gene usage was the following: VH1 17%, VH2 2%, VH3 45%, VH4 32%, VH5 2%, VH6 1% and VH7 1%. Mutated VH genes (<98% homology to germline sequence) were 74% of the total VH genes rearranged, whereas unmutated VH genes (>98% homology to germline sequence) were 26%. Within the VH4 gene family, the most frequent gene was VH4-34 (56%); only one of the 23 cases expressing VH4-34 was unmutated. The majority of mutated VH4-34 (16/22 cases, 73%) had homology <96%, whereas only six out of 22 (27%) mutated VH4-34 showed homology >96% and <98%. Eighteen of the 23 VH4-34 cases (78%) expressed IgK light chains. The VH4-34 heavy and light chain somatic mutation status was concordant in all cases but one (22/23 concordant cases, 96%). Cluster analysis of the putative translated HCDR3 protein sequences allowed the identification of a VH4-34 CLL subset. This subset comprised 7 cases (30%), all expressing a stereotyped IgKV2-30 light chain, displaying a highly homologous LCDR3 sequence (MQGTHWPWT) associated to a highly homologous HCDR3 sequence (GYPDTAVVKRYYFYGMDV). The HCDR3 sequence length was 17.8±0.3 codons and the LCDR3 sequence length was 11.5±0.5 codons. The expression of the prognostic factors ZAP-70 and CD38 was examined by flow cytometry in the VH4-34 cases: 22 of the 23 cases (96%) were ZAP-70 and CD38 negative. In conclusion, in CLL the VH4-34 subset displaying HCDR3 and LCDR3 restriction is a remarkable indication of Ig selection by antigen(s) and may contribute to the prognostic stratification at the time of disease presentation.

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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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Background. 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 filgrastim is administered from day 0 to day 5 to induce cell cycling and sensitization to chemotherapy, then from day 12 to enhance recovery of neutrophils. PEG-filgrastim 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. After standard chemotherapy for non-myeloid malignancies, one single dose of PEG-filgrastim showed to be equivalent to daily filgrastim in enhancing neutrophil recovery; notably, the single injection was largely preferred by patients (pts). *Aims*. to retrospectively evaluate the hematological recovery and the frequence of infections in MDS and AML pts. after FLA regimens followed by a single PEG-filgrastim injection given at day 12 from the beginning of the chemo cycle. *Methods*. we compared data from two groups of pts treated between 01/1999 and 01/2007. Group PEG: 27 pts; (diagnosis AML/MS 7, AMLMD/tAML 17, MDS/tMDS 3) who received 31 FLA cycles (7 FLA, 24 FLA+Ida), in which PEG-filgrastim s.c. injection was administered at day 12; status before chemo: untreated 15 (48.4%), CR/PR 7 (22.6%), active disease 9 (29.0%). Group G-CSF, 58 pts (diagnosis AML/MS 13,

AMLMD/tAML 27, MDS/tMDS 18) who received 71 FLA cycles, (10 FLA, 61 FLA+Ida) in which unconjugated filgrastim (dosage: 300 mcg/sqm/day) was given from day 12 until neutrophils recovery (>500/mmc); status before chemo: untreated 41 (57.7%), CR/PR 14 (19.7%), active disease 16 (22.6%). Hematological recovery was evaluated only in pts with a documented response to treatment (complete remission, CR, or partial remission, PR). 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.

Table 1.

Results. full results are shown in the Table 1; in one case a second PEG-filgrastim injection was administered at day 32 for delayed recovery. *Conclusions*. our preliminary results suggest that a single PEG-filgrastim injection after FLA regimens is equivalent to daily unconjugated filgastrim; in fact, haematological recovery and incidence of infective/FUO episodes during grade 4 neutropenia resulted comparable in the two groups of pts. Confirmation in a larger population of pts is warranted. Moreover, as the single dose is preferred by pts to daily injections, the overall cost-effectiveness of the PEG-filgrastim formulation could prove favourable.

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HYPEREOSINOPHILIC SYNDROMES (HESS)-CLINICAL PRESENTATION AND VARIABLE RESPONSE TO TREATMENT-A CASE SERIES

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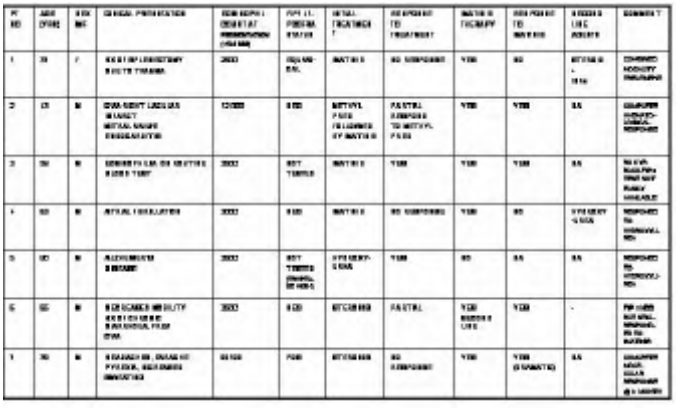
Background. Hypereosinophilic syndromes (HESs) refers to a heterogenous group of disorders characterised by marked blood eosinophilia (>1500/cu mm) and tissue eosinophilia (lasting for more than 6 months),in the absence of other eitiologies for eosinophilia, resulting in end organ damage. Eosinophilias may be a reactive condition or due to a chronic myeloprolifer- ative disorder(with evidence of clonal proliferation).Reactive eosino- philias are due to release of cytokines(IL-3,IL-5,GM-CSF etc) and the common causes are parasitic infections, allergic diseases, vasculitides, drug reactions and malignancies .Clonal eosinophilias are those in which the eosinophilia is a part of a clonal haema- tological malignancy, which is very often associated with the fusion gene FIP1L1-PDGFR α which causes the generation of a constitutively active Tyrosine Kinase.Several visceral complications like cardiomyopathies, nervous system involvement (eg paraparesis, cerebral infarction, eosinophilic meningitis etc) are often fatal illnesses. Treatment modalities for HES includes corticosteroids, chemotherapeutic agents (hydroxyurea cyclophosphamide , vincristine) and α-interferon. Newer treatment modalities including tyrosine kinase inhibitors (eg Imatinib mesylate) and monoclonal anti-IL5 antibodies are now available. Patients carrying this fusion gene respond well to the Tyrosine Kinase Inhibitor,Imatinib. Some patients with HES,that are negative for this fusion gene may also respond to Imatinib, suggesting that in such cases other Tyrosine Kinases may be dysregulated. *Aims.* Retrospective review of the variable response of 7 patients with HES (over a period of 6 months),to current treatment modalities. *Methods*.The 7 patients (6 Male, 1 Female; age range 37-80 yrs; mean age 56 yr) presented with eosinophilia in the range 2600-73,000 /cu mm. A response to treatment was defined as Eosinophil count<1500 /cumm or Eosinophil count < 5% of the total leucocyte count in the peripheral blood. Four of the 7 patients received Imatinib as initial treatment. Two patient initially had steroids (Prednisolone/Methyl Prednisolone) followed by Imatinib and 1 patient (aged >80 yr) was treated with Hydroxyurea initially.

Table 1. HES-Patient Characteristics.



Results. Four of the six patients receiving Imatinib responded to it. Of the 2 patients not responding to Imatinib,1 responded par- tially to Hydroxyurea and the other did not respond to monotherapy with steroid or α-interferon (but eventually responded to a combination of the two). The patient who had initial treatment with Hydroxyurea responded well. Of the 7 patients 1 was positive for the FIP1L1-PDGFRa fusion gene,1 result was equivocal, 3 were negative and 2 were not tested. Of the 2 that were negative 1 responded to Imatinib. *Conclusions*. Thus response of HES patients to the various treatment modalities, is variable and often unpredictable. A trial of Imatinib is worthwhile in all cases,including the FIP1 negative cases.

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CHOICE OF ENDOTHELIAL MARKER IS CRUCIAL FOR QUANTIFICATION OF BONE MARROW MICROVESSEL DENSITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. Angiogenesis is a potential prognostic factor in chronic lymphocytic leukemia (CLL). Elevated levels of angiogenic factors have been found in peripheral blood of CLL patients. However, results of studies assessing bone marrow neovascularization in CLL are controversial, in part due to different antibodies used for immunohistochemical identification of endothelial cells and different methods of assessing microvessel density (MVD). Moreover, there are insufficient data regarding relationship of marrow angiogenesis to prognostic markers in CLL such as clinical stage, pattern of marrow infiltration, genetic abnormalities detected by FISH, or mutation status of immunoglobulin heavy-chain variable-region genes (IgVH). *Aims*. 1. To quantify MVD in bone marrow biopsies from CLL patients and control group using two different monoclonal antibodies and reproducible method of vessel counting; 2. To assess relationship of MVD to most important prognostic factors. *Methods*. We analyzed MVD in bone marrow biopsies from untreated patients with CLL using immunohistochemical staining of endothelial cells with monoclonal antibodies against CD34 (n=22) and von Willebrand factor (vWF, n=18). Control group consisted of 17 biopsies from age- and sex-matched individuals without evidence of malignancy in bone marrow. Microvessel density was assessed under light microscope equipped with image analysis software and calculated using hot spot method, i.e. identification of three loci with highest accumulation of microvessels under low (100x) magnification and counting microvessels in three high-power fields (400x) per hot spot. MVD was expressed as mean number of microvessels per mm^2. CLL cohort was further subdivided according to clinical course (stable vs. progressive), Rai stage (0 vs. I-IV), pattern of marrow infiltration (non-diffuse vs. diffuse), genetic abnormalities (favourable, i.e. no abnormality or del13q14 as a sole aberration vs. unfavourable, i.e. del 17p / del11q / +12), and IgVH mutation status (mutated vs. unmutated). *Results*. MVD was significantly elevated in CLL group in comparison to controls using either antibody (CD34, mean±standard deviation [SD], 75.6±50.6, 95% confidence interval of mean [CI], 53.2-98.1 vessels/mm^2 vs. 47.4±21.8, 95% CI, 36.2-58.6 vessels/mm^2, p=0.039; vWF, 21.3±16.5 vessels/mm^2, 95% CI, 13.1-29.5 vs. 11.5±8.5, 95% CI, 6.8-16.2 vessels/mm^2, p=0.017). However, no sig-

nificant MVD differences were detected between CLL subgroups with regard to clinical course, pattern of marrow infiltration, Rai stage, FISH abnormalities or IgVH mutation status. Interestingly, there was a significant difference between MVD counts according to antibody used: MVD was higher using CD34 vs.vWF in CLL as well as control group (p<0.0001 for both). *Conclusions*. This study supports the hypothesis that microvessel density is significantly elevated in CLL. However, we did not observe significant MVD differences between CLL subgroups with regard to classical or modern prognostic factors. In addition, staining with anti-CD34 resulted in significantly higher MVD counts than with anti-vWF antibody. We conclude that unified and standardized method for neovascularization is needed to allow direct comparison of results between different centers and to elucidat the real clinical significance of bone marrow angiogenesis in CLL.

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INCIDENCE AND CLINICAL CHARACTERISTICS OF IMMUNE THROMBOCYTOPENIC PURPURA IN A COHORT OF MONOCLONAL GAMMOPATHY OF UNCERTAIN SIGNIFICANCE

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Monoclonal gammopathy of uncertain significance (MGUS) may become symptomatic for autoimmune manifestations. Immune thrombocytopenic purpura (ITP) is one of the most common autoimmune manifestations of B-cell lymphoproliferative diseases. Here we report on the prevalence and clinical course of ITP in a consecutive series of 228 patients diagnosed with MGUS. After an observation period of 681.33 patient-years, 7/228 (3.1%) patients with MGUS presented with or developed ITP. The crude incidence of ITP in MGUS was 1028.53 per 100000/year. Based on our results, the crude incidence of ITP observed in MGUS is approximately 150 to 650 fold higher than the crude incidence rate of ITP reported in the adult general population. MGUS patients with ITP displayed a higher age compared to MGUS patients without ITP (median 73 vs 65 years; p=0.027). Accordingly, the incidence of ITP was higher in MGUS patients >60 years (2022.65 per 100000/year; 95% CI 813.2-4167.5 per 100000/year) than in MGUS patients <60 years (0 per 100000/year; 95% CI 0-1100.3 per 100000/year). None of the other clinical characteristics investigated at diagnosis (sex, type and size of MC, Bence-Jones proteinuria, BMPC, albumin, CRP, β-2-microglobulin, Hb, ANC) distinguished MGUS with ITP from MGUS without ITP. The clinical characteristics of MGUS-associated ITP are reported in Table 1.

Table 1. Clinical characteristic of ITP associated with MGUS.

Case	Sex	Age	MC type	MC size (g/dL)	BJ	βMPC %	Clonality	Albumin (g/dL)	B2M (mg/L)	CRP (mg/L)	WBC (x10⁹/L)	ANC (x10⁹/L)	PLT at MGUS (x10⁹/L)	PLT at ITP (x10⁹/L)	Treatment	Status
1	F	70	IgA/L	0.79	-	5	-	3.6	1.5	6.6	7.0	3.6	23	23	-	Alive
2	F	76	IgG/K	1.26	-	6	-	4.2	2.1	4.3	8.4	5.6	190	7	PDN, CTX	Alive
3	M	69	IgG/K	1.59	-	3	-	4.2	1.6	4.0	10.0	6.0	20	20	PDN, CTX splenectomy	Death
4	M	83	IgG/K	0.60	-	5	-	4.7	3.3	4.0	5.6	2.3	84	84	-	Alive
5	M	75	IgG/L	1.38	-	0	-	3,8	2,4	1,0	9,3	5,3	21	21	PDN, CTX rituximab	Alive
6	F	68	IgG/L	1.33	-	7	-	4.8	2.1	8.0	7.8	4.8	93	93	-	Alive
7	F	73	IgM/L	0.40	-	5	-	4.1	2.5	1.0	3.1	1.8	81	81	-	Alive

MC, monoclonal component; BJ, Bence-Jones proteinuria; BMPC%, percentage of bone marrow plasma cells; Clonality, B-cell clonality determined by flow cytometry on medullar blood; B2M, beta-2-microglobulin; CRP, Creative protein; WBC, white blood cell count; ANC, absolute neutrophil count; PLT at MGUS, platelets at diagnosis of MGUS; PLT at ITP, platelets at diagnosis of ITP; PDN, prednisone; CTX, cyclophoshamide

Median age at ITP diagnosis was 73 years. Four patients were female and 3 patients were male. The type of MC was IgG/kappa in 3 cases, IgG/lambda in 2 cases, IgA/lambda in one case, and IgM/lambda in one case. Bence-Jones proteinuria was absent in all cases. One case had polyclonal Ig reduction. Median BMPC infiltration was 5%. All cases tested negative for B-lymphocyte clonality by flow cytometry on BM. Median albumin, CRP, and β-2-microglobulin were 4.2 g/dL, 4.0 mg/L and 2.142 mg/L, respectively. The median PLT count at diagnosis of ITP was 23×10⁹/L. The median Hb and ANC were 14.0 g/dL and 4.8×10⁹/L, respectively. In all cases, liver cirrhosis, portal hypertension and splenomegaly were ruled out by liver function tests and liver and spleen ultrasound. All cases tested negative for HCV and HIV serology, lupus anticoagulant, and anticardiolipin and anti-β-2-glycoprotein I antibodies. None of the patients with MGUS and ITP had clinical evidence of overt lymphoproliferative disorder. Myelodysplastic syndrome was ruled out by BM aspirate and BM biopsy in all patients. After a median follow-up of 22 months (5-70 months) from the diagnosis of ITP, none of the patients with MGUS and ITP progressed to MM, NHL or other lymphoproliferative diseases. One patient died of peritonitis after splenectomy. Six patients are alive: four of them underwent regular clinical follow-up and did not require any treatment for ITP until the last follow up visit at 5, 22, 45 and 70 months from ITP diagnosis, respectively. Three patients required treatment of ITP. Steroid dependency was osbserved in 3 cases. Three cases required second line treatment and two cases required third line treatment. In two cases treatment of ITP resulted in reduction of the MC. Overall, these observations point to an association between MGUS and ITP.

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PRIMARY BREAST LYMPHOMA

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Introduction. Primary non-Hodgkin lymphoma of the breast (PBL) is extremely uncommon accounting for 0.04-0.5% of breast malignancies, 0.38-0.7% of all patients with non-Hodgkin lymphoma (NHL) and approximately 1.7-2.2% of extranodal lymphomas. *Aims*. The aim of this study was to investigate clinicopathologic features and optimal treatment of PBL. *Patients and Results*. We retrospectively analyzed the extranodal NHL cases diagnosed in our department between 1976 and 2006. Four out of 186 (2.15%) patients with extranodal NHL fulfilled the criteria for PBL (3 female and 1 male). Their median age was 52.5 years (range 44-82). Histopathologic examination revealed diffuse large B-cell lymphoma (1 case), follicular lymphoma (1 case), MALT lymphoma (1 case), peripheral T-cell lymphoma unspecified (1 case). Patients were treated according to the histological type of the lymphoma. One patient had excision of the lump only (MALT lymphoma) and the rest three patients received chemotherapy± radiotherapy. All four patients achieved complete remission (CR). One patient died because of a systemic fungal infection 11 months after the diagnosis. Two of the rest three patients relapsed 8 and 21 months after the identification of CR. They received chemotherapy and achieved a second complete remission. They both relapsed 12 months after the second CR and received chemotherapy again. One is now in CR for 24 months while the other is still on therapy. The median overall survival was 43.5 months. *Conclusions*. Primary breast lymphomas although rare should be considered in the differential diagnosis of breast malignancies and treated according to the type of the lymphoma.

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TREATMENT OF MINIMAL RESIDUAL DISEASE WITH SUBCUTANEOUS ALEMTUZUMAB IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Alemtuzumab is a humanized monoclonal antibody that specifically targets CD52, a surface proteins expressed on normal B and T cells as well as on chronic lymphocytic leukemia (CLL) B cells. Alemtuzumab can induce responses in CLL patients that achieved complete response (CR by NCI-WG) but had detectable disease by four-color flow citometry, being designated as minimal residual disease (MRD) positive CR patients. *Aims*. MRD seems to be an important factor for relapse in CLL patients who achieved CR after fludarabine, and clearance of MRD correlates with longer overall survival. Primary objective was to asses response (NCI-WG) in patients treated with alemtuzumab in CR with MRD positive as well as in patients with partial response (PR) with minimal or absent lymphadenopathy with detectable disease limited to peripheral blood (PB) or bone marrow (BM). Secondary objective was to determine the safety and toxicity of alemtuzumab administered via the subcutaneous route. *Methods*. This Phase II study evaluated for response 25 patients who received alemtuzumab as consolidative treatment in CR with MRD and PR. Treatment schedule included alemtuzumab at doses 10 and 30 mg tiw, with dose escalation and premedication with acetaminophen and prednisone. Median dose of alemtuzumab was 269 mg (138-543). Patients received prophylaxis with cotrimoxazole, acyclovir and fluconazole. Patients characteristics are showed in Table 1. 19/25 pts achieved CR with MRD positive after fludarabine therapy when starting alemtuzumab and 6/25 were treated with alemtuzumab after PR.

Table 1.

Age, y (median)	58.6 years
Rai stage	- I/II: 23 pts - III/IV: 3 pts
Cytogenetics	- del 13q: 4 pts - del ATM: 5 pts - +12: 3 pts - bcl2(PCR): 2 pts - normal: 8 pts - NA: 4 pts
CD38 status	-Positive: 15 pts -Negative: 10 pts
Response after fludarabine therapy	-CR with MRD positve: 19 pts -PR: 6 pts

Time to alemtuzumab after fludarabine was 6.3 months. Detection of MRD was performed by four-color flow cytometry with this antibody combination (CD43/CD23/CD19/CD5, CD20/CD79b/CD19/CD5 and kappa/lambda/CD45/CD19) *Results*. 1.-12/19 (60.3%) patients treated after CR with MRD positive cleared MRD and 2/6 patients with PR achieved RC with MRD positive. Responses lasted 6-9 months untill MRD detectable again in PB and 4 patients remain MRD negative after 19.2 months follow-up (range 16-24). 2.- Most common toxicity was transient injection site skin reaction (grade 1-2 in 60%, grade >2: 7%). Other toxicities: fever (grade 1-2:60%), asthenia/fatigue (grade 1:20%), bilirrubin>2.5 normal range (1 pt), anemia and thrombocytopenia (grade 1: 1pt), neutropenia (grade >2: 3 pts). Alemtuzumab was holded in 2 pts (grade 4 neutropenia and severe skin reaction) 3.- Cytomegalocirus (CMV) reactivation ocurred in 7 pts (28%), being early reactivation (within 2 months after alemtuzumab) in 6/7 pts. Half of these pts (14%) had symptoms of CMV infection. No episodes of pneumonitis was observed. Other infectious complications: c. jejunii diarrhea (1 pt) and febrile episodes resolved with antimicrobial (2 pts). 1 patient developed sepsis. 4.- Exitus ocurred in 2 pts (disease progression and sepsis). *Conclusions*. Alemtuzumab is highly effective in clearing leukemia cells from PB/BM, with responses lasting over 6-9 months in most patients. This results support possible role for maintenance with alemtuzumab in this subset of patients, but needs to be addressed with further studies. Infection is the most common cause of morbidity in patients treated with alemtuzumab. Subcutaneous administration offers better toxicity profile compared to intravenous route resulting in a major number of pts eligible for this treatment.

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CHILDREN AND ADOLESCENT ACUTE LYMPHOBLASTIC LEUKEMIA TUNISIA

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Introduction. The prognostic factors in acute lymphoblastic leukemias (ALL) are better and better clarified, which allows to individualise different risk groups and to better adapt the treatments. That is the case of the last version of the OERTC protocol (88951) which we adopted (with certain modifications) in Tunisia. We present in this paper the feasibility of this protocol, the evolution and the survival of the patients treated according to it. *Patients and Methods*. Our study is retrospective. It concerns the patients aged 2 to 20 years, treated since 2000 according to the modified version of the OERTC 588951 protocol. The latter comprises 3 risk groups: 1) mean risk 1 (MR1) : B type LAL , WBC < 100 000/ mm^3 , without t(9,22) nor t(4,11) , corticosensitive, and with complete remission after one course of chemotherapy; 2) mean risk 2 (MR2), the same parameters, with WBC > 100 000 and/or phenotype T. 3) High risk (HR) : one of the following criteria : t (9,22), t (4,11), cortico resistance, no CR after one courseof chemotherapy. A leaflet was designed to contain the data of diagnosis, evolution and survival. Statistical analysis used are the chi 2 test for studying the correlations, the Kaplan Meier method for studying the survival, and the Long-rank test for comparing the survival curves. *Results*. 133 patients are recorded (2000-2003) : 57 are female, 76 male (sex ratio: 1,33), mean age : 9 years , 46% are older than 10 years, WBC > 50 000 in 33% of the cases ; 75% are of cytological type L1 ; 63% are of the B phenotype, caryotype is abnormal in 47% of the cases, with isolated hyperdiploidy in 8% , and structural anomalies in the remaining cases ; a Philadelphia chromosome is present in 3% of the cases. The frequencies of the 3 risk groups 1,2,3 are : 47% , 27% and 26% respectively. A complete remission (CR) is obtained in 123 patients (92,5%), with 6% deaths during induction treatment , and 1,5% failure. A relapse occurred in 26% of the cases. Global survival rate at 3 years for all patients is 63%, with 73%, 58% and 47% for MR1, MR2, and MR3 respectively. The prognostic value was cheked for the following parameters: age (< or > 10 years), sex, presence or absence of tumoral syndrome, WBC number (< ou > 50 000/mm^3), cytological type, phenotype, and risk group. Are of good prognosis: the age (< 10 years) (p= 0,0003), the Hb level < 10 g/dL (p=0,0028), the platelet number : < 50 000/mm^3 (p= 0,025) and the risk group MR1 (p= 0,048). *Comments*. Our series is characterized by an elevated number of hyperleucocytic and T phenotype forms. The number of deaths during induction is high as compared with the numbers reported in western countries. The number of relapses is also high. Although the overall survival in this study is quite superior to that reported in precedent Tunisian studies, but it remains lower than that reported in recent western publications. This difference is most clear in the HR group; in which bone marrow allografting in first CR should be indicated whenever the patient has a familial HL-A identical donor.

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DNA HYPERPLOIDY IS ASSOCIATED WITH BOTH HIGH PLASMA CELL PERCENTAGE & HIGH β2 MICROGLOBULIN AND IMPAIRS SURVIVAL IN MYELOMA PATIENTS TREATED WITH OR WITHOUT TRANSPLANTATION

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Introduction. Chromosomal hyperdiploidy (HD) is the most frequent cytogenetic abnormality in myeloma. HD can be defined by conventional cytogenetics,FISH or flow cytometry(FC). DNA content has been reported by three groups (Tafuri A 1991, J.San Miguel 1996, Mayo group 2006). HD has been associated with favourable, poor or standard risk. Proliferating plasma cells (PC) can also be quantitated by FC. Aim: In this prospective study we aimed to analyze the influence of immunophenotypic phenotypes, DNA content, S phase percentage of PC in addition to ISS, age and 13q del (FISH) on clinical outcome. All multiple myeloma patients diagnosed at our center between 2004-2006 (M/F: 58/32, median age: 60, IgG/A/light: 53/17/11, lambda/kappa: 27/54, B2M: 3.8mg/dL, albumin: 3.4 mg/dL) were included. Patients received VAD(n=53) or MP (n=3) chemotherapy and subsequent autologous stem cell transplantation (n=22). Thalidomide frontline (n:4) or second line (n=21) was also given. Bone marrow aspirates, collected at diagnosis, was used for separation of PC with CD138 positive beads (Clinimacs; Milteny Biotech,UK). The panel for immunophenotyping consisted of CD38, CD138, CD56, CD19, CD28, CD44, CD45, CD117, CD33, CD34, kappa and lambda. Upon enrichment of CD138$^+$ PCs, DNA was stained using the DNA prep reagent kit (Beckman Coulter, USA) . *Results*. S phase fraction was: median 16% (1-58). DNA content analysis revealed normal diploidy(n:27) and HD(n:56) . Comparison of HD vs non-HD revealed: similar ISS stage, albumin, S phase% but higher PC% (12% vs 5%, p=0.05), more patients with >3.5 B2MG (p=0.064), male gender (M/F: 39/17 p: 0.114) and a tendency for advanced stages (p=0.06) in the HD group.There were more patients expressing CD 19 or CD44 or CD28 in the non-HD (p= 0.048, 0.024, 0.01, respectively). Response was similar between HD and Non-HD (21/37 vs 14/22) with a tendency for better response in 13qdel-patients (3/9 vs 25/41). Cox-regression analysis revealed a positive correlation between B2MG and S phase(p= 0.046) B2MG values was higher on HP group (p=0.039), >15% S phase was associated with a tendency for advanced ISS stages (p: 0.127). Kaplan Meier analysis revealed better OS with non-HD (p= 0.004) but not S phase<15 or 13 del. Impact of HD on OS was similar in patients who had or had not received transplantation (p=0.06). *Conclusions*. Compared to published reports, we were not able to confirm the association with good prognosis or kappa monoclonality in HD but were able to confirm the lack of 13q del, CD28 and CD20 expression and male predominance in HD cases (Salamanca). However the poor prognostic role of HD is in accordance with the report from Mayo Clinic. In our study, HD and high S phase% were associated with high B2 MG and tendency for advanced stages. Among the prognostic factors ie ISS, 13q del, S phase%, B2 MG and HD, ploidy was found to be the strongest pre-

dictor of OS (p=0.004). High dose therapy was not able to overcome this poor prognostic effect of HD. FC may be a useful tool in defining high risk patients.

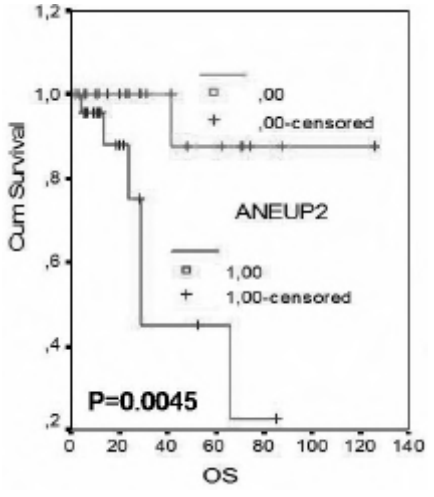

Figure 1. Survival functions.

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DYSPLASIA SCORING SYSTEM FOR ASSESSMENT OF MYELODYSPLASIA IN MDS AND SECONDARY LEUKAEMIA, AND ITS APPLICATION TO THE WHO CLASSIFICATION

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The World Health Organization (WHO) classification extends to the morphologic criteria of the FAB proposal, the recognition of the impact of multilineage dysplasia in myelodysplastic syndromes (MDS). Besides the usual epidemiological, clinical and biological data, register of the Groupe Français des Myélodysplasies (GFM) includes morphologic criteria. A structured image case of representative pictures of haematopoietic cell lineages is recorded for each patient by the diagnostic centre, following a standardized protocol. A multicentric analysis of cytologic features of blood and bone marrow cells is then performed *on line*, at least by 3 other observers. Megakaryocytic, erythroid, and myeloid dysplasia is classified into seven, four, and ten categories respectively, and a score is associated for each morphologic dysplasia criteria. Morphologic dysplasia in blood and bone marrow cells is also analysed according to the WHO criteria. This structured cytomorphological examination of recorded pictures has been performed in 120 MDS patients, from 20 French centres of the GFM. This dysplasia scoring system allowed a more structured and reproducible approach for the precise definition and quantification of dysplastic bone marrow features. Thus, it improved interobserver agreement for the WHO classification of MDS patients, particularly in the subgroups with multilineage dysplasia. Our study, using an *on line* application, confirmed the significance of multilineage dysplasia correlation with high-grade MDS and pointed out links of some dysplasia criteria with WHO classification subgroups or with blood or cytogenetic abnormalities. This suggests that a standardized dysplasia scoring system could improve diagnostic classification of MDS patients and then could help to define standards for evaluations, patients' selection and for the use of targeted drugs in the various subgroups of MDS.

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FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB UP TO FOUR COURSES AS FIRST LINE TREATMENT FOR INDOLENT NON-HODGKIN LYMPHOMAS

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Aims. Evaluate response rate and toxicity associated to fludarabine, cyclophsphamide and rituximab (FCR) up to four courses as first line treatment for indolent Non-Hodgkin Lymphomas (NHL). *Methods*. This Phase II study evaluated patients (pts) with histopathologic diagnosis of indolent NHL with clinical stages III and IV, treated with FCR combination up to four courses. Fourty seven pts were included (37 pts with Follicular lymphoma, 6 pts with Marginal Zone Lymphoma and 4 pts with Lymphoplasmacytic Lymphoma/Waldenström). Median age was 51.6 years (34-78). Treatment schedule: rituximab 375 mg/m^2 day 1, and fludarabine 25 mg/m^2 cyclophosphamide 300 mg/m^2 days 2-4. All pts received prophylaxis with cotrimoxazole, acyclovir and fluconazole. G-CSF (5 g/kg/day) was added if absolute neutrophil count <1×10^9/L in some course. *Results*. 39 pts were evaluable for response. Complete response (CR) rate was 57.9% (23/38) and partial response (PR) rate was 36.9% (14/38) for an overall response rate (ORR) of 94.8%. Median follow-up: 23.5 months (range 6-57 months). Only 1 patient that achieved PR progressed and relapsed during treatment courses. 2 patients did not respond to initial treatment. Most common toxicity (NCI-CTC Common Terminology Criteria for Adverse Events v3.0) was hematologic: neutropenia (53% pts), mostly grade >2, with 6 episodes of febrile neutropenia. Other toxicities: Transient elevation of liver function test (>2.5-5.0 ULN) and transient grade 3 gastrointestinal toxicity (vomiting that required iv fluids). 2 pts experienced zoster reactivation. 1 pt developed grade 3 severe skin reaction after rituximab. Only 3 pts did not complete proposed schema for intolerance to rituximab (infusion related reactions) in some course. 2 pts maintain hematologic toxicity after achieved CR. *Conclusions*. FCR schema achieves significant high ORR (CR+PR: 94.8%) as first line treatment for indolent NHL stages III and IV. This results support possible role for maintenance with rituximab in this pts as intent to prolong responses. This combination up to four courses is relatively well tolerated, thoug significant citopenias were observed, with subsequent delay in treatment courses. Neutropenia was most common toxicity, but it did not result into high rate of infectious complications compared to other schedules that include six corses of this combination with similar ORR.

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METHYLATION STATUS OF THE WNT ANTAGONIST DICKKOPF-1 GENE IN ACUTE LEUKEMIAS

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Background. Wnt proteins regulate development and homeostasis by binding to membrane Frizzled-LRP5/6 receptors. Wnt signaling is through a canonical pathway involving cytosolic β-catenin stabilization, nuclear translocation and gene regulation, acting as a co-activator of T-cell factor (TCF) proteins, and noncanonical pathways that activate Rho, Rac, JNK and PKC, or modulate Ca^{2+} levels. DICKKOPF-1 (DKK-1) encodes a secreted Wnt antagonist that binds to LRP5/6 and leads to inhibition of the canonical pathway. *Aim*. to study the role of methylation of DICKKOPF-1 (DKK-1) in acute leukemia. *Methods*. DKK-1, Wnt antagonist, was studied in untreated 35 AML patient and 78 ALL patient bone marrow and/or blood samples and normal hematopoietic cells by bisulfite modification of DNA, followed by the use of the methylation-specific PCR assay (MSP). This assay was further validated *in vitro* by SSI methylase. *Results*. At diagnosis, Dkk-1 was methylated in 44.8% (30/78) of acute lymphoblastic leukemia. Interestingly, in acute myeloid leukemia (AML) Dkk-1 (an antogonist of WNT5A) gene methylation status was determined to be 52% (16/35). *Conclusion*. Here, we show for the first time that extracellular Wnt inhibitor, the Dkk-1 gene, is transcriptionally silenced by CpG island promoter hypermethylation in acute leukemias.

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EFFECTS OF HUMAN BONE MARROW STROMAL CELL LINE ON THE PROLIFERATION, DIFFERENTIATION AND APOPTOSIS OF HL-60 CELLS

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Introduction. Rapid advances have been made in elucidating the molecular mechanism of etiology and pathogenesis of leukemia, while less attention has been directed toward examining the role of the hematopoietic microenvironment(HM) in the initiation and progression of leukemia. As we know, HM can regulate hematopoiesis through interactions with progenitor cells, hematopoietic cytokines and the biosynthetic products of stromal and other cells. Acute myeloid leukemia(AML) initiates and progresses in the HM. Encounters between the HM and leukemic cells may affect the apoptosis, differentiation and proliferation of leukemia cell. The bone marrow microenvironment is presumed to play an essentially regulatory role in determining the fate of leukemic cells.Objective To investigate the effects of human bone marrow fibrob-

lastoid stromal cell line (HFCL) on the proliferation, differentiation and chemosensitivity of acute myeloid leukemia sensitive HL-60 cell line and multidrug-resistant (MDR) HL-60/VCR cell line *in vitro* co-culture. *Methods*. By setting up co-culture system of HL-60 or HL-60/VCR cells in direct contact with HFCL cells, or with HFCL cells separated by transwell, the cell growth curves were detected by cell counting, cell cycle by flow cytometery(FCM). Cell differentiation was determined by morphologic observation ability of NBT cells and flow cytometric detection of expression of CD11b, CD14, CD13 and CD33.Exposing HL-60 or HL60/VCR cells to different concentrations of topotecan(TPT), morphologic evidence for apoptosis was determined by Wright-Giemsa and Acridine Orange/ethidium bromide(AO/EB) staining. Cell cycle, Sub-G1 and Annexin V FITC staining were detected by FCM. To further study mechanism of HFCL cells on leukemic cells, we compared the gene expression profiles of HL-60 cells without or in direct contact with HFCL cells by Affymetrix GeneChip Human Genome U133 setA. The expression of proliferation cell nucleus antigen(PCNA), active caspase-3, bcl-2 and Pgp was detected by Western blot. VEGF levels were evaluated by using commercial ELISA Kits. *Results*. Compared with leukemic cells alone, the proliferation of HL-60 and HL-60/VCR cells cocultured with HFCL cells was inhibited. And NBT positive cells increased slightly. The percentage of G1 phase cells of HL-60 or HL-60/VCR cells cocultured with HFCL cells was higher than that without HFCL cells, and that of S phase cells was lower. The expression of CD11b and CD14 increased. The expression of PCNA was lower. HL-60 or HL60/VCR cells treated by TPT were observed to have apoptosis characteristic morphological changes by Wright-Giemsa and AO/EB staining. The percentage of Annexin V-positive cells and apoptotic cells decreased when they were cocultured with HFCL cells.The proportion of G0/G1 HL-60 or HL60/VCR cells treated with TPT increased and the sub-G1 was 33.43% or 21.9%, but sub-G1 reduced after in direct contact with HFCL cells. In the study of mechanism, after direct contact with HFCL cells for 96h, the expression levels of 582 genes were up-regulated, and 1,323 genes were down-regulated at least twofold. The expression change in some genes such as HL14, VEGF was comfirmed by RT-PCR, Northern blot and ELISA, respectively. Meanwhile, with treatment with TPT *in vitro*, the expression of activated caspase-3 was reduced and the expression of bcl-2 increased in HL-60 or HL-60/VCR cells by co-culture of leukemic cells in direct contact with HFCL cells. However, the expression of Pgp showed no change. *Conclusions*. HFCL stromal cells could inhibit the proliferation, induce the differentiation of HL-60 and HL-60/VCR cells, and prevent TPT-induced apoptosis in HL-60 and HL-60/VCR cells via modulation of Bcl-2 and active caspase-3. Many genes might take part in the influence of HFCL cells on HL-60 cells, which may give important insights into the interaction of bone marrow stromal cells and leukemic cells.

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MESENCHYMAL STEM CELLS FROM UMBILICAL CORD VEIN, PLACENTA, AND BONE MARROW DEMONSTRATE SIMILAR PHENOTYPE AND IMMUNOSUPPRESSIVE ACTIVITY WHICH IS NOT MEDIATED BY APOPTOSIS

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Introduction. Human mesenchymal stem progenitor cells (MSC) are non-hematopoietic multipotent stem cells that are able to differentiate along several pathways. MSC are widely distributed in a variety of tissues in the adult human body; these cells are also present in the fetal environment. MSC from different sources exhibit very similar but probably not identical characteristics. *in vitro* and *in vivo* studies indicate immunosuppressive activity of MSC. In the present study, our aim was to compare phenotype and immunosuppressive activity of MSC from umbilical cord vein (UC), adult bone marrow (BM) and placenta (P), with a special focus on MSC of fetal origin. *Methods*. Ten placentas were obtained from term deliveries after each mother signed a donation form according to a protocol approved by the Research Ethics Committee of our institution. The MSC from UC vein lumen or homogenized fetal part of the placenta treated with collagenase, and cultured in DMEM supplemented with 15% fetal bovine serum. BM-MSC used as a control. *Results*. Initial results of our study demonstrate the following: 1) MSC can be effectively isolated and grown both from placenta and umbilical cord vein (7/10). MSC cultured from all three sources had indistinguishable fibroblast-like morphology. 2) Similarly to BM-MSC, UC-MSC and P-MSC exhibited characteristic immunophenotype with the expression of CD90, CD166, HLA ABC, CD105, CD166, CD9 and the lack of CD3, CD4, CD8, CD45, CD56, CD117, HLA DR, and Stro1. 3) Immuno-stimulatory activity of MSC was low, and UC-MSC caused comparable mild activation of both allogeneic and autologous lymphocytes (3.9±4.4 vs 2.7±1.5 fold higher cpm compared with controls, ND). 4) All three types of cells demonstrated uniform reduction of 3HT incorporation by PHA-activated lymphocytes in range 40% to 90% (p<0.00004). 5) Using UC-MSC we found that the above immunosuppression is cell dose dependent, in the range 10 to 50×10^3 MSC per well (Spearman rank correlation r=0.833; p=0.003). 6) The immunosuppressive effect was similar with both allogeneic and autologous UC-MSC. 7) Immunosuppression was not mediated by lymphocyte apoptosis. *Conclusion*. As 3rd party fully mismatched MSC can be effective for GVHD treatment, and UC-MSC demonstrate properties similar to BM, these cells can be an effective and pre-fabricated readily available tool for clinical use, e.g. in treating resistant GVHD.

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INTERIM RESULTS OF TREATMENT OF PROMYELOCYTIC LEUKEMIA (APL) IN CHILDREN WITH REDUCED DOSES OF ATRA AND ANTRACYCLINE

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Introduction of ATRA in treatment protocols of APL improved dramatically both short and long-term outcomes in adults and in children, but its side effects are significant. High doses of anthracyclines had been considered essential for achievement of cure of APL treatment, but they produce considerable late cardiotoxicity, especially hazardous in children. We hypothesized that when combined, reduction of dosage of both ATRA and anthracyclines will not result in worsening of treatment outcome . We present the results of prospective study of ATRA-based treatment of APL with reduced doses of anthracyclines (daunorubicin Σ405 mg/m^2) and ATRA (25 mg/m^2 in induction, intensification and maintenance) in children and adolescents. The study is ongoing from 02.2003 in 22 centers of pediatric hematology of Russia and Belarus. Patients: 49 pts (23M/26F) with a median age 10,0 y (range 1,5-18 y) with APL entered into the study. Diagnosis was confirmed by detection of t(15;17) and/or chimeric PML/RAR-α RNA. Median WBC at diagnosis was 4.7×10^9/L (0.9-777.0), Ten pts presented with WBC > 10×10^9/L and platelets <40×10^9/L. Treatment. Induction was-7+3- with conventional doses of AraC+daunorubicin Σ 180 mg/m^2, followed by consolidation with 7+3 (DNR Σ 135 mg/m^2) and intensification with AraC 1 g/m^2 bid x 8 doses + DNR 30 mg/m^2 x 3 doses. ATRA 25 mg/m^2 in was administered from 1-st day of induction course until CR, during intensification and every 3mo by 14-days pulses during the 1-st year of maintenance with daily 6-MP and weekly Mtx till 24 mo from the start of protocol. PCR of BM samples was used for molecular monitoring of PML/RAR-α before every course of CT and in 3 mo interval during maintenance. *Results*. Seven pts died of bleeding within 3 days from admission and are not included in the analysis of efficacy. Of 42 remaining pts one died in induction, and CR was achieved in 41 pts (97,6%). ATRA syndrome developed in 3 pts but was not severe. 4 pts relapsed site of relapse was bone marrow: 2 pts in 1-2 mo after molecular relapse and 2 were not monitored. All relapsed pts belong to the high risk group (WBC >10×10^9/L, delayed achievement of molecular remission). Second remission was achieved in 3 of them with treatment with As_2O_3, one pt is in reinduction course now. With a median follow-up of 36 mo DFS is 0.80±0.09 and OS is 0.95±0.04. One pt died later in remission (accident).*Conclusions*. Excellent DFS and OS were achieved in children and adolescents with APL with treatment according the protocol with chemotherapy with ATRA 25 mg/m^2 and reduced dose of anthracyclines. Molecular monitoring of MRD is warranted during such treatment of APL.

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ALTERATIONS IN THE NATURAL KILLER CELL REPERTOIRE IN MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic Syndromes (MDS) constitute a group of clonal stem cell disorders characterized by ineffective hematopoiesis and pancytopenia. The only curative treatment for MDS is allogeneic stem cell transplantation (SCT). Several studies have shown that NK cells play an important role for the outcome of SCT in patients with myeloid malignancies. These results suggest that NK cells may constitute an important therapeutic tool in the treatment of hematological diseases such as MDS. *Aim*. The aim of this study was to investigate the NK cell repertoire in MDS patients with regard to receptor expression, function and cytogenetic aberrations. *Methods*. Bone marrow (BM) and peripheral blood (PB) was collected from 25 patients of different MDS subgroups and 16 healthy control donors. NK cells were analyzed for their receptor repertoire using multicolor flow cytometry. Interphase fluorescence *in situ* hybridization (FISH) was performed on purified NK cells from patients with 5q- syndrome to detect deletions in chromosome 5. *Results*. MDS patients of all subgroups displayed severe alterations in their NK cell receptor repertoire with decreased expression of several activating NK cell receptors including DNAM-1, NKp46, and CD16. These alterations were confined to BM-derived NK cells and did not affect NK cells in PB. Two patients had abnormally high levels of CD56bright NK cells displaying a reversed ratio between CD56bright and CD56dim NK cells with 75% and 50% regulatory CD56bright NK cells in BM and PB, respectively. Interphase FISH revealed that a subgroup of NK cells in 5q- syndrome patients displayed the cytogenetic anomaly existing in myeloid cells. *Conclusions*. Our results show that MDS patients display several phenotypic aberrations in their NK cell repertoire with a predominant loss of activating NK cell receptors. In patients with 5q- syndrome, the deletion of chromosome 5q was found in a subgroup of NK cells. Altogether, these results may have implications for pathogenesis of MDS and response to immunomodulatory treatments.

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LIPOSOMAL DAUNORUBICIN AND POLYETHYLATED GLYCOL CONJUGATED ASPARAGINASE (PEG-ASPA) IN CHILDREN WITH RELAPSED AND REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED ON COMPASSIONATE BASIS

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Background. Daunoxome (DNX) is an encapsulated form of daunorubicin in liposomal vesicles with suggested better pharmacokinetics, pharmacodynamics and lesser cardiotoxicity than the free form. Polyethyl glycol (PEG) asparaginase (PEG-ASPA), a modified form of L-asparaginase, has a better activity and a lesser immunogenicity than its native molecule. *Aim*. To evaluate on a compassionate basis, the combination of Daunoxome and PEG asparaginase as regard to efficacy and toxicity as a salvage treatment in refractory/relapsed childhood ALL *Methods*. The combination of these 2 drugs has been used in 9 relapsed or refractory ALL children. One child was refractory after 2 lines of chemotherapy, 7 children and one child were in 2nd and 1st relapse respectively. A salvage therapy used DNX on the basis of weekly 3 doses Day (D) 1, 8, 15: 100 mg/m^2/dose. PEG-ASPA single dose 2500 IU/ m^2/dose, D 15. Vinca alkaloids and corticosteroids were associated. *Results*.The median duration of hospitalization was 4 days (0-55). Complications were mainly neutropenia grade IV n=4, grade III infection n = 6, grade II cardiac toxicity n = 1, grade III allergy, grade III hemostasis disorders, thrombosis, n = 1 respectively. All patients achieved CR except one. Eight out of 9 were subjected to hematopoietic stem cell transplantation (HSCT). In total, one patient died before transplant from disease progression and out of the remaining 8 undergoing HSCT. Two patients are alive and well, 4 died from transplant related causes and 2 from disease progression. **Conclusion*.* Salvage treatment of advanced childhood ALL by DNX and PEG-ASPA with steroids and vinca-alcaloids is feasible as regard to toxicity. This small patient sample with very advanced disease suggests an interesting response rate, allowing to proceed to HSCT and possible cure.

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CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHERNOBYL NPP ACCIDENT WITH FOCUS ON IMMUNOGLOBULIN HEAVY CHAIN GENE ANALYSIS

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Some epidemiological data suggest about weak relation between development of chronic lymphocytic leukemia (CLL) and influence of ionizing radiation (IR). Since restricted number of mechanisms should realize assumed influence of IR on CLL development we hypothesized that CLL in IR-exposed patients may be a more homogeneous disease with regard to its immunoglobulin variable heavy chain (IGHV) gene usage and somatic hypermutation status. Using sequence analysis of polymerase chain reaction products, the IGHV gene's configuration was analyzed in 44 CLL patients previously exposed to IR due to Chernobyl NPP accident (32 clean-up workers, 8 inhabitants of radionuclide contaminated areas and 4 evacuees) in comparison with 119 IR non-exposed patients of control group. It was found the prevalence of unmutated cases in both groups (76.2% and 70.6%, respectively). Whereas IR-exposed patients were unselected and the most of them (82%) observed from the beginning of disease, control group was presented by more severe in-patients cases, that was confirmed by such data as shorter period from diagnosis and beginning of therapy (median 7 months in comparison with 24 months in IR-exposed patients; p=0.04), higher initial WBC counts (73.4 and 45.8×10^9/L ,respectively; p=0.007), tendency for worse overall survival (medians 96 and 137 months, respectively; p=0.053). The higher number of unmutated cases was found in clean-up workers of 1986 year (94.1%), who belong to the most suffered contingents due to Chernobyl NPP accident. The distribution of IGHV gene family in IR-exposed patients did not differ from others CLL cohorts in USA and Europe, while in control group increased number of IGHV1 genes usage was revealed. Cases with homologous sequences were found in 28.5% of IR-exposed and 29.4% of IR non-exposed patients that related to subsets #1, 2, 3, 5, 6, 7, 9, 11, 12, 19, 25, 28, 34. Another feature of IR-exposed cases was increased number of the secondary development or coexistence of solid tumors and CLL (7 cases, 15.9%) in comparison with the control group (3 cases, 2.5%). Six solid tumors were diagnosed in clean-up workers of 1986 year. In the whole, 11 IR-exposed patients had solid tumor or Richter transformation (one patient had basal cell carcinoma and then Richter transformation was developed) compared to 8 patients in control group (p=0.001). These preliminary data suggest that CLL in IR-exposed persons, especially in clean-up workers of 1986 year has some distinguish features from CLL in IR non-exposed patients that it is necessary to confirm in more extensive study.

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INFLUENCE OF TREATMENT FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND NON- HODGKIN LYMPHOMA (NHL) DURING CHILDHOOD ON TESTICULAR FUNCTION

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Endocrine abnormalities including gonadal dysfunction are common late effects of anticancer treatment. Chemo- and radiotherapy affect both components of gonadal function: steroidogenesis and spermatogenesis resulting in infertility and sexual dysfunction which may compromise quality of life of the survivors.This problem is well known in patients treated for Hodgkin lymphoma with the use of the alkyling agents and radiotherapy for abdomen; less is known about the patients treated for ALL and NHL. There are also unambiguous opinions on the problem: if the testicular damage after anticancer treatment is similar in boys treated in prepubertal and pubertal stage. In this study we evaluated the gonadal function in young adults after the treatment for hematological malignancies; especially we analysed the values of inhibin B as a marker of spermatogenesis. *Patients and Methods*. Thirty one adolescents at mean age 17.7±2.2 (Tanner IV and V) treated for ALL (n=19) and NHL (n=12) 6.0±3.5years before; twenty of them were treated before puberty (at mean age 7.05±3.33) and eleven-during puberty (14.93±1.5).Thirteen received radiotherapy for central nervous system (CNS) -12 or 18Gy. We evaluated serum levels of inhibin B, testosterone, follicle-stimulating hormone (FSH) and lutenizing hormone (LH) and

compared the values to15 control males at the same age. *Results*. 1. In all group we found lower values of inhibin B than in control (116.85±107.71 ng/mL vs. 196.53 ng/mL±66.8, p=0.02). The patients treated before puberty also presented low levels of inhibin B (111.72±77.26 ng/mL). The values of testosterone, FSH and LH were normal. 2. In patients treated for ALL, the values of inhibin B were lower (91.39±77.26 ng/mL, p=0.0002) than in control and lower than in patients treated for NHL (157.17±137.77 ng/mL). The lowest inhibin B values were found in patients received radiotherapy for CNS (87.26±37.88 ng/mL, p=0.0001). 3. The values of inhibin B lower than 2 SD were observed in 10 patients treated for ALL and 5 patients treated for NHL. Nine were treated before puberty. *Conclusions*. Antileukemic treatment disturbs germinal cell function without influence on steroidogenesis. Prepubertal state does not protect the gonads from their failure by anticancer treatment.

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CD39 EXPRESSION IN T-LYMPHOCYTES IS ABNORMAL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background/Aims. Alterations in the T-lymphocyte population in patients with the B-lymphocyte malignancy chronic lymphocytic leukemia (CLL) have been identified. Early studies noted an increase in the $CD8^+$ subpopulation, leading to an abnormal CD4:CD8 ratio. More recent studies have demonstrated an abnormal response to antigens, increased $CD45RO^+$ cells, and a variety of alterations in CD25 expression in the T-lymphocytes of CLL patients. CD39 (NTDPase-1) is a cell surface molecule that mediates platelet reactivity and immune function via metabolism of ATP and ADP. CD39 is observed on the majority of B-lymphocytes and a T-lymphocyte sub-population, including subsets of both $CD4^+$ and $CD8^+$ cells. CD39 expression is associated with activation and memory cells in T-lymphocytes. We previously demonstrated that CD39 is present and active on the neoplastic cells in CLL. Our observations suggested that CD39 activity on B-lymphocytes decreases as the disease progresses. We now report on changes in CD39 expression on T-lymphocytes in CLL patients. *Methods*. Whole blood from patients with CLL and controls was stained with antibodies against CD3, CD4, CD8, CD19, CD25, CD39, and CD45RO and incubated for 1 hour in dark with shaking. Samples were lysed with FACS lysis solution and washed twice with phosphate buffered sodium (PBS). Cells were resuspended in 0.5 mL PBS and analyzed on a FACS Canto using FACS Diva software. Statistics were derived using Student's 2-way T-test with unequal variances. *Results*. Blood samples from 25 patients with CLL and 23 controls were analyzed. CD39 expression was increased on T-lymphocytes from patients with CLL as compared to controls in all T-lymphocytes (Table 1), in both the $CD4^+$ and $CD8^+$ population (Table 1).

Table 1. CD39 positivity as % all cells of given sub-type.

Parameter	Normals N=23	CLL Pts N=25	P-value (nl vs CLL)
CD3+	5.7%	19.6%	P<0.0001
CD4+	7.5%	21.4%	P<0.005
CD8+	5%	16.4%	P<0.01
CD19+	94%	96.3%	P>0.1

Subgroup analysis of CLL patients showed that T-lymphocyte CD39 expression was higher in patients with Rai stage III-IV vs 0-II disease (13.8% vs 28.2%, p=0.05) and those who had received chemotherapy (12% vs 27%, p=0.01). We found a trend toward decreased CD25 expression in CLL patients versus controls; however, the differences were not statistically significant. The CLL patients had fewer CD3/CD25/CD39 triple positives compared with controls (18% vs 42%, p=0.006). *Conclusions*. T-lymphocyte CD39 expression is increased in patients with CLL. In contrast to B-lymphocytes, increased T-lymphocyte CD39 expression is associated with more advanced disease in CLL. CD39 is associated with activation and memory cells; therefore, the increase in CD39 seen in patients may be due to an increase in memory T-lymphocytes. However, CD39 expression was seen in some CD25-/CD45RO- T-lymphocytes in patients, where it is rarely observed in controls, suggesting that some of the CD39 expression seen in T-lymphocytes may be aberrant. It has been reported that secretion of IL-2 requires extracellular ATP. High CD39 expression may decrease extracellular ATP thereby decreasing IL-2 secretion from T-lymphocytes. This could explain the previously reported observation that IL-2 secretion is decreased in a mixed lymphocyte reaction of CLL T-lymphocytes and the CD25 aberrations seen in CLL patients. These findings provide further support for the thesis that T-lymphocyte abnormalities are involved in the pathogenesis of CLL and suggest further avenues of research into the etiology of CLL.

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DIFFERENT METHODS FOR ESTIMATION OF ANGIOGENESIS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Angiogenesis is an essential component in the growth and progression of myeloma plasma cells. Different methods were used to estimate the intensity of angiogenesis in patients with multiple myeloma (MM). *Aims*. The purpose of the study was to establish the intensity of angiogenesis in patients with MM and to compare two methods for angiogenesis estimation. In addition, clinical significance of angiogenesis in patients with MM was particularly analyzed. *Patients and Methods*. We analyzed bone marrow biopsy specimens obtained from 59 patients with *de novo* MM. The clinical staging was done according to the Durie and Salmon classification (four patients had disease stage I, 16 patients stage II and 39 patients stage III). Bone marrow angiogenesis was analyzed using standard imunohistochemical analysis of B5-fixed and routinely processed, paraffin-embedded bone marrow specimens with antibody against CD34. Bone marrow angiogenesis was estimated by two different methods. Microvessel density (MVD) was estimated by counting number of microvessels in three *hot spots* at magnification x400, according to the method of Weidner *et al.* (N Engl J Med 1991; 324:1-8). Semiquantitative estimation of angiogenesis was based on visual assessment of slides at magnification x100, according to the method of Rajkumar *et al.* (Clin Canc Res 2000; 6:3111-16). Each slide was assigned as low, intermediate or high angiogenesis intensity. *Results*. Median MVD was 15 (range: 1-89). Intensity of angiogenesis estimated by semiquantitative method was assigned as low in 24 (40.67%) patients, intermediate in 17 (28.81%) patients and high in 18 (30.50%) patients. There was a statistically significant association between MVD and semiquantitatively estimated intensity of angiogenesis (p<0.001). Significant correlation between intensity of angiogenesis and histological grade, the extent of bone marrow infiltration, proliferative activity of myeloma cells and poor survival was found. *Conclusions*. The intensity of angiogenesis can predict clinical outcome in myeloma and be helpful in choosing optimal therapeutic modality in every individual patient. Since significant correlation between intensity of angiogenesis estimated by semiquantitative method and MVD, simple semiquantitative method can be recommended for daily clinical practice, as an alternative and reliable method for complicated and time consuming MVD estimation.

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CYTOGENETIC ABNORMALITIES IN 350 PATIENTS DIAGNOSED WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND RELATIONSHIP WITH IGVH MUTATIONAL STATUS

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Background. In the last years, a better characterization of the biology of CLL based on molecular studies has been ascertained. Thus, the presence of some chromosomal abnormalities and IgVH mutational status identify patients with different clinical and prognostic course. *Aims*. To determine by FISH the most prevalent cytogenetic aberrations of CLL-B. To correlate the results with the mutational status of the immunoglobulin VH genes. To analyze survival and time to the first therapy. *Methods*. A total of 350 patients diagnosed with CLL-B (233 male; ratio: 2,0) were included. Median age was 65 years (range: 29-90). Median WBC: 20×10^9/L (range: 5.3-370). LDH > 1n = 13.4%. β2microglobulin > 1n = 33.7%. Diffuse bone marrow pattern: 28.3%. Binet A stage: 79% of patients. In FISH study, specific probes of 13q14, +12, 11q21, 14q32 and 17p13 were used. Mutational status was performed in 155 cases. Unmutated cases were considered when a VH homology ≥ 98% was present. *Results*. A total of 235 patients (66.2%) presented with, at least, a cytogenetic abnormality: del (13q): 156 pts (45.6%), +12: 39 pts (11.3%), del (11q): 28 pts (8.3%), del (17p): 15 pts (4.4%), IgH rearrangements: 18 pts (6.2%) (with BCL1/IGH fusion negative in all cases), others: 3 pts (< 1%). In 48 cases (13.7%), ≥2 cytogenetic aberrations were observed. Eighty-two patients showed a mutated pattern (52.5%). Median follow-up was 38 months. The variables related to shorter survival in the univariate analysis were: del(11q), del(13q), del(17p), unmutated status, sex (male), Coombs test+, high LDH, high β2microglobulin, splenomegaly, advanced Binet stage, diffuse pattern and CD38+. By contrast, in the multivariate analysis only a high β2microglobulin level and del(17p) were associated with a poor survival. Del(11q) or del(17p) were associated with an unmutated status or CD38+, while cases with del(13q) had a mutated IGH or CD38-. Median survival times (in months) of the different cytogenetic categories were: del(17p): 50 m, del(11q): 74 m, +12: 99 m, del(13q): 154 m, and normal karyotype: not reached. Survival of mutated patients was better (p=0.018) (median survival time nor reached in both groups). The multivariate analysis showed the following factors as negatively related to the survival (Odds Ratio): del(17p) (6.83), del(11q) (2.69), del(13q) (0.26), IGH mutation (0.24), while the parameters influencing the time (in months) to first therapy were: del(17p): 3 m, del(11q): 10 m, +12: 26 m, normal karyotype: 65 m, del(13q): 87 m; unmutated cases: 22 m, mutated cases: 107 m. Mutation status, Binet stage, diffuse pattern and p53 status were selected in multivariate analysis. *Summary*. The associations between del(13q)/mutated status and del(11q) or del(17p)/unmutated cases were confirmed. The presence of IGH abnormalities (6.2%) supports the inclusion of this parameter at the diagnosis of CLL patients. Overall survival and therapy free interval were significative related with the genetic abnormalitites of CLL.

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AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION AS AN UP-FRONT THERAPY IN MULTIPLE MYELOMA: THE LEBANESE EXPERIENCE

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Background. Autologous stem cell transplantation (ASCT) has become the gold standard therapy for young patients with multiple myeloma. *Aims and Methods*. We report the results of ASCT used as up-front therapy in 71 patients with stage III (Durie-Salmon) multiple myeloma autotransplanted between March 1997 and October 2006 in the two major bone marrow transplantation units in Lebanon. *Results*. Median age of the patients was 49 years (29-70). There were 49 males and 22 females. Twelve patients (17%) were in stage IIIB. IgG, IgA and light chain were the most frequent types (58%, 21% and 17% respectively). Sixty-six patients (93%) received VAD protocol (3-6 cycles) and 4 patients (6%) received thalidomide + dexamethasone. Seventy patients received autologous peripheral stem cell transplantation (APSCT). Cyclophosphamide (4-5 g/m^2) followed by G-CSF (10μg/kg/day) were given for peripheral stem cell mobilization. The median number of CD34+ cells collected was 10.24×10^6/kg (1.36-60). High dose chemotherapy consisted of Melphalan which was given at a dose of 200 mg/m^2 in 60 patients (85%); the others received Melphalan at a dose of 140 mg/m^2. Fifty-nine patients (83%) underwent one ASCT and 12 patients (17%) underwent tandem ASCT. The median number of CD34+ cells transplanted was 5.79×10^6/kg (1.36-20) per transplant for the patients who underwent APSCT. Bisphosphonates were given IV to all patients for about 2 years after ASCT. Response was assessed according to the EBMT criteria (Blade J. *et al.*, Br J Haematol, 1998. 102: 1115-1123). Disease status before ASCT was as follows: 8 patients (11%) were refractory, 4 patients (6%) had minimal response, 54 patients (76%) had partial response and 5 patients (7%) were in complete remission (CR). Transplantation-related mortality rate was 1.4%. Twenty-one patients (30%) were in CR after ASCT. Thirty-six patients (51%) who relapsed or progressed after ASCT received thalidomide±dexamethasone (30 patients), a second ASCT (4 patients) and Velcade (2 patients). The median overall survival (OS) and the median progression-free survival (PFS) were 60 and 32 months respectively (Figure 1). *Conclusion*. The feasibility of APSCT for multiple myeloma in Lebanon was acceptable with an OS and PFS rates comparable to the EBMT registry.

Figure 1. Overall survival curve.

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THE EXPRESSION OF MDR1 PROTEIN IN DE NOVO ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE

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Introduction. The normal karyotype is detected in 40-45% of adults with acute myeloid leukemia (AML). These patients belong to an intermediate cytogenetic group. The additional factors are needed to predict the therapeutic outcome. The well established risk factor for treatment failure in patients with AML is expression of MDR1 (Multidrug Resistance 1) gene in leucemic blasts, that encodes a 170-kd transmembrane glycoprotein, known as P-glycoprotein (P-gp). The aim of this study was evaluation of MDR1 expression significance as a factor discriminating prognostic subgroups in AML with normal karyotype. *Material and methods*: One hundred and twenty one patients (pts: 60 males and 61 females; ranged from 18 to 82 years-mean 51.2) were included in the study. They were treated with induction therapy acc. Polish Adult Leukemia Group (PALG) programme. Cytogenetic analysis was performed on bone marrow aspirates by tripsin-Giemsa banding technique and the ambiguous cases were additionally analysed by FISH method with using probes for: t(15;17), t(8;21), inv(16), t(v, 11q23), aberrations of chromosome 5, 7, 8 and 20. MDR1 (Pgp-170) expression was measured by labelling fresh viable bone marrow cells with UIC2 PE monoclonal antibody (Immunotech) and analysis in FACSCalibur flow cytometer. *Results*. Among 121 pts, 109 (90.1%) had information from a cytogenetic analy-

sis. There were 30 pts with good, 70 pts with intermediate and 9 pts with unfavorable prognosis. Among 70 pts with interemediate risk cytogenetics, 67 pts had normal karyotype. The expression of P-gp in the whole group was from 0.2% to 84.3% (mean: 14.5% ±16.1). Pts with unfovorable prognosis had higher Pgp expression (24.4%, SD=21.3) than pts with interemediate (15.6%, SD=15.7), and pts with good risk cytogenetics (10.7%, SD=14.4). Using the Student's t-test there was statistical significant difference in MDR1 expression between unfovorable and good risk group (p=0.033). The statistical analysis showed significant differences (p<0.05) between P-gp expression in pts with inv(16) (p=0.01), t(8;21) (p=0.001), t(15;17) (p=0.012), normal karyotype (p=0.006) and *the rest of aberrations*, mostly with unfovarable changes. The expression of P-gp in the group of pts with normal karyotype was 14.485%. Seventeen pts obtained CR after one or more induction therapy, but 38 pts never had CR. Using the Student's t-test there was statistical significant difference in MDR1 expression between pts with CR and pts without CR (p=0.011). *Conclusions*. Our results confirm the negative role of MDR1 expression in patients with AML and normal karyotype, especially because of influence on an achievement of CR and entitles us to introduce the proposition of individualization of induction therapy.

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HEMATOGONES AFTER INDUCTION THERAPY FOR ACUTE MYELOBLASTIC LEUKEMIA (AML) PREDICTS RELAPSE AND OVERALL SURVIVAL

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Background. Treatment of acute myeloid leukemia (AML) is a therapeutic challenge due to relapsing disease in a significant number of patients. New prognostic parameters are needed to allow assignment of patients to a high or low risk of treatment failure. Hematogones (HGs) are normal B-lymphocyte precursors found in bone marrow of healthy subject. HGs decrease with age and in case of bone marrow involvement with neoplastic cells. Increased HGs have been described in patients with lymphoma, marrow regenerative states, immune cytopenias, and acquired immunodeficiency syndrome. *Aims*. To evaluate the prognostic impact of HGs on leukemia free (LFS) and overall survival (OS) in patient with AML. *Methods*. Consecutive AML patients with first complete remission (CR) were included between 1999 and 2006. Fresh bone marrow specimens were processed by Ficoll Hypaque density gradient and analyzed for HGs by four-color flow-cytometry. HGs can be easily identified with four-color flow cytometry with coexpression of CD10/CD19 and low intensity of CD45 expression. The presence of HGs was defined with a cut-off of 0.1% of the total number of cells. Each prognostic variable was compared in terms of LFS and OS by log rank test and Kaplan-Meier curves. Multivariate analysis was performed with a Cox model.

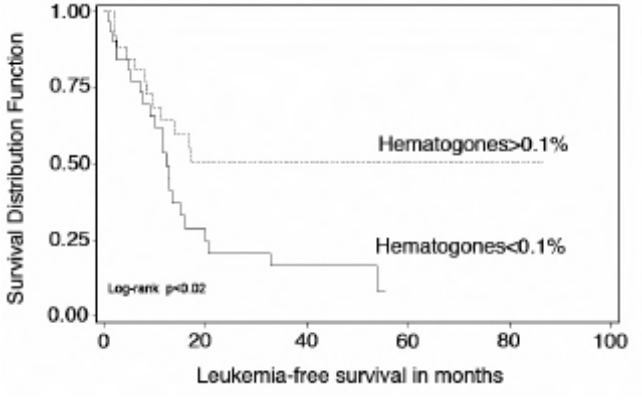

Figure 1. Leukemia free survival.

Results. 63 patients were included with median age of 57 years (range: 16-78 years). The median follow up was 19 months (range: 1-88 months). AML have been classified according to the French-American-British (FAB) classification : M0 : n = 4, M1 : n=13, M2: n=16, M3 : n=3, M4 : n=11, M5 : n=9, M6 : n=4 , unclassified : n=3. Pre-treatment cytogenetics were available for 61 out of 63 patients. According to Medical Research Council (MRC) classification, favourable, intermediate and unfavourable prognosis were found in 7 (11%), 38 (62%) and 16 patients (26%), respectively. Mutation of FLT3 was found in 11 of 55 patients (20%). The median LFS and OS were 14 and 24.6 months, respectively for the whole group. 31 patients (49%) died most of the time because of relapse. In univariate analysis age (″50), favourable cytogenetics and HGs>0.1% were associated with improved LFS and OS. AML patients with HGs in marrow sample in first CR have significant higher LFS (p<0.02) and OS (p<0.03) than AML patients without (Figure 1). Furthermore predictive value of HGs for a better LFS and OS is still significant, when adjusted for cytogenetics, in a multivariate analysis (p<0.03). *Conclusions*. Presence of HGs in first CR with a cut-off of 0.1% can be a useful tool to predict relapse and survival in acute myeloblastic leukemia. Further studies are clearly required to confirm these data in larger patient cohorts.

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TOXIC CARDIAC INTERACTION BETWEEN ARSENIC TRIOXIDE AND ANTIFUNGAL AZOLE: ANALYSES IN CHICK EMBRYOS AND IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Aims. Arsenic trioxide (ATO) has cardiac adverse effects including prolongation of the QT interval and arrhythmias in patients with acute promyelocytic leukemia (APL). On the other hand, some authors reported prolongation of the QT interval and torsades points (TdP) associated with fluconazole (FCZ) and other antifungal azole agents, which are sometimes used concomitantly with ATO. We previously reported a patient with APL in whom TdP developed during treatment with ATO concomitantly with FCZ. In this study, we tried to clarify the toxic cardiac interaction between ATO and antifungal azole using the chick embryo model and in patients with APL. *Methods*. The chick embryonic heart, which has a similar atrioventricular system to that in humans, has been used in pharmacologic and toxicologic experiments. ATO (0.25 mg-1.0mg/egg) was injected with or without FCZ (0.4 mg/egg) into the air sac of each fertilized egg on the 16th day of incubation. Five hours after injection of each drug alone or in combination, the heart rate (HR) was measured by electrocardiograms (ECG). In the clinical study, we analyzed 38 patients treated with ATO. All 38 patients had relapsed after previous extensive therapy with ATRA and chemotherapy. All patients were hospitalized while receiving ATO, which was administered daily for a maximum of 60 days. Fifteen patients were concomitantly administered an antifungal azole (Azole group), and the remaining 23 patients were not administered an azole (Non-azole group). Patients were continuously monitored by ambulatory ECG. The QT intervals were calculated in the standard 12-lead ECG weekly. *Results*. In the study on chick embryos, the HR after administration of ATO 0.25 mg/egg or FCZ 0.4 mg/egg did not differ from that of the untreated controls. Upon injection of larger doses of ATO (>0.5 mg/egg), the HR significantly decreased in a dose- and time-dependent manner (p<0.05). The HR also significantly decreased upon administration of ATO 0.25 mg/egg with FCZ 0.4 mg/egg (p<0.05) in a time-dependent manner. In addition, arrhythmia was produced by the combined treatment. In the clinical study, prolongation of the QT interval was observed in 37 of the 38 patients. The degree of prolongation did not significantly differ between the Azole group and Non-azole group. The frequency of ventricular premature contraction (VPC) was increased in 69.6% (16/23) of the cases in the Non-azole group and in 73.3% (11/15) of the cases in the Azole group. Short run or ventricular tachycardia was observed in 43.5% of the cases in the Non-azole group and in 46.7% of the cases in the Azole group. TdP was observed in one case in each group. *Summary*. Although the exact mechanism of the interaction of these drugs was not clarified, toxic cardiac interaction between ATO and FCZ was observed in chick embryos. We could not confirm the toxic interaction in the clinical cases. One of the reasons might be due to the heterogeneity of APL cases, which includes the dose of anthracycline previously administered, levels of electrolytes and other drug interactions. Our study indicates that we should still pay attention to the concomitant usage of these drugs.

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CONSTITUTIVE ACTIVATION OF STAT1 AND STAT3 PROTEINS WITHOUT INTERFERON INDUCIBILITY: RELEVANCE TO THERAPY IN IGVH-UNMUTATED CLL PATIENTS

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Background. STAT proteins play an important role in regulation of proliferation, differentiation, apoptosis, and immune response. STAT proteins are constitutively activated in hematopoetic cells, which are transformed by oncogenic tyrosine kinases and are also connected to pathology of variety leukemias and lymphomas. Tyrosine phosphorylation of STAT proteins allows formation of dimers, translocation to the nucleus and binding to specific DNA sequences within promoters of target genes, which leads to activation of transcription. Serine phosphorylation is not necessary to activate STATs, but it modulates gene activation induced by tyrosine phosphorylation. Constitutive phosphorylation of serine but not tyrosine in both STAT proteins was found in B-CLL cells. *Aims*. The aim of our study was to examine defects in STAT1 and STAT3 pathways in our CLL patients. *Methods*. We analyzed defects in STAT1 and STAT3 pathways after stimulation of cells with interferon γ (IFNγ) (10 ng/mL) and interferon α (IFNα) (5000 IU/mL). We evaluated 59 primary cultures derived from CLL patients with prevalency of B-CLL cells. Presence of STAT1 and STAT3 protein isoforms was detected by Western-blotting analysis using specific polyclonal and monoclonal antibodies. *Results*. Constitutive phosphorylation of serine and tyrosine residues of both STAT proteins was found in majority of CLL patients. STAT1 protein showed higher ratio of defect in phosphorylation of serine (69,6%) and tyrosine (43,5%) after induction by interferons compared to phosphorylation of serine (15,8%) and tyrosine (50%) of STAT3 protein. IFNα is more effective inductor of both phosphorylations in STAT1 and STAT3 proteins. Only tyrosine phosphorylation of STAT1 protein was markedly induced by IFNγ. Analysis of relationship STAT proteins and IgVH-mutation status was performed. We did not found significant differences in phoshporylation of STAT1 and STAT3 proteins. IFNα induced only tyrosine phosphorylation of STAT1 protein in IgVH-unmutated patients compared to IgVH-mutated patients where the phosphorylation was induced by both IFNs. Analysis of IgVH-unmutated patients who required therapy showed higher proportion of constitutive phosphorylation without IFN inducibility in both STAT proteins compared to untreated patients. Consequently, the significant difference was found among IgVH-unmutated patients with or without therapy. Patients with therapy showed markedly higher proportion of constitutive phosphorylation without IFN inducibility of serine and tyrosine residues in STAT1 and serine residue in STAT3 proteins. On the contrary, these patients manifested a decrease of constitutive phosphorylation without IFN inducibility on tyrosine residue in STAT3 protein. *Conclusions*. Our results show that majority of CLL patients have constitutive phosphorylation of both STAT proteins. IFNα was shown to be a more effective inductor in comparison with IFNγ. Presence of constitutive phosphorylation without IFN inducibility of STAT1 and STAT3 proteins is increased in IgVH-unmutated CLL patients who required therapy.

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CD28 AND ICOS GENE POLYMORPHISMS ARE ASSOCIATED WITH BCELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background. There are strong evidences that altered immunological function entails an increased risk of B- CLL. T cell-specific surface receptors CD28 and *inducible co-stimulator* (ICOS) are main costimulatory molecules expressed on cell surfaces and provide regulatory signals for T-cell activation. CD28 potently enhances T-cell functions essential for effective antigen-specific immune responses. Unlike the constitutively expressed CD28, ICOS is induced on the T-cell surface and does not upregulate the production of IL-2, but induces the synthesis of IL-4. *Aim*. The extended study was undertaken to evaluate the association between the T17int3C CD28 gene polymorphism, microsatelite (GT)n polymorphism in intron 4 of the ICOS gene and susceptibility to B-CLL in a Polish population. *Methods*. One hundred twenty four B-CLL patients and 202 healthy subjects were studied. Allele identification was achieved by PCR amplification. The amplified product for SNP (single-nucleotide polymorphism) loci was purified and minisequenced using the commercial kit SnapShot of PE Applied Biosystems. The dinucleotide repeat polymorphism was studied by PCR and fuorescence based technique. The products were analyzed on the ABI PRISM 310 Genetic Analyzer (ABI PRISM 310 capilary elctrophoresis system). *Results*. The distribution of the CD28IVS3+17C/T alleles and genotypes presented significant differences in B-CLL patients and healthy controls. The presence of CD28IVS3+17C allele and CD28IVS3+17(C/C+C/T) genotype increased the odds of B-CLL by 1.97 and 2.07 [p=0.0019, 1.2755~95%CI ~3.0313 and p=0.0033, 1.2686~95%CI ~3.3778, respectively]. Global distribution of ICOS alleles was significantly different in B-CLL patients and healthy controls (p= 0.000005). The results showed that (GT)9 allele increased risk of B-CLL 2,3 times [p=0.0294, OR =2.345, 1.0700~95%CI~5.1419] while frequency of the (GT)12 was significantly decreased in patients [p=0.0002, OR =0.4307, 0.4307]. Moreover, distribution of genotypes differed between patients and healthy controls (p=0.0076). The GT9/GT11 genotype was overexpressed in B-CLL patients compared with control subjects (p=0.03, OR= 2.3457, 1.0700~95%CI ~5.1419), while genotypes GT12/GT12, was more frequent in control subjects [p=0.0024, OR=0.0831, 0.0195~95%CI ~0.6306] *Summary*. Our results suggest that the CD28 T17int3C gene polymorphism and microsatelite ICOS gene (GT)n polymorphism contribute to genetic susceptibility to B-CLL

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TRYPTASE AS NOVEL TUMORMARKER IN CLINICAL HEMATOLOGY: EVALUATION OF 1041 PATIENTS

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Background. Tryptase is a term used for a family of trypsin-like serine proteases primarily produced and stored in mast cells. Recent data suggest, that tryptase is expressed in neoplastic cells in various myeloid neoplasms. The aim of the present study was to determine whether tryptase may serve as a marker of disease in patients with hematologic neoplasms. *Aims*. The aim of this study was to determine the diagnostic and prognostic significance of tryptase in patients (pts) with hematologic neoplasms. *Methodes*. Tryptase levels were determined in 906 pts with hematologic malignancies, including myeloproliferative disorders (MPD, n=153), myelodysplastic syndromes (MDS, n=233), acute myeloid leukaemia (AML, n=317), and systemic mastocytosis (SM, n=71), and in 137 pts with reactive leukocytosis/thrombocytosis or idiopathic cytopenia, by fluoroenzyme-immunoassay. Moreover, tryptase was analyzed in 165 healthy subjects, 80 pts with non hematologic disorders. *Results*. For this purpose, serum tryptase levels were determined in 906 patients (pts) with hematologic malignancies, including myeloproliferative disorders (MPD, n=153), myelodysplastic syndromes (MDS, n=233), acute myeloid leukaemia (AML, n=317), and systemic mastocytosis (SM, n=71), and in 137 pts with reactive leukocytosis/thrombocytosis or idiopathic cytopenia, by fluoroenzyme-immunoassay. Moreover, tryptase was analyzed in 165 healthy subjects, 80 pts with non hematologic disorders. In 90% of healthy controls serum tryptase levels ranged between 2.4 ng/mL and 9.5 ng/mL (median: 5.7 ng/mL; range in all pts: >0.1-18 ng/mL). Among pts with non hematologic disorders, slightly elevated serum tryptase levels (up to 24 ng/mL) were detectable in 6/18 pts with severe renal failure and in 3/29 pts with helminth infections. In the majority of pts with reactive leukocytosis/thrombocytosis or idiopathic cytopenia serum tryptase was within the normal range (″15 ng/mL), as were most pts with lymphoid neoplasms. Among myeloid neoplasms, elevated tryptase levels were recorded in 83% of the pts with SM, 37% of pts with AML, 34% of the pts with CML, and 24% of the pts with MDS. The highest tryptase levels were found in pts with SM or AML-M4eo. In pts with CML treated with imatinib, elevated tryptase levels were found to be associated with an unfavourable prognosis concerning survival (tryptase ″15 ng/mL: 100%; tryptase >15 ng/mL: 71%, after 60 months, p<0.007). In AML with intermediate karyotype, an increased serum tryptase was associated with an increased risk of relapse. In most pts with AML, SM, or CML, the initially enhanced serum tryptase levels decreased in response to successful cytoreductive therapy. In AML, a persistently elevated serum tryptase despite complete hematologic remission was also found to be associated with an increased risk of relapse. *Summary/conclusions*.Tryptase is a new and simple diagnostic marker of myeloid neoplasms. In SM, AML, and CML, tryptase is also a useful marker to monitor (minimal

residual) disease during treatment. Moreover, in AML and CML, measurement of tryptase is of prognostic significance. All in all, tryptase is recommended as a new important serum-marker in clinical hematology.

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RELATIONSHIP OF SERUM FREE LIGHT CHAIN LEVELS AND SELECTED BIOLOGICAL PARAMETERS IN PATIENTS WITH MULTIPLE MYELOMA

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Backround. The presented study is focused upon the evaluation of relation of serum free light chain levels (FLC) kappa, lambda and their ratio (K/L ratio) and levels of selected biological parameters in group of patients with multiple myeloma assesed at the time of diagnosis. *Methods*. Prospective study included 102 patients with multiple myeloma, examined during two year period. Serum FLC levels were assesed using Freelite Immunotech system, for the evaluation of serum levels of analysed parameters were used following methods: radioenzymatic assay (thymidinekinase), radioimmunoanalysis (β-2-microglobulin, ICTP, PINP), enzymoimmunoassay (sIL-6R, sVCAM, sICAM-1, sOPG) and quantitative enzymatic immunoassay (sHGF, sVEGF, syndecan-1/CD138 and sFas). Spearmanãs test was used for statistical evaluation. Kappa and lambda secretion were evaluated separately. *Results*. In kappa group was found a significant correlation between serum levels of dominant chain kappa and serum values of ,-2- microglobulin (r=0,344, *p*=0,005), thymidinekinase (r=0,263, *p*=0,035), ICTP (r=0,402, *p*=0,001), PINP (r=0,264, *p*=0,039), sOPG (r=0,328, *p*=0,028), syndecan-1/CD138 (r=0,255, *p*=0,046) and sFas (r=0,418, *p*=0,001). Also significant correlation between K/L ratio and levels of ,-2-microglobulin (r=0,316, *p*=0,01), thymidinekinase (r=0,274, *p*=0,027), ICTP (r=0,346, *p*=0,006), PINP (r=0,261, *p*=0,042), syndecan-1/CD138 (r=0,283, *p*=0,026) and sFas (r= 0,377, *p*=0,002) was found. In lambda group correlation analysis revealed a mutual relationship between serum levels of dominant chain lambda and ,-2-microglobulin (r= 0,476, *p*=0,003), ICTP (r=0,375, *p*=0,022), sVCAM (r= 0,383, *p*=0,019), sHGF (r=0,441, *p*=0,006) and sFas (r=0,334, *p*=0,040). Correlation of K/L index was found to ,-2-microglobulin (r=-0,473, *p*=0,003), thymidinekinase (r=-0,412, *p*=0,011), ICTP (r=-0,331, *p*=0,045), PINP (r=-0,409, *p*=0,012), sHGF (r=-0,357, *p*=0,028), syndecan-1/CD138 (r=-0,449, *p*=0,005) and sFas (r=- 0,371, *p*=0,022). There was found no relation between alternative light chains and analysed parameters in both groups. *Conclusions*. The above study showed a correlation of serum free light chain levels and serum values of some selected biological parameters, especially ,-2-microglobulin, thymidinekinase, ICTP, PINP, syndecan-1/CD138 and sFas at the time of diagnosis. It confirms close relation between bone marrow microenviroment, biological properties of clonal plasma cells and intensity of free light chain production. K/L index seems to be more sensitive, than only kappa or lambda chain serum levels.

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ISOFLAVONES: EFFECTS ON THE COAGULATION AND FIBRINOLYTIC SYSTEM AND LIPID PROFILE IN POSTMENOPAUSAL WOMEN

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Background. The incidence of cardiovascular disease (CVD) is associated with unfavorable alterations of the lipid profile and haemostasis, proceeding from decrease of the estrogenic activity in postmenopausal women. Hormonal Replacement Therapy (HRT) has been much debated because some studies show HRT increases breast cancer predisposition and it does not protect against cardiac diseases. Attempting to reach treatment to menopause symptoms, minimizing side effects, we suggest isoflavone, a natural compound extracted from soy (Glycine max) protein that has a effect estrogen like. *Aims*. To evaluate the effects of the isoflavone on haemostasis and lipids levels in healthy postmenopausal women. *Methods*. In this double blind placebo-controlled study, 47 postmenopausal women of age 47-66 years received 40mg Isoflavone-GALENA® (n=25) or 40mg casein placebo (n=22). Levels of hemostatic factors PT, APTT, activity factors VII and X, and fibrinogen, TAT, F1+2, antithrombin, protein C, total and free protein S, plasminogen, PAI-1 and D-dimers and lipids [total cholesterol/TC, triglycerides/TG, High Density Lipoprotein/HDL, Low Density Lipoprotein/LDL and Very Low Density Lipoprotein/VLDL] were measured at baseline and 6 months. The therapy were examined for FSH e ,-estradiol levels. Urinary isoflavone concentrations (genistein and daidzein) were measured as marker of both compliance and absorption using high performance liquid chromatography. *Results*. Levels of hemostatic variables did not change significantly throughout the study in isoflavone group; however isoflavone group experienced statistically significant reduction in plasma concentration of F1+2 (11,5%); both groups experienced statistically significant reduction in antithrombin, protein C and free protein S levels. Significant increase in D-dimers were observed only in the isoflavone group. PAI-1 levels increased significantly in the placebo group. Levels of lipids (LDL and TC) decreased no significant similarly in both groups. HDL decreased significantly in the isoflavone group (8,1%), but the change was not different to the placebo group (13,81%). Furthermore, in both groups were increased no significant of levels VLDL and TG. *Conclusions*. The results of the current study do not support biologically significant estrogenic effects of isoflavone on parameters assessed, however further research will be necessary to definitively asses the safety and efficacy of isoflavone.

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LIFE EXPECTANCY IN TYPE 1 (NON-NEURONOPATHIC) GAUCHER DISEASE

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Aims. Analyses were conducted to estimate life expectancy at birth of patients with type 1 (non-neuronopathic) Gaucher disease (GD), the most frequent lysosomal storage disease. *Methods*. The GD population included all patients with type 1 GD registered in the International Collaborative Gaucher Group (ICGG) Gaucher Registry. Life expectancy was calculated according to the standard life table method (Palmore and Gardner, 1996), and compared with the United States as a reference population (World Population Prospects, 2002, UN). Approximately 40% of GD patients came from the U.S. The life expectancy of the reference population was similar to that for developed nations (as defined by the UN). The gender distribution in GD was similar to that of the general population. *Results*. The type 1 Gaucher population of 2,876 patients had 99 reported deaths in 13,499 person-years of follow-up. The average life expectancy of the type 1 GD population was 69.7 years, and the life expectancy of the reference population was 77.1 years. Analyses restricted to the US population of patients with type 1 GD yielded a life expectancy of 71.5 years, based on 65 deaths in 6,400 person-years. *Summary and Conclusions*. The ICGG Gaucher Registry represents the single largest dataset on GD patients worldwide. The current life expectancy at birth of people with type 1 GD is about 7.4 years less than the reference population. Additional analyses on causes of death and the influence of factors such as enzyme replacement therapy with imiglucerase and therapeutic splenectomy on life expectancy are planned.

Reference

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IMATINIB PLUS CHEMOTHERAPY IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL): AN EXPLORATORY COST-EFFECTIVENESS ANALYSIS FOR THE UNITED KINGDOM

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Background. Patients diagnosed with Ph+ALL have few effective treatment options and poor prognosis. Imatinib combined with conventional chemotherapy (CC) in Ph+ALL patients has produced encouraging

efficacy results with a well-tolerated safety profile. *Aims*. This study explores the cost effectiveness of imatinib plus CC versus CC alone in adult Ph+ALL patients. *Methods*. A Markov model simulated a hypothetical cohort of adult Ph+ALL patients receiving imatinib plus CC or CC alone. The model included three states: alive without disease progression (DFS), alive with disease progression (DS), and death. State transition probabilities were derived from the published literature. In the absence of relevant data pertaining to Ph+ALL, assumptions about costs and utilities were derived from a cost analysis of CML. Only direct medical costs were included, adopting a UK healthcare payer perspective. All outcomes were discounted. Costs were adjusted to £2006. *Results*. The model projects that the total discounted survival was 1.10 years for CC and 4.31 years for imatinib+CC. Total discounted disease free survival was 0.76 year for CC and 2.77 years for imatinib+CC. The total discounted quality adjusted life years (QALY) were 0.85 v. 3.28 for CC and imatinib+CC, respectively. Thus, the net incremental gain in discounted quality adjusted survival was 2.43 QALYs. The monthly costs of DFS and DS were estimated at £123 and £417, respectively. The net costs associated with imatinib were £51,757 for the UK. The incremental cost per QALY of imatinib+CC v. CC alone was approximately £21,299 (i.e., £51,757 divided by 2.43 QALYs). *Summary / Conclusions*. For adult ALL patients with poor prognosis due to Ph+ALL, our exploratory analysis suggests that, given the underlying data and assumptions, adding imatinib to conventional chemotherapy regimens is cost-effective compared to chemotherapy alone from the UK perspective.

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BORTEZOMIB PLUS DEXAMETHASONE AS INDUCTION THERAPY IN NEWLY DIAGNOSED MULTIPLE MYELOMA: A PRELIMINARY STUDY IN THAI PATIENTS

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Background. Standard induction chemotherapy in myeloma can induce complete remission in only about 5-10% of cases. The most effective treatment, so far, is high-dose chemotherapy followed by autologous stem cell transplant. Incorporation of novel antimyeloma agents into the induction regimen should improve the outcomes, especially in patients who cannot receive high-dose chemotherapy. *Aims*. We assessed the response rate and factors that may predict the response in 20 newly diagnosed myeloma patients who were treated with bortezomib plus dexamethasone (VelDEX) as an induction therapy before undergoing peripheral stem cell collection and autologous stem cell transplant. *Methods*. Bortezomib 1.3 mg/m^2 was administered intravenously on days 1, 4, 8 and 11, along with dexamethasone 40 mg orally on day 1-4 and 8-11 of a 21-day cycle for 4 cycles. The complete evaluation was done after the fourth cycle of VelDEX. Responses were defined according to the European Group for Blood and Marrow Transplantation criteria. The response rate was then compared with the historical cohort of patients who were treated with VAD (vincristine, adriamycin and dexamethsone) regimen at the same institution. *Results*. 19 out of 20 patients enrolled were eligible for evaluation. All but one completed four cycles of VelDEX. The objective responses were achieved in 15 patients (79%), including complete remission in 7 patients. On an intent-to-treat basis, response rate for complete remission, partial response, minimal response, stable disease, progressive disease were 37%, 42%, 11%, 5%, and 5% respectively. Comparing with the cohort of 30 patients treated with VAD regimen, VelDEX is superior in inducing complete remission (37% vs. 10%, $p<0.001$). No significant factors were found to be associated with responses in these patients. The most common side effects were fatigue, diarrhea and peripheral neuropathy. Two patients developed colonic pseudoobstruction that could be related to bortezomib. No deep vein thrombosis or serious hematologic toxicity was observed. Five patients has undergone autologous stem cell transplantation successfully and remains in complete remission. *Conclusions*. Bortezomib plus dexamethasone is a highly effective induction regimen for newly diagnosed myeloma patients.

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NEW BIOCHEMICAL MARKERS AND ASSESSMENT OF CARDIOTOXICITY DURING HEMATOPOIETIC CELL TRANSPLANTATION IN ACUTE LEUKEMIA

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Background. Cardiotoxicity is a potentially serious complication of treatment in hematooncology. Myeloablative preparative regimen (PR) followed by hematopoietic cell transplantation (HCT) represents a high risk for development of cardiotoxicity. Various methods including biochemical markers have been recommended for monitoring of cardiotoxicity. There is not much experience with new cardiac biomarkers in this field. *Aims*. Monitoring of cardiotoxicity during PR and HCT with biochemical markers of cardiac damage-N-terminal pro brain natriuretic peptide (NT-proBNP), creatine kinase MB (CK-MB mass), cardiac troponin T (cTnT), cardiac troponin I (cTnI), fatty acid binding protein (FABP), glycogen phosphorylase BB (GPBB). *Methods*: 23 patients treated with anthracyclines for acute leukemia (mean age 44.5±10.6 years, 15 males) were studied. PR consisted of Cyclophosphamide (HD-C) and Busulphan in 17 patients, HD-C and total body irradiation (TBI) in 6 patients, followed by HCT. Biochemical markers of cardiac damage were measured the day before PR, the day after PR, the day after HCT and at the time of bone marrow recovery (after circa 14 days). *Results*. The results are summarized in the Table 1. Changes in NT-proBNP and GPBB concentrations were statistically significant in comparison with the baseline values ($p<0.01$). *Conclusions*. Our results suggest that administration of PR and HCT is in most acute leukemia patients associated with acute neurohumoral activation (significant rise in NT-proBNP). In our study, NT-proBNP remained markedly elevated in 7 (30.4%) patients at the time of bone marrow recovery. These persistent NT-proBNP elevations indicate subclinical cardiotoxicity (risk for development of heart failure) and require further follow-up. From markers of myocardial ischemia and necrosis, only GPBB became elevated after PR and HCT. These changes could be considered a sign of subclinical cardiotoxicity. CK-MB mass, cTnT, cTnI, FABP does not seem to be of value in detection of cardiotoxicity in peritransplant period. These findings need to be confirmed in larger studies with longer follow-up.

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Table 1. Biochemical markers of cardiac damage during PR and HCT in acute leukemia.

biochemical markers	1 day before PR	1 day after PR	1 day after HCT	14 days after HCT
NT-proBNP above 100/150 ng/l	4 (17.4 %)	14 (60.9 %)	16 (69.6 %)	16 (69.6 %)
NT-proBNP above 500 ng/l	0	6 (26.1 %)	9 (39.1 %)	7 (30.4 %)
CK-MB mass above 4.8 µg/l	0	0	0	0
cTnT above 0.01 µg/l	0	0	0	0
cTnI above 0.40 µg/l	0	0	0	0
FABP above 4.5 µg/l	0	0	0	1 (4.3 %)
GPBB above 7.3 µg/l	0	5 (21.7 %)	5 (21.7 %)	2 (8.7 %)

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EFFICACY AND SAFETY OF THALIDOMIDE AND DEXAMETHASONE COMBINATION WITH OR WITHOUT CYCLOPHOSPHAMIDE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background. Thalidomide is a potent anti-myeloma drug which can produce up to a 30-50% overall response rates in refractory multiple myeloma. Several studies have also shown encouraging results for its use in first-line therapy for myeloma. *Aims*. The aim of this study was to assess the efficacy and safety of thalidomide and dexamethasone combination regimens with or without cyclophosphamide as a first-line therapy in patients with multiple myeloma. *Methods*. A total of 66 patients with multiple myeloma were initially treated with thalidomide-containing regimens between June 2003 and October 2006. Thalidomide was given with the two different regimens repeated every 28 days: (A) TD regimen: thalidomide (50 mg/day, daily) and dexamethasone (20 mg/m^2, I.V., on D1-4, D9-12, D17-20); (B) TCD regimen: thalidomide (50 mg/day, daily), cyclophosphamide (150 mg/m^2 P.O. on D1-4), and dex-

amethasone (20 mg/m^2 I.V. on D1-5, D15-19). All patients except severe thrombocytopenia received aspirin daily for the prophylaxis of deep vein thrombosis. Autologous peripheral blood stem cells (PBSC) were collected after mobilizing with G-CSF with or without cyclophosphamide. *Results*. 66 patients (TD regimen: 31 patients, TCD regimen: 35 patients) who received at least 4 cycles or more were evaluated for response and toxicity. There were 33 males (50%) and 33 females (50%). The median age of patients was 66 years (range, 39-80 years). The overall response rate for thalidomide-containing regimens was 84.8%. There were 13 (41.9%) complete responses and 14 (45.2%) partial responses for TD regimen and 11 (31.4%) complete responses and 18 (51.4%) partial responses for TCD regimen, respectively. There was no significant difference in overall response rate between two treatment groups (TD: 87.1% vs. TCD: 82.8%, p=0.63). However, the progression-free survival (PFS) was significantly shorter in patients treated with TD regimen than those treated with TCD regimen (8.6±1.2 ms. vs. 19.4±4.8 ms. p<0.05). There was no significant difference in overall survival (OS) between two treatment groups (p=0.13). Toxicity by NCI-CTC (grade 3/4) included neutropenia in 8 patients (12.1%), thrombocytopenia in 5 patient (7.6%), infection in 9 patients (13.6%) and neuropathy in 10 patients (15.2%). In addition, there were 2 patients (3%) with thrombosis. 21 patients who achieved more than partial response to the thalidomide-containing regimens proceeded to PBSC collection and the median number of CD34$^+$ cells collected was 3.8×10^6/kg (range, 1.0-18.5×10^6/kg). *Conclusions*. Low dose thalidomide-based combination regimen, especially TCD, is effective and well tolerable regimen in patients with newly diagnosed multiple myeloma showing high response rates and sufficient collection of PBSC.

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EFFECT OF CLORETAZINE ON ACUTE MYELOID LEUKAEMIA BLASTS IN VITRO AS A SINGLE AGENT AND COMBINED WITH CYTARABINE AND DAUNORUBICIN

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Background. Current chemotherapy strategies induce remission in the majority of AML patients; however up to 70% subsequently relapse, highlighting the need for new therapies. Cloretazine (VNP40101M) is a novel alkylating agent (Vion Pharmaceuticals Inc, New Haven CT, USA). On administration, Cloretazine undergoes activation to form 90CE, a DNA chloroethylating species which cloroethylates the O6 position in guanine residues, resulting in inter-strand DNA cross linkage, cytotoxicity and ultimately cell death. *Aims*. We have investigated the effect of Cloretazine on cell proliferation, viability and apoptosis of AML blasts *in vitro*, both alone and in combination with other chemotherapy agents (Cytarabine/Daunorubicin). *Methods*. Blast cells were isolated by density centrifugation from 10 patients at presentation (BM or PB). Cells were cultured in 96-well plates at 1×10^{6}cells/mL in McCoy's 5A medium supplemented with 15% FCS, GM-CSF (100 ng/mL), SCF and IL-3 (10ng/mL). Cloretazine (0, 1, 5, 10 or 20 ug/mL) was added alone or in combination with Cytarabine/Daunorubicin (10,100 or 500ng/mL) at establishment of cultures. Cultures were incubated at 370C in 5% CO2, 5% O2, 90% N2 for 72-96h before cell proliferation, viability and apoptosis were measured using tritiated thymidine uptake, WST-1 and Annexin V staining methods respectively. *Results*. All patients studied showed a dose dependent response to Cloretazine alone. Combination with Cytarabine or Daunorubicin at a range of concentrations showed increased effects e.g inhibition of proliferation 50%(24-92) (mean (range) with Cloretazine(5ug/mL) alone), 71%(32-98) Cytarabine(100ng/mL) alone increasing to 86%(58-100) when combined, 57%(20-89) Daunorubicin(10ng/mL) alone increasing to 73%(28-96) combined. Similarly increases in cytotoxicity were observed, 28%(0-50) (Cloretazine alone), 43%(0-86) Cytarabine alone to 52%(15-92) in combination. 22%(1-70) Daunorubicin alone to 36%(0-78) combined. Apoptosis levels also showed increases with combinations. *Conclusions*. This pilot study indicates Cloretazine is effective on AML blasts *in vitro* both alone and in combination with other chemotherapy agents. Cloretazine is currently undergoing clinical trials.

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KARYOTYPIC EVOLUTION OCCURING EITHER PRE OR POST DIAGNOSIS CONFERS A POOR PROGNOSIS IN CLL

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Background. Sequential studies of genomic abnormalities in CLL show an incidence of karyotypic evolution (KE) varying from 1.4 to 43%. Factors potentially influencing the incidence and reported clinical significance of KE include; clinical stage, risk profile of the patient population studied, frequency and interval between tests, methods used to identify genomic abnormalities and the definition of KE. Previous studies have shown loss of chromosome regions 11q23 or 17p13, and the presence of translocations to be poor prognostic indicators in CLL. While the former are rarely detected in stage A0 patients, the timing of acquisition of other chromosome abnormalities, including translocations is less clear. *Aims*. Using both traditional and molecular cytogenetics to examine the incidence and nature of KE and correlate our findings with other genetic and clinical factors. *Methods*. We selected 342 patients with stored material to allow evaluation of KE. Two cohorts were identified. The of 314 patients had 2 or more samples, taken with a minimal interval of 1 year, median 4.5yrs(1.2-20.1). The second group, 28 patients, had either clonal evolution in a single sample or multiple abnormalities in a single clone at presentation, indicating that KE could already have occurred prior to diagnosis. However, many patients had further samples analysed during the disease course. G-banded karyotype and interphase FISH analyses (probes; 13q14, ATM, P53, and a 12 centromere)were undertaken. If metaphase analysis showed new abnormalities in the last, earlier samples were re-examined by FISH, and/or G-banding.In 14 cases the abnormalities were present at diagnosis. *Results*. 155 patients (45%) showed no evidence of KE, of whom 57 had a normal karyotype. Evolution was detected in 55% of patients, of whom 62% showed KE at presentation, 29% over time, and 9% both. Of patients showing KE over time, 19 had abnormalities that were detected by FISH, (4x delATM, 2x +11, 3x delP53, 12x del13q14) and 38 had abnormalities detected by karyotype. 21/38 had a normal 1st karyotype. In those with an abnormal 1st karyotype, acquired abnormalities were almost exclusively structural, +12 was detected in only 1 case.

Table 1.

Overall, del13q14 and del11q were the most commonly acquired abnormalities. Although patterns of evolution were observed for numerical abnormalities and translocations involving the immunoglobulin locii, no other recurrent translocations were found. 78% of KE resulted in an unbalanced karyotype. A significant difference was found in OS between patients who had KE compared with those without, whether patients with del11q23 and/or del17p13 were included or excluded from analysis. No difference was found in OS between KE over time compared with KE at presentation. Clinical characteristics of patients correlated with KE are shown in Table 1. Patients with both mutated and

unmutated VH genes, and high and low expression of CD38 and ZAP-70 showed KE. Unmutated VH genes and increased expression of CD38 correlated with KE (p=0.0162 and 0.0299 respectively). *Conclusions*. Our data suggest that the effect of KE on prognosis may be a consequence of *genomic instability* rather than the acquisition of specific genomic abnormalities, but this requires confirmation in a large study.

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SURVIVAL OF THE LEUKEMIA PATIENTS AMONG CHERNOBYL CLEAN-UP WORKERS IN UKRAINE

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Background. Great medical and public concern in Ukraine after Chernobyl catastrophe was about leukemia as the earliest and the most sensitive effect of irradiation. The largest epidemiological study on leukemia risk was initiated in Ukraine by the Research Center for Radiation Medicine (Ukraine) and National Cancer Institute (USA) to study the leukemia risks in clean-up workers in Ukraine. One of the important characteristics of the cases, identified in the framework of the study, is presented further. *Study goal*. To study the peculiarities of the leukemia patients survival among Chernobyl clean-up workers, including life expectancy, 1-year mortality and 5-year survival rates. *Study population*. 110 645 male clean-up workers after Chernobyl catastrophe residing in 6 administrative regions of the Ukraine. *Period of observation*. 1987-2000 *Diseases under study*. All types of acute and chronic leukemia. *Results*. The survival of the 84 of 86 leukemia cases, identified in the study cohort and confirmed by the International Diagnostic Review Panel in the framework of the Ukrainian-American retrospective case-control study was analyzed. Median of the Chronic Lymphocyte Leukemia (CLL) cases survival was 5 years, of the CML Chronic Myeloid Leukemia (CML) cases-4 years and Acute Leukemia (AL) cases-3 months. 1-year mortality rate may be considered as an indicator of the aggressive character of the disease course. It composed 24% in total and varied from 78% for AL to 6% for CLL. The proportion has more positive value in compare with the analogues one for the total Ukrainian population (44% in total). 5-year survival rate composed in total 43% with highest value (53%) for CLL and lowest (1 of 18) for AL. The same value was identified for the total Ukrainian population (41,7-45,9% in total). *Conclusions*. Relatively lower 1-year mortality rate in leukemia cases among clean up workers in compare to Ukrainian population does not prove the less aggressive disease course. It may demonstrate more high level of the diagnostic and treatment of sufferers following Chernobyl catastrophe. Similar 5-year survival rate in clean-up workers and general population may serve as proof of this.

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IDENTIFICATION OF A NOVEL, TRANSACTIVATION-DEFECTIVE SPLICING VARIANT OF P53 GENE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. p53 is an important protein implemented in many cellular processes controlling the cell fate. It has been reported that besides the well-studied regulation of p53 at the levels of protein-protein interactions and posttranslational modifications, the activity of p53 is substantially controlled at transcriptional level, too. *Aims and methods*: In our group of 397 patients with chronic lymphocytic leukemia (CLL), we have investigated mutational status of p53 gene using direct sequencing. All identified mutations were tested by a functional assay FASAY (Functional Analysis of Separated Alleles in Yeast). *Results*. In 25 out of 397 cases (6.3%), point mutations or short deletions were found. Using cDNA sequencing, a novel p53 splicing variant, lacking the whole coding sequence of exon 6 was identified. This splicing p53 isoform (*delta ex6*) lacks the original 113 nucleotides of exon 6, thus leading to a frame shift mutation and premature STOP codon after 189th amino acid. The *delta ex6* p53 variant is devoid of transactivational activity and is differentially expressed in CLL patients as compared to healthy controls. *Summary and conclusions*. Herein, we present evidence of a novel *delta ex6* p53 splicing variant that is differentially expressed in patients with CLL. This finding supports the recent data on dysregulation of p53 splicing pattern in malignancies.

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ASSOCIATION BETWEEN PREVALENCE OF FRACTURES AND BONE MINERAL DENSITY IN SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Since children and adolescents are at the most rapidly developing stages of their lives when childhood cancer is diagnosed, the disease and its therapy can severely disturb the normal growth, bone mineral acquisition and skeletal development. In adults a deficit of 1 SD in bone mass is associated with a 1,5-3-fold increased incidence of fracture. So far, no correlation between BMD and fracture risk in children, especially in cancer survivors, has been determined. The aim of the study was to determine the prevalence of fractures and to asess an association between bone mineral density and fractures in survivors of acute lymphoblastic leukemia in childhood *Material and methods*. One hundred and seven (70 males) survivors of acute lymphoblastic leukemia (median age at diagnosis 7.3 years) were assessed in this study, they recevied: chemotherapy with methotrexate and antymetabolities, glucocorticoids and in parts (n=62) cranial irradiation (12 Gy). We acknowledged only the treatment elements of a previously evidenced negative influence of bones. All fractures events were documented by x-ray. Information on age at the time of fractures, trauma severity and anatomical location was obtained from a detailed questionnaire. Using dual-energy x-ray absorptiometry bone mineral density (BMD) of spine and total body were performed twice : after a completed treatment (median age 10.3 years) and after a 1,5 year follow-up period (median age 11.8 years). The references values were obtained from the 473 age- and gender-matched healthy children from the north-east region of Poland. The results were expressed as Z-score. *Results*. Forty one subjects (38.1% of the studied population) had a history of fractures. Six of them were patients presenting with vertebral compression fractures at the time they were diagnosed of ALL. Twenty eight subjects reported their fractures had occurred after treatment. All fractures (except for those of vertebral bodies) were localized in the extremities (65.6% in upper: wrist, radius, ulna or ankle and 34.3% in lower extremities: tibia, humerus); all of them were results of injuries, including low-energy trauma. BMD Z-score for total body (mean -0.11 and '0.012) and BMD Z-score for lumbar spine (mean 0.03 and '0.10) were not significantly different from reference values in both examinations. No significant difference in BMD Z-score between patients reporting fractures compared to the non-fractured subjects was found. No associations were found between BMD and cranial irradiation, cumulative dose of steroids, high dose of metothrexate or fracture prevalence. Changes in BMD accrual did not correlate with any parameters of treatment. *Conclusions*. 1. In survivors of childhood ALL prevalence of fractures was not associated with lower BMD. 2. Pattern of fractures was similar to the published data in healthy population. 3. Further studies in this area are needed to determine a long-term effect of cancer therapy on bone mass and bone fragility.

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LENALIDOMIDE TREATMENT AND THE EFFECTS ON T-CELL RECEPTOR REPERTOIRE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background and aims. The myelodysplastic syndrome (MDS) is a multifactorial pathogenetic disease and therefore therapeutic decisions in patients with MDS are very complex. A novel immunomodulatory drug for the management of 5q-subtype of MDS is lenalidomide. This agent shows encouraging results in MDS treatment. The involvement of a T cell-mediated autoimmune process in the pathogenesis of the cytopenia in myelodysplastic syndromes is still under evaluation. To further investigate a T-cell involvement in the pathogenesis of MDS we have studied the complementarity-determining region 3 (CDR3) size distribution of the CD3, CD4 and CD8 T-cell receptor (TCR) V-β-chain subfamilies in the bone marrow and peripheral blood samples of 5q-subtype of MDS patients before and after therapy with lenalidomide (n=10). 6/7 MDS patients treated with lenalidomide and evaluable to date achieved a CR (complete remission), one a PR (partially remission). *Methods*. We used the multiplex PCR based technique TCR spectratyping based on the size heterogeneity of the CDR3 region and compared the results with age-matched controls (n=10). At the same time we collected urine samples of these patients for proteome analysis. *Summary*. We confirmed that TCR V-β skewing of T-cell repertoire is more frequent in the bone mar-

row samples of MDS patients than in the whole blood samples. The most frequently skewing of TCR V-β fragments before lenalidomide treatment occurs in V-β short 1 (50%), V-β short 3 (50%) and V-β long 13 (50%). CD8 T-cells harbour the most pronounced deviations from a Gaussian distribution of TCR fragment length compared to CD4 T-cells. Three of the patients are now far enough after lenalidomide therapy to evaluate the changes of the spectratyping pattern. *Discussion*. We conclude that TCR V-β skewing is frequent in MDS especially in CD8 T-cells. Normalization of at least 1 initially skewed V-β profile after lenalidomide therapy occurred in all three 5q-MDS patients. These encouraging results need further evaluation in a larger patient population. These studies are currently ongoing in our laboratory, first data on the proteomic screening of urine of MDS patients will be presented.

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NO THROMBOPOIETIN (TPO) OR ITS RECEPTOR (MPL) MUTATION IN 6 FAMILIES WITH MYELOPROLIFERATIVE DISORDERS

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Background. Most myeloproliferative disorders (MPD) are sporadic, although, in the past, patients with familial MPD have been published through case reports. The casual occurrence of 2 cases of MPD in the same family is extremely rare. Anecdotal familial cases of MPD have been described along the last 25 years. In 4 families with a thrombocytotic pattern TPO gene mutation has been found (in 1 family, females were polyclonal). In 1 other family a mutation of Mpl exon 10 (S505N) has been reported always interesting clinical affected members. Most affected patients of 62 families recently published carried V617FJak2 mutation. Two recent papers evaluated the occurrence of V617FJak2 mutation in 42 and 20 families respectively. *Aims*. We also searched V617FJak2 mutation in familial cases with MPD but we searched also alteration of thrombopoietin (TPO) and its receptor (Mpl) genes. *Methods*. Our study comprehends 14 affected members of 6 families (3 polycythemia vera PV and 11 essential thrombocythemia ET, 11 females and 3 males aging 30-75 years). The diagnostic criteria adopted are those of the PVSG. For the detection of the V617FJak2 in peripheral blood granulocytes DNA, sequence analysis and allele- specific PCR was used. In 6 females the state of activation of the X-chromosome was determined using HUMARA gene. Sequence analysis of the whole TPO gene and exons 9 and 10 of Mpl, using primers in agreement with the literature (available on request) have been performed. *Results*. No mutation of TPO and Mpl has been found, while already known polymorphisms of TPO were observed. All but 2 patients carried V617FJak2 mutation and all the interpretable females displayed a polyclonal pattern. Our results are summarized in the Table 1. *Conclusions*. None of our patients had alteration of TPO and Mpl gene while most of our patients carried Jak2 mutation. A germinal mutation is undue because in 2 families both V617F and WT Jak2 has been observed. We surmise that Jak2 mutation may be a predisposing condition for MPD phenotype.

Table 1.

Family	Pats	Clinical Phenotype	Clonality	Jak 2	Plasma TPO (ELISA)	TPO Polymorphis
A	1	ET	Not Performed	V617F	/	A784G
	2	PV	Not Performed	V617F	/	A784G
	3	ET	homozygous	V617F	normal	A784G
B	1	ET	polyclonal	V617F	normal	C3767T
	2	ET	(male)	V617F	normal	C3767T A7684G
	3	ET	polyclonal	V617F	normal	C3767T
C	1	PV	polyclonal	V617F	/	C3767T
	2	ET	skewing	WT	/	C3767T
D	1	ET	polyclonal	V617F	increased	C3767T A7684G
	2	ET	polyclonal	V617F	normal	C3767T A7684G
E	1	ET	(male)	V617F	normal	none
	2	ET	(male)	WT	increased	none
F	1	PV	polyclonal	V617F	increased	none
	2	ET	polyclonal	WT	normal	none

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LEUKOCYTE ALKALINE PHOSPHATASE EXPRESSION PREDICTS THE JAK2 V617F MUTATION STATUS IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background. A single, somatic mutation in the Janus Kinase 2 (JAK2) gene (JAK2V617F) 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.

Figure 1.

Aims. Aim of this study was to predict the presence of the JAK2V617F mutation by a simple flow cytometry analysis of LAP expression. *Methods*. Patients with Polycythemia Vera (PV, n=146), Essential Thrombocythemia (ET, n=329) and Idiopathic Erythrocytosis (IE, n= 56) were selected among those regularly followed up in our out patient clinic. Healthy volunteers were selected from those referred to our institutional blood bank for blood donation (n=40). The JAK2V617F mutation screening was performed by allele specific Polymerase Chain Reaction. The surface expression of Leukocyte Alkaline Phosphatase was quantified as arbitrary units of Mean Fluorescence Intensity (MFI) by flow cytometry using the lB12.1 (IgG1) antibody and a FACS analyzer. PRV-1 gene expression was performed by Real Time Quantitative PCR (RQ-PCR) using RNA from purified granulocytes and a 7700 ABI platform. The discriminant ability of LAP and PRV-1 expression in identifying the JAK2V617F mutation, was performed by a Receiving Operating Characteristic (ROC) analysis, using each value of the rating variable (LAP and PRV-1) as a possible discriminant cut-point. *Results*. The mutational analysis for JAK2V617F showed that 132/146 PV patients (90%) and 174/329 ET patients (53%) were mutated, while none of the IE patients proved positive. The LAP expression detected on granulocytes isolated from ET patients lacking the JAK2V617F mutation was not different from that of normal donors and IE patients. On the contrary, a progressive and highly significant increase of the LAP Mean Fluorescence Intensity was registered in ET and PV patients carrying a heterozygous or homozygous JAK2V617F mutation (Figure1 A). A ROC curve analysis allowed to identify a LAP MFI value of 90 (AUC=0.8295; 95% confidence interval: 0.7965-0.8625) as the optimal cut-off to discriminate, with a good score of sensitivity (76.87%) and specificity (75.76%), patients carrying a JAK2 wild type gene as compared to those with a JAK2V617F mutation (Figure 1 B). The presence of a homozygous JAK2V617F mutation strongly correlates with the highest values of LAP expression (Figure 1 C). 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 (β-coefficient: 399.85, $p<0.0001$). The ROC curve analysis by plotting results of both the LAP and the PRV-1 tests gave superimposable results (Figure 1 D). *Conclusions*. Flow cytometry of LAP on peripheral blood granulocytes is an easy and reproducible assay to predict the JAK2V617F mutation.

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2-DEOXYGLUCOSE: A POTENTIAL CANDIDATE IN THE THERAPY OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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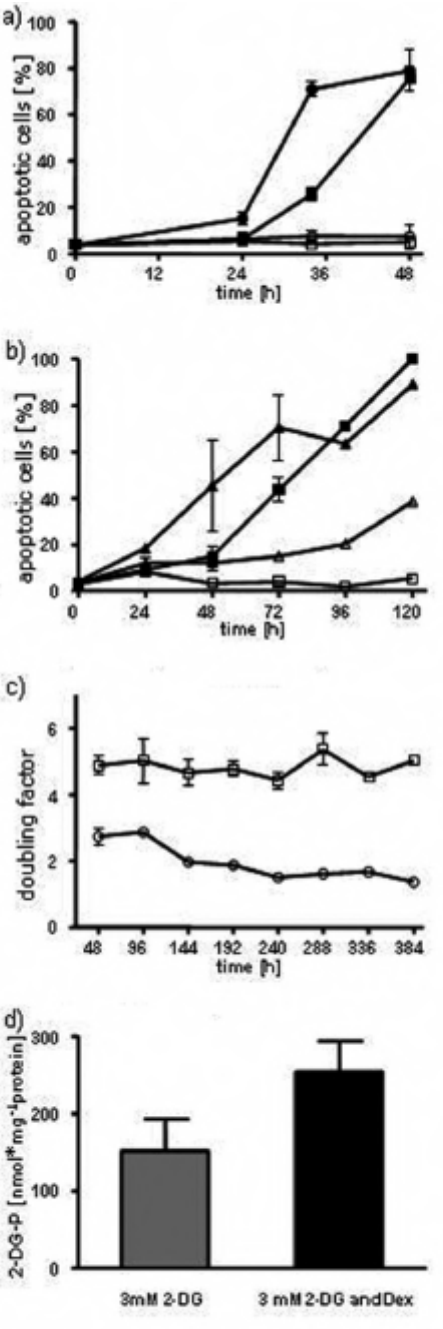
Background. Glucocorticoids (GC) are used in the therapy of lymphoid malignancies in combination with other chemotherapeutics because of their ability to induce cell cycle arrest and apoptosis. Despite a relatively high survival rate, the therapy has severe side effects and resistance develops frequently. There is a demand for improved combinatory protocols with less adverse effects and more efficiency. Our gene expression profiles in children with acute lymphoblastic leukemia (ALL) in the initial phase of GC mono-therapy revealed phosphofructokinase/fructose-biphosphatase-2 as a promising candidate gene. The induction of this key regulator of glycolysis suggests metabolic alterations as a key event in GC-induced cell death. Therefore, we explored the use of a non-toxic dose of the glucose analog 2-deoxy-glucose (2-DG), which accumulates as 2-deoxy-glucose-6-phosphate (2-DG-P) and can´t be further metabolized by either the glycolytic or the pentose-phosphate pathway, in combination with GC. *Aims*. Investigation of the effect of the combined administration of GC and 2-DG on the kinetics of GC-triggered apoptosis in ALL cells as a mean of reducing the effective GC dose. *Methods*. Acute lymphoblastic T-cells (CEM-CCRF-C7H2) and pre-B ALL cells (pre-B 697) were treated with 100 nM dexamethasone and either 3 mM or 1 mM 2-DG, respectively. Apoptosis was determined by Annexin V/PI stain and sub G1-peak detection. Intracellular accumulation of 2-DG-P was determined by CE-ESI-MS-TOF, using PolyE-323 coated capillaries and internal standards for quantification. Lactate and glucose quantification in the supernatant were analyzed by enzymatic assays (R-Biopharm, Sigma). Mitochondrial function was examined by high-resolution respirometry analyzing the respiratory enzymes coupled and uncoupled to oxidative phosphorylation. *Results*. T- and preB-ALL cells were dramatically sensitized to GC-triggered apoptosis by the co-administration of 2-DG, (Figure 1 a, b), which was not observed in combination with other chemotherapeutics like vincristine or gemcitabine. The effective dose of GC could be reduced by a factor of 20 in T-ALL cells and preliminary data suggest a partial reversion of resistance in B-ALL cells. 2-DG alone did not induce cell death during GC treatment, but we observed a pronounced increase in the doubling time (Figure 1c).



Acceleration of GC-induced apoptosis by the co-administration of 2-DG in T-ALL cells (a) and pre-B cells (b, control □, 100 nM dexamethasone ■, 1 mM 2-DG △, 1 mM 2-DG + 100 nM dexamethasone ▲, 3 mM 2-DG ○, 3 mM 2-DG and 100 nM dexamethasone ●). c) Decline in doubling factor by 3 mM 2-DG in T-ALL cells. d) Accumulation of 2-DG-P after 12 hours in T-ALL cells. n = 4 independent experiments

Figure 1. Acceleration of GC induced apoptosis.

Glucose up-take and the glucose to lactate ratio were unchanged in dexamethasone treated cells compared to controls. Sole 2-DG treatment lowered glucose up-take by 14% and glucose to lactate ratio slightly, but had no impact on mitochondrial activity. Combination with dexamethasone did not result in an additional disturbance of glycolysis, but mitochondrial function was reduced and membrane alterations were observed. 2-DG-P accumulation was increased by 70% when 2-DG was

combined with dexamethasone compared to 2-DG alone (Figure 1d). *Conclusions*. Since glycoltic alterations are observed under sole 2-DG treatment, but had no effect on cell viability, these changes are not sufficient to explain the strong effect of the co-administration. Although the mode of action of the combined effects of GC and 2-DG remains to be delineated, the strong effect on the kinetics of apoptosis induction in two independent childhood ALL models makes this combination a promising candidate for an improved treatment option for childhood acute lymphoblastic leukemia and perhaps other lymphoid malignancies.

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A MODIFIED DHPLC ASSAY FOR A RAPID SCREENING OF JAK2 EXON 14 MUTATION

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Background. V617F mutation in exon 14, within JH2 domain of the JAK2 gene, occurs in 90% of patients with polycythemia vera (PV) and 50% essential thrombocythemia (ET). The mutated clone contributes to the myeloid lineage but sometimes it is found in a very low proportion of circulating leukocytes, requiring sensitive assays for its detection. The molecular basis of V617F negative myeloproliferative diseases is unknown, but two novel nucleotide changes in the JH2 domain of the JAK2 gene have been recently described. The allele-specific (AS)-PCR is still the most sensitive assay for the detection of V617F; nevertheless, this conventional method is unable to identify other mutations. DHPLC could be an attractive alternative for the detection of new mutations, but a previous study reported a sensitivity of 50% in identifying V617F positive cases. *Aim*. Development of a novel, fast and high-throughput Temperature Modulated Heteroduplex Analysis (TMHA) DHPLC assay for the detection of mutations in the JAK2 exon 14. *Methods*. 160 PV and 174 ET patients, diagnosed according to WHO criteria, were tested by AS-PCR for the V617F mutation. 141 (88%) PV and 94 (54%) ET patients were positive. V617F negative cases (19 PV, 80 ET) and 10 randomly selected heterozygous V617F positive patients were studied with TMHA DHPLC searching for mutations in JAK2 exon 14. For the detection of the heteroduplex by DHPLC, partial denaturation of the amplicon was obtained by temperature modulation. In the case of JAK2 V617F, the melting profile of the region of interest was very unstable (Figure 1, A), hence a short stretch of nucleotides (GC-clamp) was added to the forward primer, creating an additional stable domain at the 5' end of the fragment, resulting in amplicon stabilization. All amplifications were performed under standard conditions using the Jak2F clamp (5'-cgccgccgccgccctgcatctttattatggcagagaga-3') and Jak2R (5'-cactgacacctagctgtgatcc-3') primers. For the heteroduplex creation sample:wild-type JAK2 ratio was of 3:1. 7 µL of mixed products were loaded on a WAVE fragment analysis system. *Results*. The new predicted melting curve with GC clamp was uniform along the target sequence (Figure 1 B). The presence of the V617F mutation in positive PV samples was confirmed by an abnormal profile at 60°C (Figure 1 C).

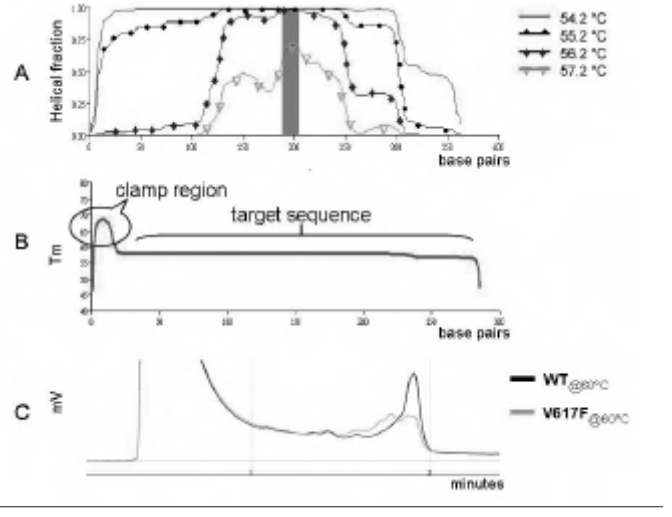

Figure 1.

Serial dilutions of 100% homozygous sample allowed a 7-10% detection rate of purified granulocytes. V617F positive controls were missed by DHPLC in the absence of the clamp. No additional allelic variants were found, confirming the very low frequency of mutations other than V617F. *Conclusions*. A significative proportion of myeloproliferative disorder patients remains without an identified genetic defect. At variance with conventional methods, like AS-PCR or restriction enzyme digestion, DHPLC is suitable in identifying genetic abnormalities outside the specific mutational hot-spot. The addition of a GC-clamp to the amplicon allowed us to employ this technology performing a rapid, cheap, reliable and sensitive assay for the detection of allelic variant involving JAK2 exon 14. These preliminary results need to be confirmed in a larger patients population.

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ANTITUMOR EFFECTS OF SOME ANIMAL AND PLANT NUCLEASES AND THEIR POLYETHYLENE GLYCOL (PEG)-CONJUGATES

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Aims. The antitumor and immunosuppressive effects of Bovine pancreatic ribonuclease (RNase A), Bovine seminal ribonuclease (BS-RNase), wheat leaf neutral ribonuclease (WLN-RNase) and Mung been nuclease (PhA) both *in vitro* and *in vivo* were studied. *Method*. RNase A and PhA enzymes were obtained from comercial sources whereas BS RNase and WLN-RNase were isolated in our laboratories from bull seminal vesicles and weat leaves. Antiproliferative activity was tested on ML-2 cell line and immunosuppressive effect on human lymphocytes in mixed lymphocyte culture. The antitumor activity *in vivo* was tested in athymic nude mice bearing human melanoma tumor. *Results*. The free RNase A and PhA-nuclease exerted very low antiproliferative and immunosuppressive activity in comparison to the effect of BS-RNase. Immunosuppressive effect of WLN-nuclease reached the same level as that of BS-RNase. However, in the experiments *in vivo* a significant decrease of the tumor size was observed in the mice treated with WLN- and PhA-nucleases. Furthermore conjugate of PhA with PEG injected seven times at the dose of 10 µg i.p. showed identical antitumor activity as that of BS-RNase. The tumor size after treatment with PEG-RNase A decreased by 70%. The both free or PEG-conjugated BS-RNases injected into the human melanoma tumors intraperitoneally decreased the tumor size by the same way. The strongest aspermatogenic, embryotoxic and immunosuppressive effects, and antigenicity were proved by BS-RNase enzyme. The smallest side effects were demonstrated by PhA nuclease. *Conclusion*. This paper shows for the first time antitumor activity of some polymer conjugated plant RNases and their potential use as agents in treatmant of human malignancies.

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FARNESYLTRANSFERASE INHIBITORS AS A NEW THERAPEUTIC APPROACH IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Adult acute leukemias remain formidable therapeutic challenge. Only 70% of adults with newly diagnosed adult acute lymphoblastic leukemias (ALLs) achieve complete remission (CR) after cytotoxic induction chemotherapy. Although these CRs may be prolonged in 35% to 40% of younger adults, the remainder have a relapse and die. The prognosis is particularly poor in Philadelphia chromosome (Ph1) disease. Thus, new approaches are needed to improve the outcome for adults with refractory leukemias. Improved understanding of signal transduction pathways has resulted in identification of a panoply of potential therapeutic targets. Novel agents in the early phase of clinical testing include farnesyltransferase inhibitors (FTIs), are also being investigated as potential therapies. FTIs are drugs that target the farnesyltransferase enzyme. This enzyme initially developed to inhibit the prenylation necessary for Ras activation, adds the farnesyl moiety to Ras protein, among other targets, enabling Ras to attach to the cell membrane. It is believed that inhibition of this enzymatic activity would interfere with Ras function, altering cell signaling in tumor cells. FTIs have been developed and

tested across a wide range of human cancers namely in hematologic malignancies. Besides preliminary results from clinical trials demonstrate enzyme target inhibition, a favorable toxicity profile and promising efficacy, with this study we hope to contribute to determine their mechanism of action and the role of combination with other agents, to define their place in the therapeutic arsenal of hematologic disorders, namely in ALLs. For this purpose CEM cells were incubate in absence and presence of an FTIs, α-Hydroxyfarnesylphosphonic acid (α-HFPA) in different concentrations (rangin from 10 nM to 300 μM), as single agent and/or with combination with Doxorrubicin (DOX) and/or MG262, a proteasome inhibitor during 72 h. Cell growth and viability were evaluated by Trypan blue exclusion. Cell death was performed by morphological studies using optic microscopy and by flow cytometry (FC), using annexin-V and/or propidium iodine incorporation. The proteins related with apoptotic cell death, BAX, BAD e BCL-2 were evaluated by FC using monoclonal antibodies. As a marker of FT inhibition we evaluate, by FC, the levels of lamin A/C before and after α-HFPA administration. To determine the inhibition of RAS-MAPK pathway we analysed by FC the levels of ciclin D1. Our results show that α-HFPA induces a decrease in cell density and viability, as single agent, in a dose and time dependent manner (IC50 ≥300 μM), inducing cell death by apoptosis. However, the anti-proliferative effect seems to be higher than the cytotoxic one, witch may be related with Ras-MAPK pathway inhibition once we detected a decrease in ciclin D1 levels. The observed decrease in lamin A/C levels confirm the farnesyltransferase inhibition and may also translate the inhibition of Ras-MAPK pathway. This study suggests that FTIs may be a usefull therapeutic approach in ALLs as single agent. However, the future of treatment for leukemia resides in defining the molecular pathways underlying the pathogenesis of this disease and in further elucidating the pharmacogenetic factors of the host.

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SURVIVIN AND VEGF MRNA EXPRESSION IN ELDERLY PATIENTS WITH DE NOVO OR SECONDARY AML

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Background. Several biologic features of AML differ between older and younger individuals. Older patients often express multidrug resistance phenotype and cytogenetic abnormalities, which may be responsible in large part for the poor outcomes observed in older-aged subgroups. Traditional cytotoxic chemotherapy is associated with a low complete response rate and a high treatment-related mortality in older patients, which explains in part the poorer outcomes in individuals over 60 years of age. For that reason, treatment for the elderly AML patients should be based on biologic and prognostic factors. Research into the pathophysiology of AML has revealed an abundance of intracellular signalling events that govern angiogenesis and apoptosis of the malignant cell. VEGF plays an important role in angiogenesis by acting as a potent inducer of vascular permeability and serving as an endothelial cell mitogen. Furthermore, the anti-apoptotic properties of VEGF during angiogenesis are primarily mediated by the induced expression of Survivin. Survivin is the smallest member of the inhibitory of apoptosis protein family (IAP family) and a putative oncogene that is aberrantly expressed in cancer cells. Survivin is also highly expressed in neoangiogenesis. The AIM of this study is to examine the mRNA expression of Survivin and VEGF in elderly patients with de novo or secondary AML. *Methods*. Total RNA was isolated from peripheral blood cells of 16 elderly AML patients (median age 73.8±8.7 years, 10 men and 6 women) and 16 individuals, of the same age, with normal haemopoiesis. Seven patients were diagnosed with *de novo* AML, while 9 had secondary AML, derived from MDS. All of our patients had excess of peripheral blasts (range 60-80%). Real Time Semi-Quantitative RT-PCR assay was performed in order to detect the mRNA levels of Survivin and VEGF. Abl was used as a reference gene. The regulation of the target genes was estimated as an expression rate. *Results*. Survivin and VEGF were both up-regulated in all AML patients that were examined [4.5 fold (p>0.05) and 2.5 fold (p>0.05), respectively]. More specifically, Survivin was up-regulated by the factor 2.8 (p>0.05), in patients with *de novo* AML and by the factor 6.8 (p<0.05), in MDS-->AML patients. VEGF mRNA expression was 1.5 times (p>0.05) higher in *de novo* AML patients and 3.2 times (p<0.05) higher, in MDS-->AML patients. The mRNA expression of both genes in the elderly patients with secondary AML is statistically significant. *Conclusion*. Our data and the findings of other authors, suggest that Survivin and VEGF may play an important role in the pathophysiology of hematopoietic malignancies and the progression of leukemia. Antisense approaches, which could be used to target Survivin or VEGF or inhibit their common pathway, would be expected to be useful for the treatment of AML, as well as carry limited toxicity for normal cells. These approaches could be therefore, more tolerable by elderly patients. Further studies are needed in order to determine whether Survivin and VEGF could be used as prognostic markers, minimal residual disease (MRD) indicators or promising cancer therapeutic targets.

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INTRATHECAL LIPOSOMAL CYTARABINE AS THERAPY OF LYMPHOMATOUS MENINGITIS

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Background and aims. 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. Currently, the systemic therapy with high doses of Methotrexate (HD-MTX) (3 g/sqm) followed by radiotherapy (WBRT: 45 Gy) represents the elective treatment. The role of the intratecal chemotherapy (IT-CHT) isn't well defined. Recently, an important role both in the prophylaxis and in the therapy of LM is played by the intratecal administration of Cytarabine in liposomal formulation (DepoCyte): randomized studies have shown a significant better effectiveness and a reduced toxicity of liposomal formulation treatment with respect to the traditional one. *Methods and results*. In this work 11 cases of LM treated with systemic chemotherapy and DepoCyte (50 mg IT every 15 days x 6 times) are shown. Male/Female ratio is 7/4; average age is 41 years (range 24-78). In all patients neurological symptoms were present; lymphomatous cells were identified in the liquor of 6 patients and Central Nervous System (CNS) localizations were detected by NMR in 9 patients. The 6 CSF-positive cases were heterogeneous, as follows: 1) B-lineage CD10$^+$ ALL meningeal relapse (after two marrow relapses), already undertaken to allogeneic staminal cells transplantation, treated only with DepoCyte; 2) Lymphoblastic T Lymphoma meningeal localization, with mediastinic mass, treated with DepoCyte associated to systemic chemotherapy (LSA2L2 Protocol) and autologous stem cell tranplantation; 3) DLBCL meningeal localization treated with DepoCyte associated to systemic chemotherapy (R-CHOP Protocol); 4) LM at diagnosis in a 78 years old patient with inadequate compliance to systemic therapy, treated with DepoCyte; 5) Mantle Cell Lymphoma meningeal (and systemic) relapse treated with DepoCyte associated to systemic chemotherapy (Codox-M Protocol); 6) Acute Myeloid Leukemia M3 FAB with meningeal relapse during manintenance therapy treated with DepoCyte and HAM + RT protocol. In patients 1) to 4) the C.R. was obtained and they are still alive; only the latter patient, namely, case 5), died for disease progression. The patient 6) is still in treatment. The remaining 5 CSF-negative cases had been diagnosed as DLBCL (3 at diagnosis and 2 at relapse) CNS solitary localizations. In all cases the systemic therapy with L-VAMP Protocol (modified with HD-MTX) was associated to IT-CHT with DepoCyte. 4 patients had a good response to treatment and 3 are still alive and in C.R. *Conclusions*. 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 synergic effects. Studies with higher number of patients might validate the effectiveness of DepoCyte also in those CSF-negative cases with solitary CNS localization.

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USE OF INTRATHECAL LIPOSOMAL CYTARABINE DRAMATICALLY IMPROVED NEUROLYMPHOMATOSIS IN WALDENSTROM MACROGLOBINEMIA

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Neurolymphomatosis, a rare complication of Waldenström's macroglobinemia, is characterized by meningeal and root nerve infiltration by lymphoplasmacytic cells. Combined intravenous and intrathecal chemotherapy including methotrexate and cytosine arabinoside is usually effective but poorly tolerated. We report a 61 year-old patient with waldenström's macroglobinemia who presented with rapidly progressive distal leg weakness. He had been previously treated by six courses of COP associated with good partial response (IgM serum level decrased

from 42 g/L to 25 g/L) Electrophysiological study showed features of sensorimotor neuropathy with axonal injury. Cerebrospinal fluid (CSF) examination revealed meningeal lymphoplasmocytic cell infiltration (40 cells/mm^3 with 96% lymphoplasmocytes ; protein level of 1,81g/l). Magnetic resonance imaging of the lumbar spine suggested infiltration of nerve roots. Intravenous chemotherapy with high dose of methotrexate was interrupted because of acute renal toxicity; subsequent four cycles of intravenous chemotherapy with cytosine arabinoside and dexamethasone were ineffective. Intrathecal liposomal cytarabine (DepoCyte®) was then administrated according to the induction and consolidation treatment schedule (9 injections). Clinical improvement as well as normalization of CSF cellularity (2/mm^3) and disappearance of electrophysiological signs of neuropathy were obtained at the end of the DepoCyte® treatment. Following this good neurological response, the patient received six courses of purin analogues (fludarabin®) to achieve a better response on systemic disease. One year later, the patient remained asymptomatic (monoclonal Ig M is 8 g/L and CSF is normal). In conclusion, use of intrathecal depot liposomal cytarabine may be a well-tolerated and appropriate treatment of meningeal infiltration by low-grade lymphoma such as waldenström's macroglobinemia. In combination with a systemic treatment, one can expect to potentiate this drug'effect.

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MULTIPLE MOLECULAR MECHANISM MAY ACCOUNT FOR RESISTANCE TO IMATINIB IN RESISTANT CELL LINES

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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 this disease. However, a part of patients in chronic-phase CML and even more in advanced-phase, demonstrate primary resistance to imatinib or develop secondary resistance during treatment. In addition, imatinib is able to induce molecular remission only in a limited proportion of CML patients who achieve a complete cytogenetic remission. Therefore, a second level of resistance to the treatment in patients may be considered minimal residual of Ph$^+$ cells. The best known cause of resistance is point mutations in the Abl kinase domain. Others putative mechanisms include genomic amplification of BCR-ABL and modulation of drug efflux or influx from the cells. However, in a sizeable number of patients the resistance mechanisms are still unknown. In this study we investigate imatinib-sensitive and -resistant cells derived from the original Kcl22 line. In these cells the resistance is innate and is not associated to mutations of Abl catalytic domain. The aim of our work is the study of the complex intracellular pathway of signal transduction and gene expression involved in the molecular mechanisms of resistance to imatinib. To this aim, we combined a proteomics and a gene expression profile approach. On one hand, we studied by 2D-DIGE technology the protein expression both in sensitive and resistant KCL22 cells to the imatinib. On the other hand, we studied the pattern of gene expression in the same cells by microarrays technology. Using Agilent microarray analysis we detected differential expression of 256 genes, many of these are associates to imatinib-resistant phenotype. Among genes down regulated in Kcl22r, there are phosphatase like SHP1 and DUSP6. They negatively regulate JAK/STAT and MAP Kinase signalling pathway which are associated with cellular proliferation and differentiation. Interestingly we also found down expression of the heat shock protein like HSP70-1B, HSP27 and HSP70-2; these molecules stabilize the structure of intracellular proteins and mediate the folding of newly translated proteins. The HSP70 protein has been recently shown to stabilize the inactive form of the oncogenic SHP-2 phosphatase. 2D-DIGE analysis detected 27 proteins whose levels are highly different in the couple of Ima- resistant and sensitive Kcl22 cell lines. Among these, Annexin A1 a protein involved in several cellular functions such as trafficking, exocytosis, apoptosis and cellular differentiation, seems to be implicated in the resistance to different drugs used in chemotherapy, including imatinib. These data indicate that the activation of alternative pathway(s), independently of Bcr/Abl kinase activity, may help CML cell to escape from imatinib action and may, therefore, correlate with disease progression.

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RADIOIMMUNOTHERAPY WITH 90Y IBRITUMOMAB TIUXETAN IN REFRACTORY/RELAPSED FOLLICULAR NON-HODGKIN LYMPHOMA RESULTS IN AN SPANISH CENTER

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Background. We are present our experience on the therapy with 90Y ibritumomab tiuxetan (90Y-IT) in an every day clinical practice protocol applied to refractory/relapsed Follicular non Hodgkin Lymphoma (NHLF), since commercial availability in Spain. *Methods*. Observational retrospective analysis of the efficacy and safety of 90Y-IT. Patients: 18 NHLF Period of study: september 2005 to december 2006. Variables: age, gender, time from diagnosis, number of previous schedulles, FLIPI, relapsed and stage at baseline, haemoglobine, leucocytes, platelets,. bone-marrow infiltration 90Y-IT was applied according an establish coordinate and uniform protocol. The effectiveness endpoints was evaluated by type of response: CR, PR, F, relapsed free survival (RFS) and overall survival (OS) and safety. *Results*. 18 patients were included, three of them were from other hospitals. M/F, 9/9; mean age 57.6 y (37-77); ECOG 0-1, 73.3%. Mean time from diagnosis: 4.2 y. 85% grade 1, 10% grade 2, 5% grade 3 NHLF. FLIPI distribution was: low-risk 53.1%, intermediate-risk 15.5% and high-risk 31.4%. Mean of previous schedules 3(1-7), administered dose 0.4 mCi/kg (12) and 0.3 mCi/kg (6). OR 85%. With a maximum follow-up time of 15 months, only 1 grade 3 NHLF relapsed at 5 months. Median OS has not been achieved. Safety: non immediate adverse events were appeared. Grade 2-3 neutropenia (nadir: +4±7 week; mean: 1.1×10^9/L range: 0.4-2.2), G-CSF were administered in 4 patients, grade 3-4 trombocytopenia (nadir: +4±8 week; mean: 70×10^9/L; range: 7-162), 3 patients required red blood cell packet (12 U) and 5 required platelet transfusions (40 U). Mean time to normalized blood parameters 20 days (range: 7-60). In two patients a neoplasia of colon and prostate were diagnosed in two and 4 months after received 90Y-IT and were considered as not related it. Any patient requires hospitalization. *Conclusions*. Despite the limitations of the small number of cases, our experience with 90Y-RIT for NHLF patients treated in an every day clinical practice setting are similar to that obtained in clinical trials

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REFRACTORY ANAEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH THROMBOCYTOSIS: RESULTS FROM A SPANISH MULTICENTRIC STUDY

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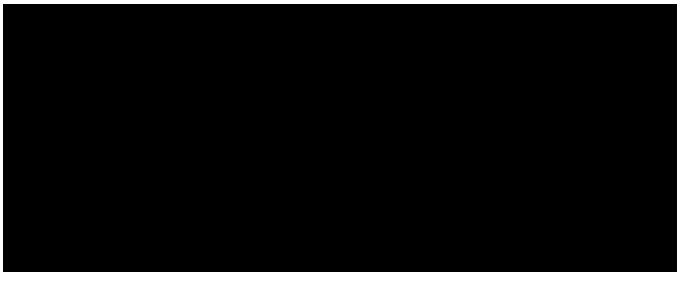
Introduction. Refractory anaemia with ringed sideroblasts associated with marked thrombocytosis (RARS-MT) constitutes a provisional entity among unclassifiable myelodysplastic/myeloproliferative disorders (WHO classification, 2001). A platelet (Plt) count above 600 ×10^9/L is necessary for its diagnosis. Our aim was to analyze the clinical and analytical features of a group of patients with RARS-MT, and to compare with those of patients with RARS associated with not-marked thombocytosis (RARS-nMT, Plt between 400 and 600×10^9/L). *Methods*. We retrospectively reviewed RARS cases associated with thrombocytosis, differentiating RARS-MT and RARS-nMT. Philadelphia chromosome and BCR/ABL fusion gene were excluded prior to diagnosis. Analyzed data included main features at presentation (clinical, biological and analytical), as well as clinical course (treatment, complications, survival). The whole studied data and parameters are described. *Result*. From 59 patients with RARS associated with thrombocytosis, collected from 16 Spanish hospitals, 30 cases fulfilled the diagnostic criteria of RARS-MT (74,6±8,2 years; 18 male, 12 female) and 29 were assigned as RARS-nMT (70,2±12,1 years; 16 male, 13 female). The main differences between both groups are expressed in the Table 1. Main motive for initial consultation in RARS-nMT patients was anaemic symptoms (50%), while most of patients with RARS-MT

were incidentally diagnosed (52%). There were not significant differences in age, presence of splenomegaly, presence of cytogenetic aberrations, basophil count, blasts (%) in bone marrow (BM), ferritin and B12 vitamin levels, transfusion requirements, and treatment strategies. All the results and statistical analysis are showed. *Conclusions*. In our experience, characteristics of patients with RARS-MT are nearer to that of myeloproliferative disorders (an elevated proportion of JAK2 mutations, a higher leukocyte count, BM megakaryocytic hyperplasia and increased fibrosis, a higher LDH concentration), while RARS-nMT cases showed more myelodysplastic features (none JAK2 mutation, a lower Hb level and a higher MCV, a higher percentage of ringed sideroblasts). Although we did not find differences in terms of survival, it seems that the number of platelets demanded for RARS-MT (>600 $\times 10^9$/L) clearly separate two distinct disorders.

Table 1.



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EVALUATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR, FIBROBLAST GROWTH FACTOR AND THROMBOPOIETIN SERUM LEVELS IN MYELOPROLIFERATIVE DISEASES

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Background. Nowadays, angiogenesis seems to be also involved, other than in solid tumors, in the pathogenesis of hematological malignancies, including acute and chronic leukemias, non-Hodgkin's lymphomas, Hodgkin's disease, multiple myeloma and chronic myeloproliferative diseases (CMD). Moreover, in these last pathologies an increased bone marrow angiogenesis seems to have important prognostic and therapeutic implications. In fact, a number of growth factors are involved in clonal hematopoietic expansion and their clinical significance in patients with CMD must be carefully evaluated. *Aims*. The aim of this study was to analyze some angiogenic factor levels in 80 patients suffering from CMD. Moreover, a correlation with clinical and laboratory parameters of the patients was investigated. *Methods*. Serum levels of Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (b-FGF) and Thrombopoietin (TPO) were assayed, by enzyme-linked immunosorbent assays, in 80 patients, with a median age of 56 years (range 29-65), nursed in Caserta's Hospital. 29 of them were affected by essential thrombocythemia (ET), 15 by chronic myeloid leukemia (CML), 19 by polycythemia vera (PV) and 17 by primary myelofibrosis with myeloid metaplasia (MMM). These patients were compared to 80 healthy sex-age-matched blood donors as control group (CG). *Results*. Serum VEGF levels were significantly increased in patients (mean±SD = 478.25±125.22 pg/mL) respect to the CG (134.17±42.28 pg/mL, $p<0.001$). The b-FGF levels also appeared to be significantly higher in CMD compared to CG ($p<0.05$). In the same way, TPO was significantly higher in CMD (9.4±1.3 pg/mL) than in CG (6.7±0.6 pg/mL, $p<0.05$). The highest concentration of VEGF, between the four groups of patients, was found in ET. Moreover, VEGF levels were significantly higher in CMD patients with vascular complications than in CMD without complications ($p<0.01$). A significant positive correlation was found between VEGF and platelet count in ET (r=0.54, $p<0.05$) and spleen index in CML. No correlation with other laboratory or clinical parameters was found. *Conclusions*. Our study confirms a significant increased angiogenic activity by measurement of circulating angiogenic factors. Thus cytokines may be useful markers for predicting clinical evolution of CMD. In particular, VEGF is a soluble, circulating, molecule that acts through receptor tyrosine kinases and it is the major proangiogenic factor that regulates multiple endothelial cell functions, including mitogenesis; but VEGF may probably have also a function distinct from its role in angiogenesis. b-FGF is a multifunctional cytokine that exerts positive regulation in hematopoiesis and that may also have a role in myelofibrosis and angiogenesis. TPO is a glycoprotein that primarily regulates megakaryocyte development and platelet production; increased TPO levels precede thrombocytosis and are caused by decreased expression of c-Mpl in ET and in idiopathic myelofibrosis. In addition, VEGF level, in this study, seems associated with increased risk of thrombotic complications; however the high TPO levels and the frequent thrombocytosis present in CMD could limit the interpretation of these data since platelets and megakaryocytes may be considered a major source for VEGF. In conclusion, a high level of cytokines are adversely associated with prognosis in this pathologies.

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ALTERNATIVE SPLICING TARGETS PBX3 MRNA FOR NONSENSE MEDIATED DECAY

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Background. The PBX family of genes encodes homeodomain-containing transcription factors which play important roles in embryonic development and haematopoiesis. PBX1, the prototypical member of the group, was first identified as an E2A fusion partner in cases of pre-B cell acute lymphoblastic leukaemia with t(1;19). Since then, three further PBX genes have been identified. It has previously been shown that PBX3 is over-expressed in acute myeloid leukaemia (AML). In particular, within the normal karyotype group of AML, PBX3 expression is higher in those cases with NPM1 exon 12 mutations. These findings indicate a potential role for PBX3 in the pathogenesis of AML. Alternative splicing (AS) of mRNA is frequently used by cells to generate protein diversity. Nonsense mediated mRNA decay (NMD) is an evolutionary conserved process that destroys mRNA transcripts containing premature termination codons (PTCs) that might otherwise generate toxic truncated proteins. In recent years it has been shown that the coupling of AS and NMD can provide an additional level of expression regulation for certain genes. Alternative splicing of PBX3 mRNA generates three shorter isoforms (PBX3B, PBX3C and PBX3D) in addition to the full length sequence (PBX3A). These shorter isoforms contain premature termination codons (PTCs) and it has been suggested that re-initiation of translation downstream of some of these PTCs allows truncated homeodomain-containing proteins to be generated. It is not known if these shorter transcripts are targets of the NMD machinery. *Aims*. 1. To study the expression patterns of PBX3 isoforms in AML cell lines and primary AML samples. 2. To look for evidence of re-initiation of translation by PTC-containing PBX3 isoforms. 3. To investigate if PBX3 isoforms are targets of the nonsense mediated mRNA decay machinery. *Methods*. The patterns of PBX3 isoform expression were studied using RT-PCR and gel electrophoresis. We highlight the limitations of real-time PCR in this situation. Protein synthesis by each isoform has been studied by overexpressing C- and N-terminal tagged PBX3 isoform constructs in the 293T cell line. Using Cycloheximide to inhibit the nonsense mediated mRNA decay machinery we have studied the effects on PBX3 isoform expression. *Results*. We have identified a fifth novel isoform of PBX3 which we have designated PBX3E. We show that only PBX3A and PBX3B generate homeodomain-containing proteins. Most importantly we show that both PBX3C and PBX3D are targets of the nonsense mediated mRNA decay machinery. *Summary/Conclusions*. We show that AS of PBX3 mRNA generates protein diversity and, by targeting specific isoforms for destruction by the NMD machinery, allows an additional level in the regulation of PBX3 gene expression. We believe this to be the first example of NMD regulating the expression of a homeobox gene.

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USE OF BLOOD PRODUCTS IN THE MANAGEMENT OF CHEMOTHERAPY INDUCED HEMATOTOXICITY IN NSCLC AND LYMPHOMA PATIENTS IN GERMANY

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Background. The growing number of cancer patients and the broad spectrum of emerging treatment options will further increase transfusion needs in Haematooncology, while the number of blood donors decreases. Therefore optimal use of blood is indispensable. Yet there are no studies illuminating transfusion practice in this group of patients. *Aims*. We examined the use of blood products for the management of chemotherapy induced hematotoxicity in the daily routine of an academic cancer center in Germany. *Methods*. Prospective observational

study with consecutive patients receiving single or multiple drug (immuno)chemotherapy as first or second line treatment for non-small cell lung cancer (NSCLC) or lymphoma. Patients undergoing high dose chemotherapy with PBSC support were excluded. Hematotoxicity (anemia, thrombocytopenia, leucocytopenia/ neutropenia) was monitored at each chemotherapy visit using the Common Terminology Criteria for Adverse Events. Consumption of blood products was collected from preplanned chart reviews. *Results*. 180 patients receiving a total of 633 chemotherapy cycles were evaluable. 85 patients (47,2%) had NSCLC and 95 (52,8%) lymphoma. Mean age was 58,7 years (range 17-84, median 61,5 years). During 67 chemotherapy cycles (10,6%) blood products were transfused to 49 patients (27,2%). Of the 310 units transfused 289 (93,2%) were standard preparations and 21 (6,8%) were cmv negative. With a total of 210 units red blood cells were substituted most frequently (64 cycles, 10,1%). Platelets and fresh frozen plasma were only transfused in 10 cycles (1,6%) and 4 cycles (0,6%) with a total of 49 and 51 units respectively. Most transfused patients (55,1%) received 1-2 units during the course of treatment. 17 patients (34,7%) were transfused with 3-10 units and 2 patients (4,1%) with 11-20 units. 3 patients (6,1%) received more than 20 units and accounted for 34,5% of the overall blood consumption. In two patients who were transfused with 24 and 74 units, a septic shock was the underlying cause of the high transfusion need during one chemotherapy cycle while in the third patient a dose intense chemotherapy regimen for T-lymphoblastic lymphoma caused a chronic transfusion need with a total of 27 units over 4 chemotherapy cycles. Red blood cell units (RBU) were mostly (54,8%) transfused at haemoglobin levels of 8,0-8,9g/dL. 78 of 210 RBU (37,5%) were given at a haemoglobin levels 6,4-8g/dL and 16 (7,7%) at haemoglobin levels 9,0-10,1 g/dL. 75,0% of all transfused platelet units were given when the patient´s platelet count was less than 11000/µL. 20,8% were transfused at a platelet count of 11-20000/µL and 4,2% at higher platelet counts. *Summary/Conclusions*. After chemotherapy for NSCLC and lymphoma blood products are transfused in more than 10% of chemotherapy cycles. While most of the transfused patients receive 1-2 TU, a small percentage of patients has a very high blood product consumption. In the episodes investigated transfusion of ≥20 units occurred related to sepsis or because of above-average dose intense chemotherapy. Under current practice patterns transfusion triggers for red blood cell and platelet transfusion seem to be in accordance with national and international guideline recommendations.

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EVALUATION OF ENDOTHELIAL FUNCTION IN PATIENT WITH SHEEHANS SYNDROME AND THE EFFECT OF HORMONE REPLACEMENT TREATMENT EXCEPT GROWTH HORMONE ON ENDOTHELIAL DYSFUNCTION

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Background and Aim. We aimed to examine the endothelial functions of patients with Sheehan syndrome (SS), a common cause of panhypopituitarism, and to eveluate the effects of *hormone replacement treatment except growth hormone* (HRTeGH) on endothelial functions. *Methods*. Twenty-four patients with SS aged with 40.83±6.43 years and 25 healthy control women aged with 41.13±6.51 years were included to study. Baseline endothelial functions evaluated in both patient and control groups. After treatment with prednisolon 5-7.5 mg/d, L-thyroxin 100-200 µg/d, and sex hormone replacement for patients under 40 years of age (conjugated estradiol 0.625 mg/d and medroxyprogesteron acetate 5 mg/d) for 15 months, the patient group reevaluated for endothelial functions. Endothelial functions were determined radiologically with high resolution ultrasond by evaluation of flow mediated dilatation (FMD) and biochemically with serum nitric oxide (NO) level. These data were compared by paired samples and independent t tests. *Results*. Before of HRTeGH blood pressure were lower and increased with HRTeGH (p=0,02 for systolic; and p=0,01 for diastolic), but it could not reach to the level of healthy control (p=0.001). The high pretreatment serum VLDL-cholesterol and tryglyceride levels of patients than controls (p=0,001 and p=0,01 respectively) did not change with HRTeGH (p=0,002 and p=0,04 respectively). Serum CRP level was higher in pretreatment patients than healthy control group (p=0,02), but it decreased to level of control group after HRTeGH. Pretreatment baseline and stimulated by FMD NO levels of patients were higher and baseline arterial diameter was smaller than healthy control group (p=0,0001). FMD stimulated NO level increment ratio and arterial diameter dilation ratio were also lower in SS group than healthy control group before treatment (p=0,0001 and p=0,003, respectively). The baseline and stimulated NO levels in SS group were higher than healthy control after treatment (p=0.0001). NO level increment ratio increased after treatment and reached to similar level of healthy control group. Arterial diameter was smaller in SS than healthy control group after treatment (p=0,0001); but FMD stimulated arterial diameter dilation ratio of patients increased and reached to the similar level of control group after HRTeGH. Baseline NO level of patients did not change with treatment but FMD stimulated NO level and the NO increment ratio was found as significantly higher after treatment (p=0,0001). Baseline arterial diameter did not change after treatment, but FMD stimulated arterial diameter dilation ratio increased significantly (p=0,0001). *Conclusion*. HRTeGH may have benefical effects on systolic and diastolic blood pressure, and serum CRP level in SS patients. Patients with SS appear to have endothelial dysfunction caused by inflammation, and HRTeGH may restore endothelial functions. Increased expression of eNOS caused by inflammation may responsible from endotheial dysfunction. Improvement in endothelium and decrement in CRP level have been thought that HRTeGH may have anti-inflammatory and may be anti-atherosclerotic effects.

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ANTI-2 GLYCOPROTEIN I IN CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA

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Background. ITP is referred to as autoimmune thrombocytopenic purpura because the etiology of the disease is not clarified and thrombocytopenia is caused primarily by the generation of antibodies against platelets antigens. Antibodies against phospholipid antigen (APA) have been demonstrated in the sera of ITP patients , but the precise role played by increased serum concentrations of APA in the mechanism of thrombocytopenia has remained elusive. *Objectives*. to keep an open mind about these antibodies could be an epiphenomenona or surrogate marker or directly involved in the cause-and-effect relationship of childhood immune thrombocytopenic purpura disease and study the predictive value of either elevated anti-ß2glycoprotein (anti- ß2GP1) or anticardiolipin antibodies (ACA)concentrations for secondary ITP detection and compare their levels to the steroid therapy responsiveness. *Design &Methods*. The study was conducted on 3 groups of children and adolescents . Group I consisted of 15 children with acuteITP(8males,7females) with mean age at examination 9.7+4.25 yrs., this group was sub classified into: Ia acute ITP in active disease (NO=4 cases) at the time of diagnosis, Ib acute ITP in remission stage (NO =11cases) , Group II consisted of 27 children with chronic ITP(12males,15 females), with mean age at examination 12.2+5.07 yrs, this group was sub classified according to response of steroid therapy into: II a: chronic & steroid dependent ITP (NO=17 cases) II b: chronic & steroid resistant ITP (NO =10 cases), Group III: 28 healthy controls(13 males and 15 females) matched in age and sex to patients groups.All children had thorough medical history and examination ,CBC , liver, renal function. and bone marrow aspirate was performed. Antinuclear antibodies (ANA), ACA (Ig M, G) and anti-ß2GP1 (IgG) were assessed in all studied children and adolescents. *Results*. There was a significant higher mean concentrations of ACA(IgM) (6.7±4.75 MPLU), ACA(IgG) (11.4± 5.52 GPLU) and anti-β2GP1(IgG) (79.5±62.0 U/L) in chronic ITP cases when compared to their corresponding levels in acute or control cases (p=0.000). About 77.8% of chronic ITP cases showed elevated serum concentrations of IgG ACA while increased serum levels of IgG anti-ß2GP1 in all (100%) chronic ITP cases was observed. A significant positive correlation between increased levels of IgG anti-β2GP1 and increased serum concentrations of IgG ACA was determined (r=0.42, p< 0.01). The detection of increased concentrations of IgG isotype of both ACA and anti-ß2GP1 was significantly correlated to steroid therapy resistance (r=0.54, p=0.000). About 76.1% (32/42) of ITP cases had positive APA including all chronic and only 5 cases from acute studied ITP children. Splenectomy was done in 28.1% (9/32) of ITP children with positive APA. Elevated serum concentrations of IgG isotype of either ACA or anti-β2GP1 in all 9 splenectomized ITP children with positive APA was observed, only 3 cases of them showed increased levels of IgM ACA. Interstingly, follow up of the enitially studied ITP children. During the period from 2000-2004 revealed that 16.7%(7 cases) proved to develop clinical & laboratory criteria of systemic lupus erythrematosus (SLE).The detected SLE cases consisted of one acute ITP child in remission and six children with chronic ITP. Elevated serum concentrations of IgG ACA was found at the start of the study in all 7 SLE cases ,but 6 cases (85.7%) from them had increased levels of anti-β2GP1 . *Conclusions*. In the light of the present results, it is emphasized that the assay of IgG class of both ACA and anti-

ß2GP1 may be considered as determinant cofactors for the developing risk of antiphospholipid syndrome (APS) or autoimmune diseases in ITP patients. Moreover, great attention should paid to both assay as predictors for steroid therapy response. The presence of APA in ITP patients was not be relevant to the pathogenesis of thrombocytopenia. Nevertheless, further study are needed to address this important issue because of the small number of patients in our series.

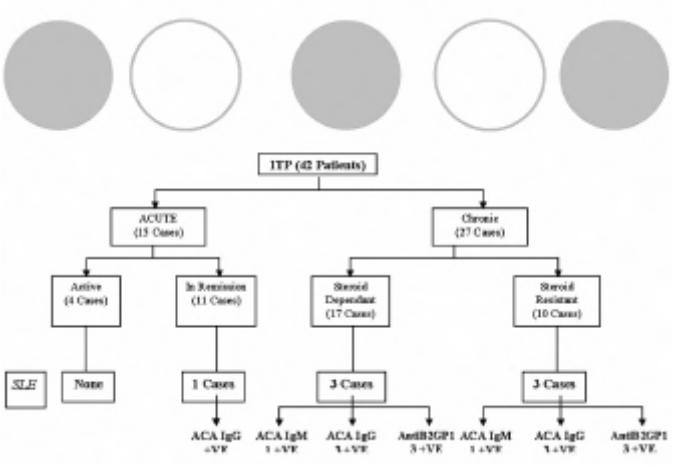

Figure 1. Results of follow up study for initially presented ITP patients to show those who developed SLE and their antiphospholipid antibosies status at start of the study.

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IN VIVO AND *EX VIVO* OXIDATIVE DAMAGE OF ERYTHROCYTE MEMBRANE PROTEINS IN HEREDITARY SPHEROCYTOSIS AND CPDA PRESERVED RBC: A COMPARATIVE STUDY

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Background. In conditions of increased cellular stress the membrane and the cytoskeleton of the RBC sustains certain modifications. HS is a heterogeneous group of disorders with regard to clinical severity, gene defects and mode of inheritance. The abnormal red cell morphology is due to a deficiency of, or a dysfunction in, spectrin, ankyrin, band 3 or pallidin. During storage in anticoagulant media, RBC membrane proteins undergo progressive alterations, oxidation, cross-linking and increased hemoglobin association, resembling HS. *Aims*. To determine the possible oxidation-related protein alterations and the oxidative index of the membrane ghosts and cytoskeletons in clinically diagnosed cases of HS and in CPDA-preserved non-leukodepleted RBCs units during storage. *Methods.* Twelve patients with clinical and laboratory diagnosis of mild (N=5) to typical HS [Sp(-)HS N=4, Sp/Ank(-) HS N=2, ank(-)HS N=1, B3(-)HS N=4, No(-)HS N=1, splenectomized N=2, concomitant carriers of α-thalassemia N=2] and twelve healthy subjects used as controls were examined in addition to RBC concentrates from six eligible blood units (each RBC unit was followed up during the whole storage period). Ghosts and cytoskeletons were analyzed by SDS-PAGE densitometry and probed for hemoglobin, IgG's and a variety of membrane proteins using human RBC-specific antibodies. Carbonylated protein content was determined after 2,4-dinitrophenylhydrazine derivatization and immunodetection of the DNP moiety. *Results.* Protein degradation, formation of high molecular weight aggregates and increased Hb and IgG's binding to the membrane were found in the majority of the HS patients examined, and in RBCs after 4 and 30 days of storage, respectively. The protein band-8 was also increased in 8/12 HS patients, half of which have membranes enriched in Hb and in RBCs after 15 days of storage. Probing of the HS and stored ghost membranes for Hb clarified that the membrane-associated globin was in the form of probably oxidized/denatured Hb or hemichromes. Subsequent analysis of the cytokeletons revealed pathologically increased amounts of skeleton-associated Hb monomers and high order aggregates, representing globin oligomers and complexes with membrane protein components, in the majority of the HS samples (10/12), and in the middle of RBCs storage period. Immunoblotting with dinitrophenol-specific antibody showed increased RBC membrane and cytoskeleton protein carbonyls in the 75% of the HS patients and soon after 10 days of storage in RBC units. *Summary/Conclusions*. The RBCs *in vivo* (HS) and *ex vivo* (CPDA storage) are characterized by oxidative alterations both in Hb and a variety of other membrane components and increased protein carbonylation levels. The increased binding of oxidized Hb to the membrane is expected to affect the flexibility of the membrane, to transmit the oxidative potential in individual membrane proteins and lipids and finally to initiate band 3 aggregation and other cell surface topography changes that are associated with events like vesiculation and erythrophagocytosis. These data corroborate the evidence for the occurrence of oxidative damage in membrane proteins both in HS and in RBCs storage, adding some new insight in the field of HS pathophysiology, as well as of the causes underlying the clinical variability of the disease.

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SEVERE THROMBOCYTOPENIA INDUCED BY PEGYLATED (PEG)-INTERFERON (IFN) PLUS RIBAVIRIN IN HEPATITIS C PATIENTS

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Background. The combinated therapies with interferon (pegylated or not) and ribavirin are the standard treatment of chronic hepatitis C and are associated with a high rate of sustained virologic response. Ribavirin is directly toxic to red blood cells and is associated with hemolysis while mild to moderate thrombocytopenia (and neutropenia) is a common adverse effect of interferon being, this circumstance, usually adscribed to bone marrow suppression. However, severe cases of thrombocytopenia have been very rarely reported in the literature. We present the cases of 2 patients who developed severe thrombocytopenia after beginning treatment with the combination of PEG-IFN and ribavirin. CASE 1: A 35-year-old man developed severe thrombocytopenia 9 months after beginning PEG-IFN and ribavirin combination for chronic hepatitis C. Platelet (Plt) counts fell up to 1×10^9/L. No hemorrhagic complications were observed. In bone marrow megakaryocytes were slightly decreased. PEG-IFN and ribavirin were stopped and i.v. immunoglobulins were started achieving a moderate but not sustained response (Plt 51×10^9/L) with subsequent fall (Plt 8×10^9/L). Corticosteroids (prednisone 1 mg/Kg/day) were later used obtaining a complete and sustained response (3 months after treatment cessation). CASE 2: A 35-year-old woman developed severe thrombocytopenia 10 months after beginning PEG-IFN and ribavirin combination for chronic hepatitis C. Plt counts fell up to 4×10^9/L. The patient presented metrorrhagia and platelet transfusions were administered twice without significant recovery. In bone marrow megakaryocytes were reduced. Antiplatelets antibodies were positive. PEG-IFN and ribavirin were stopped and i.v. immunoglobulins were started achieving a moderate but not sustained response (Plt 23×10^9/L) with subsequent fall (Plt 6×10^9/L). Corticosteroids (prednisone 1 mg/Kg/day) were later used obtaining a complete and sustained response (12 months after treatment cessation). *Conclusions*. Although very rare, PEG-IFN can produce severe thrombocytopenia. The mechanism of production could be variable but severe bone marrow suppression is rare and immune phenomena should be suspected in severe thrombocytopenia. The treatment of these cases could be performed with the classical options for idiopathic thrombocytopenic purpura however, in our experience, i.v. immunoglobulins do not produce sustained responses, and the best approach of treatment should be corticosteroids.

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IMPROVED β-THALASSEMIA GENOTYPING BY MEANS OF POPULATION-SPECIFIC REVERSE-HYBRIDIZATION TESTSTRIPS

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Background. β-thalassemia is among the most common inherited diseases throughout the Mediterranean area, parts of Africa, the Middle East, India and Southeast Asia. Mutations in the β-globin gene may lead to structural abnormalities (e.g. Hb S, Hb E, Hb C) or to haemoglobin imbalance due to the reduced synthesis or complete absence of β-globin chains. In each at-risk population β-thalassemia results from a limited number of common mutations and a larger, more variable number of rare mutations. *Aims*. Based on a previous reverse-hybridization assay (Beta-

Globin StripAssay) for Mediterranean countries, population-specific teststrips should be developed to improve β-thalassemia genotyping and make the test more globally applicable. *Methods*. Three separate teststrips, specific for the most prevalent mutations in Southeast Asia, the Middle East plus India and the Mediterranean region, have been designed. Each teststrip comprises 22 variants and represents an allele coverage of >90% in the respective area. Comprehensive β-thalassemia genotyping is achieved by a single multiplex DNA amplification reaction and subsequent hybridization to the adequate teststrip. *Results and Conclusions*. The test is simple and convenient, and requires only very small amounts of samples, which is of particular importance for prenatal diagnosis. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be automated using robotic instrumentation. Proprietary software (StripAssay Evaluator) is available to scan, interpret and electronically archive StripAssay results. The broad range of β-thalassemia mutations covered by the extended StripAssay should make it an attractive and globally useful diagnostic tool.

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PATTERN OF IRON CHELATION THERAPY IN EGYPTIAN CHILDREN WITH β THALASSEMIA: ONE YEAR MANSOURA UNIVERSITY CHILDRENS HOSPITAL EXPERIENCE

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Background. The simultaneous use of deferioxamine (DFO) and deferiprone (DFP) has an additive effect in iron excretion in transfusion-dependent thalassemic patients. *Aim of the Work*. The purpose of our study was to evaluate the effectiveness and safety of prospective combined therapy with deferoxamine (DFO) and deferiprone (DFP) in patients with β-thalassemia major and increased serum ferritin with DFO alone. *Patient and Methods*. Sixty patients with β thalassemia major (mean age±SD, 13.05±6.1, range 3'20 years) attending the outpatient clinic of the hematology unit for regular transfusional support were studied. They received packed red cells every 3-4 weeks to maintain pretransfusion hemoglobin concentration above 9 g/dL. They had been receiving DFO at a daily dose of 40 mg kg/day by subcutaneous infusion for 8-10 h on 4-5 nights each week for the past several years. However, due to various reasons, they had developed considerable transfusional iron overload. These patients were randomly assigned either to prospectively receive additional therapy with oral iron chelator DFP at 75 mg kg/day in three divided doses with food for 4-6 days per week after informed consent (combined therapy group, no= 30) or to receive treatment with DFO alone as per the above dosage (DFO group, no=30). The follow up of both groups was done for one year. The comparison between both groups was assessed by measurements of serum ferritin, echocardiography, and 24-h urine iron excretion levels. *Results*. At the start of the study, both groups were comparable as regard age, Hb level, chelation status, number of splenectomy, serum ferritin, echocardiography, and 24-h urine iron excretion levels. Thus, in the 60 evaluable patients {12 months on therapy}, the mean serum ferritin (±SD) fell dramatically from 4,150 (±1,250) ng/mL at the start of the study to 1,250 (±750) ng/mL (combined therapy group; $p<0.001$) at the end of the study. There was also a significant improvement in the myocardial function as assessed by the ejection fraction ($p<0.002$) and fractional shortening ($p<0.01$) in those patients on combined therapy for 1 year. Their mean urinary iron excretion elevated from 0.41±0.27 to 0.76±0.49 mg/24 h ($p>0.003$). Also, there was a significant difference between both groups as regard the studied parameters at the end of the study. Meanwhile, there was no statistical difference as regard the studied parameters at the start and the end of the study in the DFO group. *Conclusions*. This study emphasizes that β thalassemia major patients with transfusional iron overload can be successfully treated with a combination of DFO and DFP. Furthermore, these improvements lead to a progressive fall in the mean serum ferritin. Lastly, the study also demonstrates significant improvement in the echocardiographic parameters of myocardial performance in these patients receiving combination therapy.

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MANAGEMENT OF IRON OVERLOAD IN β-THALASSEMIA PATIENTS: SAFETY AND EFFICACY OF SUBCUTANEOUS BOLUS INJECTION OF DESFERRIOXAMINE AND BLOODLETTING

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Background. Iron overload remain a substantial challenge in thalassemia major. Standard iron chelation therapy with Desferrioxamine (DFO) is the reference treatment and it has to be infused for 8-10 hours, 5-7times a week. However this chelation is limited in developing countries, related to a lack of access to drugs and pumps and a poor compliance. *Aims*. This prospective study was designed to evaluate safety and efficacy of bolus injection of DFO and bloodletting in patients with β-thalassaemia intermedia (TI) and major (TM) with good response to Hydroxyurea (HU) , in order to insure good chelation, improving acceptance of this therapy and maintaining a good quality of life. *Patients and Methods*. We enrolled 23 patients (14 male, median ages: 19yrs, range: 11-55). Twenty patients received previously Hydroxyurea treatment, 18 with TM and 2 with S/B-thalassaemia, associated with decrease of transfusion requirements. Stopped Reed Blood Cell transfusion (RBCt) was obtained in 13 of them. Two patients with TI had received only sporadic transfusions (less 10 blood units throughout their life), and 1 patient with hereditary hemochromatosis. Starting chelation is recommends: serum ferritin >1000 ng/mL, patients who have received > 20 RBCt. Bloodletting is allowed when Hemoglobin level>8 g/dL. Median follow-up was 18± 8months. Mean ferritin was 5216±3973 ng/mL (range: 1300-18360). Thirteen patients received DFO alone; mean dose was 30±6 mg/kg/day, 2 injections/d, 3 to 5 d/week. Six patients underwent bloodletting (mean: 20, 200 to 300 mL/session) and 4 patients received combination DFO and bloodletting. Serum ferritin has been monitored every 3 months, performed by Elecsys 2010(Roche, Hitachi). *Results*. After 1 year therapy the assessment of all patients shown that the serum ferritin had fallen from 5216 ng/mL to 3080ng/mL (p: 0.03).The ferritin decrease below 1000 ng/mL in 4 patients. The serum ferritin level was lower in 13 patients receiving DFO alone: from 6278 to 4777ng/mL. A clear decrease showed in 6 patients with bloodletting alone: from 2634 to 1408 ng/mL and also with combination in the others 4 patients: from 2840 to 1769 ng/mL. The compliance was good. Side effects were observed: local reactions such indurations 3, skin nods 2, skin abscess 1.Transient arterial hypotension 1 after bloodletting. *Conclusions*. The reduction of transfusional needs in β-thalassaemia patients treated with HU, the use of subcutaneous bolus injection of DFO instead of cutaneous infusion and bloodletting performed in some patients, could be a good alternative for the management of iron overload. Add of oral chelating drugs may improve the quality of this chelation.

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MOLECULAR CHARACTERIZATION OF HETEROZYGOUS B-THALASSAEMIA IN LANZAROTE (CANARY ISLANDS)

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Background. β thalassaemia is a common genetic disease but its incidence and the relative frequency of different alleles vary among the populations. Objective: To know the prevalence of β thalassaemia trait in Lanzarote and to determine the molecular defects affecting the β globin gene in the heterozygous state. Patients and *Methods*. A epidemiologic cross-sectional observational investigation has been undertaken in 22560 samples with microcytosis (mean corpuscular volume<70 fl) collected from population living in Lanzarote The haematological parameters were measured using a Coulter automated blood cell counter (Coulter Electronics, Hialeah, Fla., USA). The quantification of HbA2 and HbF was done by microcolumn chromatography and radial immunodifusion respectively. Hb analysis was carried out using haemoglobin electrophoresis at different pH, isolectrofocusing (PhastGel IEF media) and cation-exchange high-performance liquid chromatography (HPLC) (Variant™; Bio Rad Laboratories, Hercules,CA,USA). DNA was isolated from peripheral white cells with phenol-chloroform. Molecular screening of β thalassaemia was performed by a rapid real-time PCR method using specific fluorescently labelled hybridization probes (RT-PCR Lyghcycler) (Roche Diagnostics, Manheim Germany) to detect the mutations prevalent in the Mediterranean area. For samples with mutations which could not be determined by real-time PCR, DNA analysis was carried out both by PCR-ARMS and DNA sequencing using an ABI Prism™ automated DNA sequencer (Perkin-Elmer Biosystem, Norwalk, Conn,USA). *Results*. Heterozygous β thalassaemia was detected in 202 subjects from 78 unrelated families with a global prevalence in Lanzarote of 0.17%. Of 202 thalassaemic subjects, 99 (49,8%) had a familiar origin distinct from Lanzarote and 55 (27,2%) were originating from foreign countries. Four alleles were not identified due to the limited amount of DNA. A total of 12 different mutations and 1 deletion were

characterized in 198 β thalassaemia alleles (98.01%). The distribution of four mutations identified by RT-PCR in 83.6% of the thalassaemia chromosomes was: [99 carriers of codon 39 (C--T); 34 of IVS-1-nt-110 (G--A), 19 of IVS 1-nt-1 (G--A) and 17 of IVS 1-nt-6 (T--C)]. The remaining 8 mutations were represented in 33 alleles (16.4%) at frequencies ranging between 7.1% and 0.5%. These molecular defects include the deletion 619 bp (7.1%), related with population with origin in India and Pakistan and the mutations -28 (A--G) and IVS1-nt-2 (T--G) originating from China detected in two Chinese carriers, the codon 41/42 (-TTCT) was detected in a Tunisian woman and the mutation codon 8/9 (+G) previously reported in Spain. This is the first report of three following β thalassaemia mutations codon 51 (-C), codon 22 (G--T) and codon 24 (T--A) in Spain, and the first description of novel mutation frameshift CD 20/21-TGGA in anywhere before. *Conclusions*. The distribution of the mutations in Lanzarote is similar to found in Eastern Spain and Mediterranean area. The important migratory flow received in Canary Islands during last years may explain the emergence of mutations not reported before in our area. The increasing incidence of β thalassaemia trait and the information about the β thalassaemia mutations justifies the implementation of screening of heterozygote carriers and prevention programs.

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FIRST CASE OF UPSHAW-SCHULMAN SYNDROME REVEALED AT EARLY ADULTHOOD IN A PATIENT OF WHITE ARABIC NORTH AFRICAN HERITAGE

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The congenital form of thrombotic thrombocytopenic purpura (TTP) or Upshaw-Schulman Syndrome (USS) is a very rare but life-threatening disease due to autosomal recessive severe deficiency of Von-Willebrand factor-cleaving protease (ADAMTS 13).There's a striking age-dependent clustering of the first TTP attack. Half of the patient suffered from their first bout of TTP between the first day of life and the age of about five years (early onset), while the other half remained asymptomatic into early adulthood and suffered from a first acute TTP episode at the age of 20-40 years (late onset). Symptoms in adults develop in association with the stress of infection or pregnancy. We report herein a case of an USS revealed at early adulthood, misdiagnosed initially as immune thrombocytopenic purpura (ITP).A 17 year's old man was admitted in December 2002 in the Hematology department of Aziza Othmana University Hospital of Tunis with pallor, epistaxis, skin petechiae and ecchymosis.He has a sister (22 yr) with medical history of Rosai-Dorfman disease diagnosed at the age of 2 yr. Laboratory studies showed: WBC= 13,000/mm^3, regenerative anemia with hemoglobin level at 8,5g/dL and thrombocytopenia with platelet count (Plt) at 21,000/mm^3.Serum creatinine and serum bilirubin was normal.Coombs test was negative. Peripheral blood smear showed rare platelets and no schizocytes.Serology for hepatitis B, C and for VIH were negative. Bone marrow aspiration revealed increased number of megacaryocytes.The diagnosis of ITP was considered and he received prednisone :1mg/kg/d leading to complete correction of anemia and persistence of a mild thrombocytopenia between 50,000 and 100,000/mm^3 despite discontinuation of corticotherapy.In June 2005 he presented an episode of fever and diarrhea followed by hemolytic crisis (Hb=7,7 g/dL) and severe thrombocytopenia (Plt=13,000/mm^3), hyperbilirubinemia 107 µmol/L, and elevated LDH=1095UI.Coombs test remained negative and schizocytes were absent. This episode didn't respond to scheduled cycle of Dexamethasone 20mg/m^2/d1-d4,and the patient developed an ischemic cerebral accident (August 2005) salvaged with Polyvalent Immunoglobulin :1g/kg/d for 2 days and a plasmapheresis without plasma infusion leading to normalization of Plt at 245,000 mm^3.He remained in complete response until developing another hemolytic crisis (Hb=8.7g/dL) and acute renal failure :serum creatinine at 290 µmol/L in November 2006.Peripheral blood smear showed for the first time schizocytes (16%). Assay for ADAMTS13 activity taken before plasma exchange was <1% without the presence of inhibitor. Partial hematological (Hb=10 g/dL, Plt=110000/mm^3) and renal function (serum creatinine: 150µmol/l) recovery was obtained with repeated plasma-exchange and Fresh Frozen Plasma infusion. To our knowledge this is the first such case reported of USS in young adult of white Arabic North African heritage and this can lead perhaps to the diagnosis of a new mutation in ADAMTS13 gene since over than 70 mutations have been reported with a majority of Asiatic and European family.This observation also suggests that there are other factors in conjunction with severe deficiency of VW protease activity that participate in the platelet-mediated thrombotic complication and other disease manifestations of congenital TTP.

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IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD: 10 YEAR OVERVIEW

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Background. Idiopathic thrombocytopenic purpura (ITP) is a common bleeding disorder in childhood due to reduced platelets, can be either acute and self limited or refractory and chronic. *Aims, Methods*. The aim of the present study was to evaluate all patients with diagnosis of ITP treated or followed over a 10 year period at the Pediatric Clinic in Skopje which is the only institution that provides tertiary care and copes with this problem in our country. *Results*. Over the past 10 years (from 1997 to 2006) 223 children aged 2 months to 15 years have been diagnosed with ITP and followed up. Acute ITP remitting within 6 months occurred in 202, and 21 had a chronic course. Sex incidence in the acute ITP is equal (m:f=100:102) and the most cases in this group occur in children aged 2-10 years - 113 (55,9%). The mean platelet count on admission was 21×10^9/L, lowest count 0×10^9/L. Bone marrow aspiration was performed almost in all cases. Serious bleeding symptoms were registered in just 17 (8,4%). No patient suffered an ICH, and no death was reported. Initial management consisted of no drug treatment in 12 patients (5,9%), intravenous immunoglobulins (IVIG) in 19 (9,4%) and glucocorticosteroids (GS) in 171 (84,7%). IVIG were used just in infants as they were expensive form of treatment. The majority of children with acute ITP recovered without recurrence. In 13 (5,8%) of patients resolution of the initial disease was followed by recurrent episodes of thrombocytopenia. Chronic ITP occurred in 21 (9,4%) of the children with ITP, was more common in older children >10 years (33,3%) and in females (66,7%). Most received GS and/or IVIG, 3 were splenectomised. Although the course of chronic ITP was generally mild, 15 (71,4%) had some serious bleeding episodes, and 1 child died from ICH. *Conclusions*. Annual incidence of acute ITP in our country is 4,0/100000 children under 15 years (for chronic ITP it is 0,4/100000). Serious bleeding in acute ITP is uncommon. Fatal ICH in our study (in a patient with chronic ITP) is low (0,4%).

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RITUXIMAB FOR REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE

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Background. Refractory Immune Thrombocytopenic Purpura (ITP) is a difficult disease to treat effectively, with a mortality approaching 10% over 10 years. Rituximab is a chimeric monoclonal antibody targeting CD20 antigen on neoplastic and normal B cells, approved for use in B cell malignancies as well as rheumatoid arthritis. Its use in other autoimmune diseases remains investigational. In ITP it has been used in several scenarios, even as an splenectomy sparing strategy. *Methods*. We report here our experience on eight patients treated with rituximab for refractory ITP. The drug was used off label after informed consent. All patients were refractory to steroids (at a maximum dose of 2 mg/kg of prednisone per day) and intravenous immunoglobulins. Only one of the patients had undergone splenectomy before the administration of rituximab. Schedule of the drug was 375 mg/m^2 weekly for a total of four doses. A patient was considered to have reached a response when a platelet count of at least 50×10^9/L was achieved in the 120 days next to the first rituximab administration, in the absence of bleeding symptoms and provided no other effective therapy was administered in that period of time. *Results*. Responses were achieved in six out of eight patients. Median time to response was 38 days, ranging from 12 to 68 days. One of the non-responding patients was rescued with splenectomy, while the other one remains unresponsive. No significant side effects occurred with rituximab administration, allowing an outpatient approach in three patients. Four of the responding patients maintain normal platelet counts, but follow-up is too short to state a median response. To date, the longest response achieved has been 40 months in one patient. One of the two patients who relapsed is responsive to steroids, requiring high doses to maintain a safe platelet count. *Summary*. In our experience, rituximab is a safe and effective therapy for refractory ITP, and can achieve long last-

ing responses; its use can even spare splenectomy. Obviously, further studies are needed to state effectiveness in several groups of ITP patients

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CHARACTERIZATION OF A SEVERE FORM OF α-THALASSEMIA. HB AGRINIO [α2 29(B10) LEU>PRO] HOMOZYGOUS

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Background. α-thalassemias constitute a group of diseases of genetic origin, caused by a diminution or absence in the synthesis of the α chain of globin. The great variability in its expression phenotypic is determined by its molecular complexity that even includes from silent cases to dependent forms transfusion and incompatible forms with the life. *Aims*. This work presents a case of disease of severe Hb H, caused by a hyper-unstable hemoglobin variant of α chain (Hb Agrinio) in state homocigous. *Methods*. Propositus is a boy 2 years-old of gypsy ethnic group, who from her birth presents a microcytic anemia with high transfusional requirements (haematological data to the birth: Hb 8.4 g/dL, Hto 28.4%, MCV 71.1 fL, MCH 21.1 pg, MCHC 29.7 g/dL). The study extended its ancestors. The study of hemoglobins was made by electrophoretic methods at alkaline acetate of cellulose to pH (pH 8.6), isoelectrifocusing (IEF) in polyacrylamide gel (pH 5.5-8.5), in agar citrate (pH 6.0), HPLC of ionic exchange and for chains of globin by HPLC of reversed phase. The stability of the hemoglobin was determined by means of the test of isopropanol. The HbA2 was determined by chromatography of anionic exchange, the quantification of Hb F was done following the Betke's method. The inclusion bodies were determined with incubation during one hour with brilliant blue cresil. The molecular study to discard a deletion α-thalassemia was made by Southern blot, with enzymes of restriction Bam HI and Bgl II and the probes α (1.5 Kb. Pst I) and zeta (1.8 Kb. Sac I). The molecular analysis was completed with the automatic sequencing of products of amplification by PCR of the genes α1 and α2 with the Kit of reaction ABI Prism dRhodamine Terminator Cycle Sequencing Ready (PE Applied BioSystems, Foster City, AC) and the sequence of reaction was analyzed in a sequencer ABI 310 Prism Genetic Analyzer (PE Applied BioSystems). *Results*. The molecular study demonstrated mutation CTG>CCG in codon 29 of exon 1 of the gene α2 in state homozygous. This mutation determines a change of leucine by proline in the α chain globin. *Summary/Conclusions*. The leucine in position 29 is located in Helix B of the α chain that it corresponds to very deep an internal zone of the quaternary structure. The change at this level by a proline determines a high instability of the quaternary structure with a precipitation and a fast catabolism of α29Leu?Probeta, which would justify its phenotypic expression like a thalassemia and that, is not detected by the electrophoretic and chromatographic studies. This case constitutes the second case of Hb Agrinio in state homocigous described in scientific literature and the first in Spain.

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AETIOLOGICAL SPECTRUM OF HYPERFERRITINEMIA IN A UNIVERSITY HOSPITAL: RETROSPECTIVE REVIEW OF 1111 CONSECUTIVE CASES

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Background. Elevated serum ferritin level (SFL) is a commonly identified disorder. This includes iron overload but also many pathological condition where hyperferritinemia is not related to increased body iron stores. *Aims*. In this study we aimed to assess the aetiological spectrum of high ferritin levels in a university hospital (950 beds) and explore the association between the etiology and the related level of hyperferritenemia. *Methods*. During a 3 months period (April 1st to June 30th 2005) we retrospectively reviewed the medical charts of 1111 consecutive patients (M: 625 F:486; median age 58 y) with hyperferritinemia (upper normal value F: >200 ng/mL; M >300 ng/mL) and/or a transferrin saturation (TfSat) > 45%. For statistical analysis patients were divided in 6 groups according to SFL (ng/mL) and Tfsat (Normal ferritin value and Tfsat >45%; upper normal values to 500; 500 to 1000; 1000 to 1500; 1500 to 2000; 2000 and more). *Results*. The most frequent pathologies being associated with hyperferritinemia were: elevated blood pressure (32,2%); hyperlipemia (20,25%); BMI>25 (15,6%), solid tumors (14,3%), infections (12,7%), alcohol abuse (11,8%); type 2 diabetes (9,45%). Over 1111 cases reviewed 681 (61,5%) had SFL below 500 ng/mL; in this group the most frequent associated pathologies were: elevated blood pressure, hyperlipemia, overweight and diabetes mellitus, diagnostic components of the metabolic syndrome. 311 cases (38%) had SFL between 500 and 1000 ng/mL; most of them presented with metabolic syndrome, inflammatory processes and low grade tumors. SFL between 1000 and 1500 ng/mL accounted for 5,3% (59 pts) and were mainly associated with infectious or inflammatory diseases or hepatic disorders. SFL over 1500 (5,3%) were mainly associated with solid tumors (the more elevated the SFL the more extended the neoplasia). Genetic analysis were performed in only 1% of the patients (80% of classical hemochromatosis). 3/1111 cases remained unexplained. *Conclusions*. Most of the cases of elevated SFL remain below 500 ng/mL. In these patients the metabolic syndrome is the most frequent associated pathological condition.

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FEASIBILITY OF AUTOLOGOUS TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA (MM) PATIENTS RECEIVING THADD AS INDUCTION THERAPY

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Background. High-dose therapy (HDT) is now the standard care for MM patient younger than 65 years but it look potentially promising as l as effective This strategy seems to be feasible and effective in selected patients aged over 65 years. Nevertheless, in this setting it is not yet clear enough whether HDT has to be integrated or replaced with novel drug combinations. *Aim*. we assessed the feasibility and toxicity of autologous hematopoietic stem cell transplantation (HSCT) in MM patients receiving as front-line therapy the combination Thalidomide 100 mg/day, Dexamethasone 40 mg on days 1-4 and 9-12, pegylated liposomal Doxorubicin 40 mg/m^2 (ThaDD)on day 1 every 28 days. *Patients and Methods*. We studied 20 newly diagnosed MM patients treated with ThaDD regimen followed by allogeneic (2 patients) or autologous (18 patients) HSCT. Median age for 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. Median β2-microglobulin was 2.7 (range 0.3-9.3) while median CRP was 1.1 (0.5-10.7). *Results*. After induction therapy 5 patients (28%) achieved CR, 1 (5.5%) nCR, 9 (50%) VGPR and 3 (16.5%) PR. Overall, 14 patients (78%) underwent single and 4 (22%) double autologous HSCT. Conditioning regimen consisted of high-dose Melphalan (200 mg/m^2) in 10 cases (55%) and intermediate dose (100 mg/m^2) in the remaining 8 patients (45%). Median CD34$^+$ cells collected after high dose Cyclophosphamide and hematopoietic growth factors was 5.7×10^6/kg (range 2.8-11.3) whereas median CD34$^+$ cells reinfused resulted in 4.1×10^6/kg (1.2-5.6). Platelets and neutrophils recoveries were observed within 14 days; particularly, 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 13 days after first and second transplants, respectively. Only 3 patients (17%) developed neutropenic fever of unknown origin. Non hematologic toxicity consisted of grade 2 mucositis in 2 patients (11%) and grade 2 diahorrea in another one. No transplant related death was reported. Remarkably, post autologous HSCT 46% of patients showed improved response if compared with those achieved following ThaDD regimen. In particular, response to VGPR was obtained in 5 additional patients leading the final VGPR rate to 83%. After a median follow-up of 24 months, TTP, EFS, and OS at 2 years were 60%, 60% and 90%, respectively. Regarding the 2 patients undergoing allogeneic HSCT, one died at day +85 from secondary EBV lymphoma whereas the other one is still alive and in CR at day +103. *Conclusions*. Our data suggest ThaDD does not negatively impact the ability to collect stem cells allowing a rapid hematologic recovery. Both high- and intermediate-dose melphalan can be safely administered in selected older patients without severe toxicities. The good quality of response with ThaDD regimen can be further improved by transplant procedures. It will be interesting to compare the outcome of patients receiving ThaDD as front line therapy who had or had not received transplant subsequently.

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PLATELET AGGREGATION IN CHILDREN WITH HELICOBACTER PYLORI INFECTION

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Background. Helicobacter pylori (H. pylori) infection is the most common gastrointestinal bacterial disease worldwide. Although it generally causes an asymptomatic infection, H. pylori facilitates the development of various gastrointestinal pathologies and also the development of different disorders such as iron deficiency anemia, pernicious anemia, autoimmune thrombocytopenia, coronary artery disease, stroke and growth retardation. *Aims*. This study was conducted to investigate the effects of H. pylori infection on platelet aggregation. *Methods*. Platelet aggregation in platelet-rich plasma induced by ADP (adenosine diphosphate), collagen, ristocetin and epinephrine was studied in 32 patients aged between 6 to 15 years before and one month after H. pylori treatment. Tests for complete blood count, ferritin, prothrombin time, activated partial thromboplastin time, fibrinogen, C-Reactive Protein and von Willebrand Factor levels and platelet aggregation studies were performed in patient and control groups. Platelet aggregation in platelet-rich plasma was studied twice in the patients prior to and one month after the treatment and once in the control group. *Results*. The pre-treatment maximum aggregation values induced by 10 microM ADP were significantly lower than values of post-treatment and control groups (62.76±13.89 vs 78.16±15.21 and 62.76±13.89 vs 80.93±10.84, respectively) (p<0.0001). Aggregation responses to collagen, ristocetin, and epinephrine were not different from those of post-treatment and control groups. *Summary*. Our study shows that H. pylori infection causes disordered platelet response to (ADP-like defect), but could be corrected with treatment. The derangement in platelet functions caused by H. pylori infection can be explained with stimulation of nitric oxide synthesis, and inhibition of platelet function by increased production of nitric oxide by means of signal transmission through thromboxane A2 and intracellular calcium (Ca^{++}).

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IMMATURE PLATELET FRACTION (IPF) UTILITY IN FOLLOW UP ON PATIENTS RECEIVING CHEMOTERAPY

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Reticulated platelets are the youngest platelets in the blood flow, contain RNA and can be measured by flow cytometric methods. The number of reticulated platelets reflects the rate of thrombopoiesis, increasing when platelet production rises and decreasing when production falls. Reticulated platelet percentage or IPF can now be counted employing the XE-2100 blood automated cell counter with upgraded software (Sysmex, Kobe, Japan). Investigators have communicated that patients receiving cytotoxic chemotherapy or undergoing stem cell transplantation show a rise of IPF several days before engraftment. Some events, such as fever, can produce an IPF rise, and platelet transfusion a decrease.

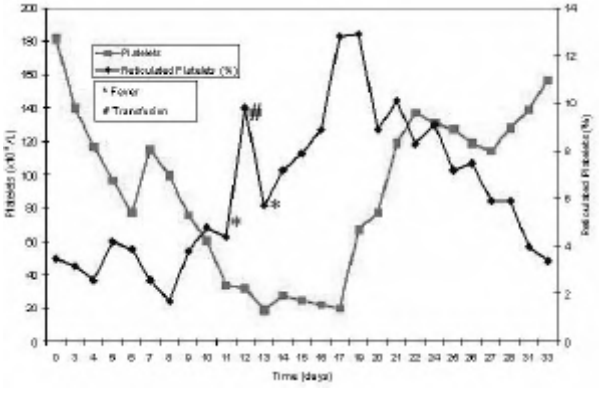

Figure 1.

The aim of the study was to determine normal range of IPF in our population, in the platelet apheresis product and to follow patients with chemotherapy regime daily in order to establish the normal IPF and compare it to oncohematologic patients evolution. *Material and methods*. 92 peripheral blood (PB) healthy donor samples and 55 platelet apheresis product samples were analysed in the Sysmex XE-2100 within 4 hours from collection. PB samples from 9 patients receiving chemotherapy were processed daily, until platelet recovery. *Results*. IPF mean and range were 1.65 (0.-7.4) and 3.0 (0.4-11) for PB and platelet apheresis respectively. We find differences between the two groups but not when comparing apheresis platelet count and apheresis IPF product. IPF was below normal range during the aplasia phase and started to rise 2-4 days before platelet recuperation. As well as other authors, an IPF decrease was found when patients receiving chemotherapy were transfused, and showed a slight increase when fever appeared in six out of the nine patients (Figure1). *Conclusions*. Our results suggest that IPF might be useful in the follow up of patients receiving chemotherapy in order to achieve a better understanding of thrombopoiesis during aplasia phase. We could guess platelet recuperation some days before rising and probably spare the last platelet concentrate transfusion. However, other factors could influence thrombopoiesis in this kind of diseases, specially in patients with stem cell transplant regime.

More samples must be studied to confirm these results.

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PHENOTYPE/GENOTYPE IN SIBLINGS WITH WISKOTT-ALDRICH SYNDROME

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Background. The Wiskott-Aldrich syndrome (WAS) is a severe X-linked disorder characterized by immune disregulation, microthrombocytopenia and eczema and, progressively, high risk of autoimmune and lymphoproliferative disorders. The researchs of pathogenesis have shown an identical gene mutation both in WAS an in a milder condition, X-linked thrombocytopenia (XLT). The protein product of gene WASp maps to chromosome Xp11.22-p11.23 and consists of 12 exons in a 9kb region. This is a cytoplasmatic protein expressed in haemopoietic cells and plays a key role in cytoskeletal organization. *Aims*. The different severity of the clinical disease and the frequent improvement of clinical course in some patients lead to verify the genotype/phenotype relationship. *Methods*. - Two siblings with WAS were studied. First patient is now 35 years old, had a severe phenotype. He showed during the first decade of life severe eczema, microthrombocytopenia with frequent bleeding, severe repeated infections, continous vasculitis in the lower extremities and glomerulitis, low IgM levels (score 4). Successively he experienced progressive improvement, moderate infections and more rare vasculitis in the extremites, rare and moderate bleeding. The brother is now 29 years old, had mild microthrombocytopenia, few petechiae, mild eczema, but no history of infections (score 2). He is now in good health and he shows moderate thrombocytopenia without bleeding.Analysis of the WASp gene mutations was performed as reported by Derry *et al*.(1995). Detection of WASp was performed by immunoblot analysis as described by MacCarty *et al*. (1998). *Results*. The mutation causing WAS in both siblings was a 6-bp insertion in exon 4 of WASp. *Conclusions*. Extensive efforts have been made to link genotype to phenotype. However, there are many patients with severe WAS who present the same genotype as individuals with milder disease (XLT). The coexistence of other biological or environmental factors could influence the biological effects of WASp gene mutations.The marked difference in clinical severity between these 2 siblings who have identical gene mutations and protein profiles, and the improvement of clinical course in the older brother suggest that other and distinct parameters must influence the phenotype.

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DEFERIPRONE (DFP) IS AN EFFECTIVE ORAL IRON CHELATOR IN PATIENTS WITH HEREDITARY HAEMOCHROMATOSIS (HH) FAILING, OR INTOLERANT TO, VENESECTIONS OR DESFERRIOXAMINE (DFO) THERAPY

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Background. Hereditary Haemochromatosis (HH) is an inherited iron loading disorder associated with excessive iron accumulation and damage to organs and tissues such as the heart, pancreas, liver, skin and joints. The commonest method employed for removing iron stores in patients with HH is by venesecion. However, some patients are unable to tolerate the intensive regimen required to achieve optimal reduction in iron stores. Those failing venesection are most commonly offered sec-

ond-line therapy with Desferrioxamine (DFO). This has several toxicities and requires considerable compliance and lifestyle modifications which many patients find unacceptable. Deferiprone (DFP) is an oral iron chelator approved by the European Regulatory Authority in 1999 as second line therapy for transfusional haemochromatosis in patients with thalassaemia unable to tolerate DFO. It is generally well tolerated but its major side effect is the idiosyncratic development of agranulocytosis. There is no literature on the off-license use of DFP to lower iron stores in patients with HH despite there being a good pharmacobiological reason to suggest this may be beneficial. *Aims*. To assess the efficacy and tolerability of DFP in lowering iron stores in patients with HH failing, or intolerant to, venesection and DFO at our institution. *Methods*. We retrospectively reviewed clinical outcomes in all patients with HH at our institution who were treated with DFP to lower iron stores following failure, or intolerance to, venesection or DFO therapy. *Results*. The results are summarised in Table 1. Seven patients with HH (M=6, F=1) were eligible for inclusion in the study which covered a period from October 2004 to February 2007. Their average age was 60 years (range 42 - 70 years). All but one were homocygous for the C282Y mutation. The remaining individual (patient 2) was an H63D heterogygote. The average serum ferritin on commencement of Deferiprone was 1718 microgram/litre (range 633 - 4929 micrograms/litre). DFP was commenced up to the recommended dose of 75 milligrams per kilogram per day, which equated to total daily doses of 0.5 grams - 6.0 grams. Doses were modified according to clinical response and side effects. Serum ferritin levels were measured every 3 months for up to 12 months. The average serum ferritin level at 3 months, 6 months, 9 months and 12 months was 1485 (n=6 evaluable patients), 1242 (n=5), 1166 (n=5) and 1067 (n=4) micrograms/litre. This represented an approximate 38% reduction in ferritin levels with DFP at 12 months. Two patients developed reversible grade 2 neutropenia which necessitated dose reduction (patient 3) and discontinuation (patient 7). No infections were noted during this period. Patient 6 developed abdominal pain and diarrhoea, opting to discontinue therapy after less than 4 weeks. *Conclusion*. DFP appears efficacious at lowering iron stores in patients with HH who fail, or are intolerant to, venesection or DFO therapy, with low toxicity. Our observations merit longer follow up and support further large scale studies to evaluate the safety, efficacy, cost effectiveness and patient quality of life using oral DFP as standard second line therapy, following venesection, in patients with HH.

Table 1.

UPN	Age /Sex	Serum Ferritin (ug/l) Baseline	At 3 months	At 6 months	At 9 months	At 12 months	Side Effects
1	70M	1312	1277	-	-	-	Nil
2	51M	1402	902	864	610	618	Nil
3	67M	2231	1623	898	940	-	NTpenia (grade2)
4	69M	709	521	495	600	515	Nil
5	60M	812	438	269	190	19	Nil
6	42F	633	-	-	-	-	GI upset (grade 2)
7	63M	4929	4147	3682	3491	3117	NTpenia (grade2)

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THE HYPERCOAGULABILITY AND THE CHRONIC VASCULAR ENDOTHELIUM SUFFERING WITH THE ABNORMAL CYTOKINES RELEASE MAY AFFECT THE PROGRESSION OF THE OSTEOPATHY IN HOMOZYGOUS BETA-THALASSEMIA PATIENTS

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It is well known that a chronic hypercoagulation and vascular endothelium dysfunction determine frequent thromboembolic events in homozygous β-thalassemia(β-Th)patients.In this scenario,although the survival of these subjects is increased through the regular blood transfusions and the iron chelation therapy,a marked osteopathy becomes certain in adult β-Th patients.Generally, the β-Th osteopathy culminating in low bone mass and osteoporosis is mainly referred to the several endocrine deficiencies,while the contribution of the hypercoagulation and abnormal vascular endothelium cytokines release has not been considered in our opinion. Sicilian homozygous β-Th patients(6 females and 5 males), aging 24-66 yrs, all splenectomized on regular blood transfusions and iron chelation regimen,without renal,hepatic and metabolic dysfunctions were considered. Sicilian heterozygous β-Th subjects and 10 healthy individuals of comparable age served as controls.Bone density scans showed severely low bone mass in 7/11 and low bone mass in 4/11 respect to the control groups.The hosteoblastic cytokines net-work as the Platelet-derived growth factor(PDGF),Transforming growth factor-β (TGF-β)and Interferon-α (IFN-α) were assayed by ELISA(R&D Systems,Genova).The osteoclastic cytokines as the Interleukin-1 (IL-1), Interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α) were determined by ELISA.Our results showed a significant (p<0.001) increase of the osteoclast cytokines in homozygous β-Th respect to those observed in the controls groups. The osteoblastic cytokines were in normal range in all groups. Keeping in mind that the thromboembolic events frequently occur in β-Th patients,we suggest that the chronic red blood cells haemolysis,blood transfusions and iron chelation *quoad vitam* would determine continuously biochemical changes leading also to an abnormal release of several cytokines from injured vascular endothelium.The osteoclastic cytokines' formation is dominant over the osteoblastic one and by reducing bone mineral density would potentiate the osteoporosis in adult homozygous β-Th patients.In this scenario the chronic vascular endothelium suffering together with the hypercoagulability,leukocytes and platelet hyper-activation could enhance further the osteoclast cells functions in β-Th.

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RECURRENT PRIAPISM ASSOCIATED WITH HEREDITARY XEROCYTOSIS AFTER SPLENECTOMY A CASE REPORT

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Background. Dehydrated Hereditary Stomatocytosis (DHS) or Xerocytosis is a rare genetic condition characterized by red blood cell (RBC) abnormal membrane permeability. These RBC lose K^+ in excess of Na^+ gained with a decrease in total cation content, generating dehydrated and rigid xerocytes. DHS patients usually have a well compensated haemolysis, with high reticulocytes count, increased mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), stomatocytes in peripheral blood smear and an increased resistance to osmotic lysis. The diagnosis is supported by osmotic gradient ektacytometry studies showing a typical left shifted curve with leftward displacement of Omin and by quantification of intraerythrocytic cation concentrations and cation flux rates across the membrane, showing markedly increased RBC Na^{++} K^+ fluxes with low total cation (Na^{++} K^+) content. DHS has been associated with an increased risk for thrombotic events after splenectomy. Priapism, defined as the presence of a persistent penile erection unrelated to sexual stimulus, has a special and long known association with sickle cell disease. Other conditions have sporadically been pointed out, such as hyperviscosity syndromes and after splenectomy in thalassemia and some unstable haemoglobins (such as haemoglobin Olmsted) patients. Although its physiopathology is still not fully understood, the classical paradigm of veno-occlusive/venous congestion remains a plausible mechanism. Several investigators consider venous outflow obstruction of the corporal bodies and increased intracavernous blood viscosity as probable cornerstone events of ischaemic priapism. *Aims*. We report a case of a man with DHS and recurrent episodes of priapism after splenectomy. CASE REPORT A 50 years old man was referred to our centre for the etiologic diagnosis of a chronic haemolysis. He reported splenomegaly, lithiasic gallbladder and episodic haemolytic crises, since age 18, with jaundice and dark urine. At the age of 45, in a car accident, he had a spleen trauma and two years later, during elective cholecystectomy, his spleen has been removed. Since then, patient reports several episodes of priapism, three of which, documented as venous-occlusive (ischaemic), required surgical treatment. These episodes decreased in frequency and intensity when he started antiaggregant therapy with Acetylsalicylic acid and Dipyridamole. Patient has no evidence of other thromboembolic events, no history of drug abuse, antidepressants or other psychoactive drugs, and no geni-

to-pelvic or perineal trauma. No metabolic disorders were detected. CT scan and MRI showed evidence of a L4-L5 lumbar hernia, without signs of spinal cord injury. No history of consanguinity and no family history suggestive of haemolysis or perinatal oedema. Hb 14.5-15.4 g/dL, MCV 104-111 fL, MCH 34-39 pg, MCHC 35-35.5 g/dL, Reticulocytes 4.8-9.2%. Spherocytes and stomatocytes in peripheral blood smear. DAT negative. Persistent hyperbilirrubinemia. Negative screen for thrombophilic risk factors. Ecktacytometry studies confirmed the DHS diagnosis. *Summary/conclusions*. Being a rare condition, to the best of our knowledge, this is the first report of recurrent venous-occlusive priapism as a thrombotic manifestation of DHS after splenectomy. It emphasizes the need of an accurate differential diagnosis between DHS and other chronic haemolytic conditions when assessing the possible benefit of splenectomy.

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IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) PRESENTING IN A PATIENT WITH UNDIAGNOSED KLINEFELTERS SYNDROME

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Background. Klinefelter's syndrome is one of the most common genetic disorders (1 in 500-1000 live male births), typically caused by a chromosome nondisjunction that results in the presence of an additional X chromosome (47, XXY or XXY syndrome). The diagnosis of the syndrome is often delayed until puberty, while several cases remain undiagnosed. Klinefelter's syndrome exhibits a well-established association with malignant germ cell tumors and breast cancer in men, yet, there are limited reports on its effects on hemopoiesis. *Aim.* To report a rare case of idiopathic thrombocytopenic purpura in a patient with a previously undiagnosed Klinefelter's syndrome. *Case report*. A 20-year-old man was admitted because of epistaxis, a large ecchymosis on the left flank and bilateral symmetrical petechiae over his distal lower extremities. His history was otherwise unremarkable and he denied any recent trauma and drug use. Clinical examination also revealed obesity (BMI: 32 kg/m^2), sparse body hair, mild gynecomastia, small testes and tall stature (1.91 m) that was 24 cm higher than the mean height of the parents. The peripheral blood count showed only thrombocytopenia (platelets: 38000/mm^3), while the bone marrow examination yielded normocellular marrow with an increased number of megakaryocytes. Hormonal tests revealed low plasma testosterone levels with concomitant increased values of LH and FSH. Bone marrow cytogenetic analysis demonstrated an abnormal male karyotype with an additional X chromosome (47, XXY). *Results*. The patient was diagnosed with idiopathic thrombocytopenic purpura and Klinefelter's syndrome. The initial treatment consisted of high doses of intravenous immunoglobulin (IVIG) for five days and glucocorticoids that were tapered over the following 4 weeks. Clinical remission of the bleeding diathesis was documented by the end of the IVIG administration and the platelet count progressively rose to almost normal levels (platelets: 120000/mm^3 to 150000/mm^3). The patient remains asymptomatic four months after the diagnosis, with platelet counts consistently over 100000/mm^3, while he was also placed on testosterone replacement therapy for the hypergonadotrophic hypogonadism. Frequent follow-ups have been scheduled to monitor for recurrence of thrombocytopenia and other possible defects of hemopoiesis. *Conclusions*. Rare reports in the literature associate Klinefelter's syndrome with various hematologic malignancies, including acute and chronic types of leukaemia, myelodysplastic syndromes and lymphomas, yet, there are only two previous published cases of this genetic disorder combined with idiopathic thrombocytopenic purpura. The variable and often subtle phenotypic expression of the Klinefelter's syndrome explains why the diagnosis of this common chromosomal defect may first be made during the diagnostic work-up for hematologic malignancies. Acute isolated thrombocytopenia in patients with Klinefelter's syndrome, apart from idiopathic, may also indicate a preleukemic state, hence, meticulous monitoring is warranted in order to promptly detect any other possible underlying hemopoietic defects.

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REMISSION INDUCED BY INTERFERON α + THALIDOMIDE+ZOLEDRONIC ACID IN A PATIENT WITH MASSIVE HAEMANGIOMATOSIS OF BONE, MEDIASTINUM AND RETROPERITONEUM IN ADULT. A CASE REPORT

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Generalized haemangiomatosis in adults is a very rare disorder. The progression of haemangiomas may cause organ impairment and disseminated intravascular coagulopathy. The treatment modalities are in this disease rather limited. We are describing treatment of a young man, that induced remission on CT scans and disappearance of coagulopathy. The young man had been complaining since his 25th year of age about pain in backbone, pelvis and hips. In his 28th year of age he was send to hematological department with anemia for examination. CT examination of thorax and abdomen has shown pathologic mass in retroperitoneum and in mediastinum. Diagnostic laparothomy was made because of pathologic mass on CT examination and consequently diagnosis of haemangiomatosis was made. Later CT examination of backbone proved destruction of vertebrae Th 8, 10, 11 a 12. The CT signal was typical for haemagiomas. The patient has a massive coagulopathy with very low fibrinogen, high D-dimers as results of DIC. Inteferon α therapy started in June 2005, the dose at the beginning was 5-6 mil. IU 3time a week. After one year of therapy only a small regression was found on CT, but he was without bone pain he suffered from before the treatment. Combined therapy with interferon α 3 mil IU 3time weekly, thalidomide 100-200 mg daily and zoledronate began in June 2006. This therapy was stopped after half a year because this young man desired to have a child. Only small residual pathologic mass has been found in retroperitoneum on the control CT evaluations. The patient has normal value of fibrinogen and D-dimmers are only slightly elevated. *Results*. Interferon α and zoledronate administered for one year have only limited effect, the combination of interferon α, zoledronate and thalidomide led to near complete regression of haemangiomas and normalization of coagulopathy. *Conclusion*. Therapy with interferon α in combination with thalidomide and zoledronate is very useful therapy for generalized heamangiomatosis with mediastinal and abdominal pathologic mass and bone destruction caused by bone-heaemangiomas.

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OUTCOME OF FIVE PATIENTS WITH CHRONIC MYELOID LEUKEMIA AFTER IMATINIB MESYLATE DISCONTINUATION

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Background. Imatinib mesylate (IM) therapy is effective and leads to a complete cytogenetic response in the majority of patients with chronic myeloid leukaemia (CML). However, wheather it should be discontinuated in patients who achieve substained molecular response is debated. We describe five patients with undetectable levels of BCR-ABL transcripts in whom IM therapy was discontinuated. *Methods*. Between January 2001 and October 2006 we treated 53 CML patients (pts) with Imatinib mesylate: 38 were IFNα pretreated pts in late chronic phase and 15 were newly diagnosed pts in early chronic phase. 39 (74%) achieved a complete cytogenetic response and in 10 of them (26%) BCR-ABL transcripts became undetectable. In 2 case Imatinib was discontinued because of the pts intolerance; in 2 pts was discontinued because of toxic effects; in 1 patient was IM discontinued because of the patient request. All pts were pretreated with IFNα, no patient had a family donor for allotransplant or was a candidate for an unrelated transplant at the time of IM withdrawal. *Results*. All pts had been in subtained CCR for 12 to 23 months and in complete molecular response (CMR) for 9 to 18 months. All pts showed normal bone marrow and none of them had additional cytogenetic abnormalities. Three pts relapsed after 6,7 and 9 months and promtly responded after restarting imatinib therapy (400 mg daily). Two pts are off therapy at the last follow-up visit after 24 and 36 months and are still in complete molecular remission. *Conclusions*. Although the follow-up of our patients is short, the improvement quality of life while off therapy and the prompt response to resumed IM therapy suggest that the subset of patients who have sustained complete molecular response may be candidates for intermittent therapy. Future studies should determine the optimal duration of BCR-ABL negativity before IM therapy can be safety discontinued.

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COMPARISON BETWEEN SERUM AND ERYTHROCYTE FOLATE IN PATIENTS WITH ANEMIA AND/OR MACROCYTOSIS

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Background. The determination of folic acid (FA) is very important in the diagnosis of megaloblalstic anemias due to the lack of this nutrient in patients with anemia and/or macrocytosis. Detection of erythrocyte folate (EF) is even more relevant because it is a more reliable value of body FA reserves than serum folate (SF), due to the fact that this nutrient can not pass through the erythrocyte membrane. Furthermore, its value is not affected by common clinical situations such as chronic liver or kidney impairment, or even cobalamin (B12) deficiency. *Aim*. The aim of this study was to determine whether discrepancies between both determinations exist in our patients, and if according to these findings, EF determination should still be carried out. *Materials and Methods*. Values of EF and SF petitioned in our laboratory were compared during the last three months (a total of 100 patients with anemia and/or macrocytosis) focusing on whether discrepancies in both determinations were observed. In these cases, possible causes for this difference were analyzed. Samples were analyzed by an external laboratory through radioimmunoanalysis (RIA) in both determinations. *Results*. Discrepancies, understood as a normal SF value (2.2-17 ng/mL) together with a low EF value (<175 ng/mL), were observed in 10% of the patients. B12 deficiency was observed in 6 cases, and on the other 3 cases chronic clinical conditions as well as hemoglobinopathies were detected. *Conclusions*. According to our results it would still be justified to determine EF concentration, specially on those cases were B12 deficiency or chronic clinical conditions coexist, because when not done, low folate levels would go unnoticed, and these patients would not receive the appropriate treatment.

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HERPES GROUP VIRUS INFECTIONS AND GRANULOCYTOTOXIC ANTIBODIES IN CHILDREN WITH NEUTROPENIAS

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Background. In the genesis of immune neutropenias virus infections play a great role. Because of high rate of infection of herpetic group in the population it is sensible to study their role in the immunepathogenetic mechanisms of immune neutropenias in children. *Aims*. To study the relationship between the duration of circulation of granulocytotoxic antibodies (GCTA) and presence of herpetic infection in children with immune neutropenias. *Methods*. 33 children in the age of 4 to 24 months were examined for presence of specific herpetic group virus antibodies of IgM and IgG classes in blood serum. The method of three-phase enzyme immuoassay was used. GCTA were found in the blood serum with the help of granulocytotoxic test. *Results*. Group 1 consisted of 15 herpes group virus-infected children with immune neutropenia aged 4 to 24 months; virus infections included cytomegalovirus (n=10), Epstein-Barr virus (n=1), herpes simplex virus 1 or 2 (n=2) and mixed infection (n=2). GCTA circulation lasted for 0.5 to 14 months (6.96 0.33). GCTA titers ranged from 1:2 to 1:64. No correlation between GCTA titers and duration of GCTA circulation has been revealed. Group 2 consisted of 18 children aged 6 to 12 months with immune neutropenia and no markers of herpes group viruses. GCTA circulation lasted for 0.5 to 5 months (1.67 1.25). GCTA titers ranged from 1:4 to 1:156 and, similarly to those in Group 1, caused no effect on the duration of GCTA circulation. *Summary / conclusions*. Thus, statistically significant difference in duration of GCTA circulation ($p< 0.001$) between the studied groups has been found; this result indicates the presence of a pathogenetic role of herpes group viruses in the immune conflict in children with immune neutropenias.

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ENHANCED BACTERIOCIDAL FUNCTION BY WKYMVM IN PATIENTS WITH ACUTE LEUKEMIA

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We evaluated the leukocyte bacteriocidal function in patients with acute leukemia (AL) and searched whether WKYMVm can enhance the bacteriocidal function in patients with AL. Cytokines were measured at the time of diagnosis. Complete blood count and bacteriocidal activities were followed during induction and first consolidation chemotherapy. Twenty eight AL patients and 31 healthy controls were enrolled. WKYMVm receptor and bacreriocidal activity did not different between patients and control. Addition of WKYMVm markedly increased the bacteriocidal activities when compared with that without WKYMVm. Baceriocidal activities by WKYMVm were increased as a dose-dependent. During induction chemotherapy, there were significant changes of bacteriocidal activities at concentration 0 and 1 nM of WKYMVm resulting in higher bacteriocidal activities at the time of complete remission when comparing at diagnosis or on day 15. During consolidation chemotherapy, none of every concentration of WKYMVm showed significant changes in bacteriocidal activities. TNF α, IL-1b, IL-6 and IL-8 were significantly higher, but IL-2, IL-4 and IL-12 were significantly lower in patients. Among them, TNF α, IL-1b and IL-6 have significant negative correlation with bacteriocidal activities of neutrophil, and IL-4 has significant positive correlation with bacteriocidal activities. In conclusion, WKYMVm can enhance the bacteriocidal activity in patients with AL.

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THE PATTERN OF BONE DISEASE AND ITS MORBIDITY AMONG THALASSEMIA TREATED AT A SINGLE INSTITUTE IN SAUDI ARABIA

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Background. Bone disease is an increasingly recognized serious cause of morbidity on young adult of both thalassemia major (TM) and thalassemia intermedia (TI). It's etiology is multifactorial, culminating with increased bone resorption and remodeling, due to the complication of the disease itself and other risk factors; low baseline hemoglobin, delayed puberty, hormonal failure, high iron stones and nutritional deficiency. The lack of early diagnosis and treatment can led to multiple problems, growth failure, osteoporosis, fractures, spinal deformities and nerve compression. *Aims*. To assess the prevalence of bone disease and its morbidity among thalassemia patients treated at our institute. *Methods*. Hundred-fourteen thalassemia patients were enrolled in the study, (104 TM and 10 TI), patients age range from 1-40 years old (59 females & 55 males) 67% were children and adolescent. These patients were treated at King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia. All patients were assessed clinically (Table 1).

Table 1. Bone disease assessment of patients with thalassemia major and thalassemia intermedia treated at KAUH, Jeddah, KSA.

Blood and urine samples were obtained for the determination of biochemical and hormonal profiles, included, PTH, 25 OH vitamin D3. Bone maturation was assessed by radiological bone age. Bone marrow density (BMD) by DEXA was determined on half of the patients. Bone formation markers (bone-specific alkaline phosphatase and osteocalcin) and bone resorption markers (Pyridinoline and deoxy pyridinoline) were analysed for patients whom had BMD and referred for treatment. *Results*. Indicate a high prevalence of hypovitaminosis D, 50% in thalassemic children, 80% among adolescents, up to 70% of adolescent and young adults had dysfunction in hypogonadotropic hypogonadism. 60% had reduced low bone mass (LBM) among adolescents, worsen by increase

in age. High prevalence of LBM among thalassemia intermedia. *Summary*. Bone assessment was found to be suboptimal in children and adolescents, bone morbidity increased with age. All thalassemia should be screened annually for bone disease. Prevention of osteoporosis is the most important priority in managing thalassemia patients by early diagnosis and treatment.

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IDENTIFICATION OF HAEMOGLOBIN O-ARAB USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Background. The HLC-723G7 (TOSOH) is a fully automated high performance liquid chromatography (HPLC). In the ,-thalassaemia analysis mode, predetermined windows are set in the software to detect the presence of haemoglobin F, A2, A, D, S, C. All other windows are for presumptive identification of various haemoglobins. Unidentified haemoglobin fractions (peaks bigger than 10%) need further investigation. *Aim*. The aim of this study was to determine the chromatogram patterns and retention times for specimens containing haemoglobin O-Arab (Hb O-Arab) in order to avoid in the future alternative, confirmatory tests. *Patients and Methods*. During a six months period, all specimens containing O-Arab and S haemoglobin variants were reanalyzed on a G7 TOSOH analyzer (HPLC method). The identification of the variants was previously achieved by alkaline / acid electrophoresis and sickle screening test. Group comparisons were performed by t-Student test. Value of $p<0.05$ were considered to indicate statistical significance. *Results*. We identified 22 Hb O-Arab (19 heterozygous, 3 homozygous), group A, and 13 HbS (12 heterozygous, 1 homozygous) group B. All specimens containing Hb O-Arab presented an unknown peak near the peak of Hb S separation. The retention times (minutes) were: 6.14 (3 specimens), 6.15 (16 specimens), 6.16 (3 specimens). The proportion of the peak was 31.8±2.2% for heterozygous and 80.1±1.7% for homozygous. The retention times (minutes) for specimens containing Hb S were: 5.68 (2 specimens), 5.70 (2 specimens), 5.72 (5 specimens), 5.73 (3 specimens), 5.76 (1 specimen). The proportion of Hb S was 30.1±2.4% for heterozygous and 72.9% for homozygous. The retention times for Hb O of group A and for Hb S of group B were 6.15±0.005 and 5.71±0.21 minutes (mean±SD) respectively and they were significantly different ($p<0.001$). *Conclusion*. According to our results the identification of haemoglobin O-Arab using G7 TOSOH analyzer (HPLC method) is accurate. The retention time is between 6.14 and 6.16 minutes. We suggest that there is no need of confirmatory tests to be performed.

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FACTOR V-LEIDEN AND PROTHROMBIN G20210 MUTATIONS AMONG IRANIAN PATIENTS WITH SICKLE CELL DISEASE

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Background. A hypercoagulable state in sickle cell disease (SCD) and β thalassemia is well documented and thrombosis is an important aspect of the clinical spectrum of sickle cell disease. Factor V Leiden and prothrombin G20210A mutations are two frequent genetic risk factors involved in venous thromboembolism. *Aims*. A case-control study was performed to determine the prevalence of Factor V Leiden and prothrombin G20210A mutations among sickle cell anemia (SS), sickle cell trait (AS) and sickle/β thalassemia (S/Thal) patients from Southern Iran compared to healthy individuals. *Methods*. Patients comprised 60 SCD patients of them 35 were SS (21 males and 14 females) aged 17.2± 8.3, 15 were AS (9 males and 6 females) aged 30±15.4 and 10 were S/Thal (3 males and 7 females) aged 24.6±10.4. Control group were 126 apparently healthy individuals (50 males and 76 females) aged 20.1 ±9.8. Seven out of 10 (70%) S/Thal patients were sickle/ β-zero Thalassemia. Genotyping was done by PCR-RFLP using Mnl I and Hind III for factor V Leiden and prothrombin G20210A, respectively. *Results*. Heterozygous factor V Leiden mutation was found in 5 of 35 (14.3%) SS patients, 2 of 15 (13.3%) AS individuals, 1 (a sickle/ βzero Thalassemia patient with IVSII.1 G:A mutation) of 10 S/Thal patients (10%) and 2 of 126 (1.6%) control subjects ($p<0.05$). However, only one AS individual was found to be carrier for prothrombin G20210A compared to 5 out of 126 (4%) healthy individuals. *Summary/conclusions*. A significantly high prevalence of factor V Leiden was found in sickle cell disease patients from Southern Iran compared to normal individuals (around 8 times). Our results indicated that there is an association between sickle cell anemia with factor V 1691G:A ($p=0.006$) in Iranian SS patients. Regarding the high frequency of FV Leiden mutation in SCD patients it was suggested that Iranian SCD patients need to be screened for this mutation.

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REVERSE-HYBRIDIZATION-BASED GENETIC TESTING FOR THE PREDICTION OF ANTICOAGULANT DOSE REQUIREMENT

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Background. Coumarin derivatives (Warfarin, Phenprocoumon, Acenocoumarol) are the most widespread oral anticoagulant drugs for the prevention and treatment of arterial and venous thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide inter-individual variability in dose requirement. Despite adjustment for clinical variables, adverse events, such as delay in achieving a stable maintenance dose or bleeding complications, are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing cytochrome P450 isozyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin. *Aims and Methods*. A genetic test (StripAssay) was developed for the detection of -1639G>A and 3730G>A in the VKORC1 gene, and 430C>T and 1075A>C in the CYP2C9 gene. The test is based on multiplex PCR, followed by reverse-hybridization of biotin-labeled amplification products to a parallel array of allele-specific oligonucleotides immobilized on membrane teststrips. *Results*. Genotyping for VKORC1 polymorphisms and the functionally defective CYP2C9 variants *2 and *3 allowed the classification of patients into high, intermediate and low dose responders to phenprocoumon (Marcumar™), the most commonly used oral anticoagulant in Central and North European countries. Favourable properties, such as the rapid DNA extraction protocol, ready-to-use reagents and teststrips, as well as the potential for automation of the hybridization/detection step, make the StripAssay convenient and easy to perform within less than 6 hours. *Conclusions*. A simple and reliable diagnostic tool was developed and evaluated for predicting the response of patients to phenprocoumon treatment. The results obtained in our study will assist clinicians to achieve a more individualized anticoagulant therapy.

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FIBRINOLYTIC ACTIVITY IN CHRONIC RENAL FAILURE PATIENTS UNDER HAEMODIALYSIS AND ITS RELATIONSHIP TO RECOMBINANT HUMAN ERYTHROPOIETIN RESISTANCE

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Cardiovascular disease is responsible for most deaths in chronic renal failure (CRF) patients. Disturbances of coagulation and fibrinolysis have been reported in patients with chronic uremia and are known to contribute to the pathogenesis of cardiovascular disease. However, studies of different coagulation and fibrinolysis parameters in regularly dialyzed patients have yielded conflicting results, with some indicating suppressed fibrinolysis and others showing increased fibrinolysis. Moreover, correlation between fibrinolytic activity markers and resistance to recombinant human erythropoietin (rhEPO) therapy were not previously studied. In order to investigate the relationship between fibrinolytic activity and resistance to rhEPO therapy in haemodialyzed patients, we studied the circulating levels of plasminogen activator inhibitor type-1 (PAI-1), tissue plasminogen activator (tPA) and D-dimers in 50 CRF patients under regular haemodialysis and rhEPO therapy (25 responders and 25 non-responders to rhEPO therapy) and in 25 healthy controls. Correlations between studied variables were assessed by using the Spearman rank correlation coefficient. Compared with controls, CRF patients presented with significantly lower levels of tPA and with higher D-dimers; no statistically significant differences were found for PAI-1. In CRF patients, the levels of D-dimers correlated positively and significantly ($r=0.359$, $p=0.01$) with rhEPO doses (rhEPO/Kg/week) and

negatively with haemoglobin levels (r=-0.335, p=0.017). No statistical differences were found between responders and non-responders to rhEPO therapy, concerning tPA and PAI-1 levels. However, the D-dimer level is significantly higher in non-responder group [137.25 (68.50-266.94) vs 214.75 (139.76-547.56), p=0.032]. Our results showed an altered haemostasis in CRF patients based on D-dimer plasma level, which is used as an index of fibrin turnover and intravascular thrombogenesis. The increased levels of this fibrinolytic marker in CRF patients, particulary in non-responders patients, associated to its correlation with haemoglobin levels and rhEPO doses suggest a relationship between abnormal haemostasis and resistance to rhEPO therapy. Moreover, as tPA is decreased in CRF patients, it is reasonable to assume that the higher levels of D-dimers are primarily a result of increased fibrin formation and that this increased thrombogenic state may be related to increased susceptibility to vascular disease in these patients, particularly in non-responders to rhEPO therapy.

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CA 15-3 : CAN USED TO DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF MEGALOBLASTIC ANEMIA ASSOCIATED WITH VITAMIN B12 DEFICIENCY?

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Background. The CA 15-3 is glycoprotein used to diagnose breast cancer and gastrointestinal carcinoma, to define their prognosis and to monitor of the treatment. However in some diseases like NHL and liver cirrhosis; the CA15-3 levels may increase slightly. A disease different from breast cancer that increase 15-3 level, is also a megaloblastic anemia associated with serum vitamin B12 deficiency. *Aims*. In this study the diagnostic value of CA15-3 at the megaloblastic anemia with vitamin B12 deficiency and its role at the differential diagnosis of disease presented with macrocytic anemia, are investigated. *Methods*. Eighty-nine patients with MCV higher than 96 fl were included in this study. Sixty-two of them had megaloblastic anemia with vitamin B12 deficiency, 9 patients had MDS, 10 patients had chronic liver disease and 8 patients had hypothyroidism. CBC, serum vitamin B12, folic acid, CA15-3 testing, thyroid hormones, liver enzymes, HbsAg, Anti HCV Ab testing, reticulocyte counting, abdominal ultrasonography and gastroscopy were applied to all patients. To eliminate breast cancer, mammography was applied to all women patients. Bone marrow biopsy and cytogenetic examination was made to the patients with MDS. Bone marrow aspiration is applied to the patients with megaloblastic anemia. Other reasons of macrocytosis were eliminated for patients with MDS; chronic liver disease and hypothyroidism. 1000 µg vitamin B12 was injected IM to the patients with vitamin B12 deficiency , per day for 10 days and then once a month. Post-treatment analysis for these patients had been constructed when the hemoglobin and MCV values decreased to normal levels. *Results*. In patients with megaloblastic anemia associated with vitamin B12 deficiency, Post-treatment hemoglobin and MCV values have been obtained as 13,0±0,9 g/dL, 87,3±11,8 fl while these values are 8,4±2,5 g/dL and 114±10,8 fl. respectively for the pretreatment case (p< 0,0001). Serum CA 15-3 values are obtained as 92,4 U/mL (N:0-25 U/mL) and 19,6 U/mL (p<0,0001) for pre and post treatments, respectively. For 59 patients of 62 (%95,1) serum CA 15-3 level was observed as high. The average serum CA 15-3 levels were obtained 20,8 U/mL, 32,0 U/mL and 23,7 U/mL for the patients with MDS; chronic liver disease and hypothyroidism, respectively. When these values are compared with the pretreatment serum CA 15-3 levels of megaloblast›c anemia associated with vitamin B12 deficiency individually and totally, it is concluded that there is a statistically significant difference (p<0,0001). No tumor occurrence for all the patients observed for breast cancer and gastrointestinal tumor occurrence for 11,4 months as average. *Conclusion*. For the cases with megaloblastic anemia associated with vitamin B12 deficiency, the serum CA 15-3 levels have increased and returned to their normal levels following treatment. It has been interpreted that in these cases, megaloblastic normoblasts in the bone marrow have produced CA 15-3. It is concluded that the level of serum CA15-3 is a reliable marker for diagnosis in these cases and for differential diagnosis from the disease presented with macrocytosis.

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IMPACT OF HETEROSIS AND GENOME-WIDE HETEROZYGOSITY ON MARKERS OF HEMOSTASIS AND INFLAMMATION IN HUMANS

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Background. Genetic architecture of human quantitative traits with relevance to health and disease is a matter of great interest. However, it has been proposed that many of these traits are highly polygenic and also caused mainly by very rare genetic variants, which could make the majority of present efforts futile in their search for improved understanding of genetic regulation of biomedical relevant quantitative traits. Heterosis and genome-wide heterozygosity are known to have effects on quantitative traits in animals and plants, summarized as *inbreeding depression*. It has been demonstrated that these effects also exist in humans, especially affecting highly polygenic traits, which could have effects on health at the population level. *Aims*. To determine whether heterosis (achieved through admixture and outbreeding) and genome-wide heterozygosity (measured by dense genome-wide scans using polymorphic markers) have significant impact on biochemical markers of hemostasis and inflammation. *Methods*. We studied 1041 individuals from an isolate island of Vis, Croatia, where consanguinity is prevalent. For each individual *personal genetic history* (PGH) was determined as being isonymous, endogamous or outbreed. We also assessed genome-wide multilocus heterozygosity (MLH) using a set of 800 short tandem repeats (STR) markers. We correlated these variables to the five biochemical markers of hemostasis and inflammation: fibrinogen, D-dimers, von Willebrandt's factor (vWF), tissue plasminogen activator (tPA) and C-reactive protein (CRP). To control for a possible confounding effects, we included a larger set of variables known to have a possible effect on the five measured traits. These covariates were: age, sex, body mass index, mean blood pressure, HDL, LDL, triglycerides, glucose, insulin, HbA1c, creatinine, calcium, uric acid, albumin, smoking status, alcohol consumption, physical activity at work and at home, level of education, socio-economic status index, ankle brachial pressure index, forced expiratory volume in 1st second, presence of chronic diseases, drugs use, several dietary habits and variables obtained from a questionnaire GHQ-30. Ethical approval for this research was obtained from appropriate research ethics committees and informed written consent was obtained from all participants in the study. *Results*. Statistical analysis showed that personal genetic history was a significant predictor only for tPA (p<0.005), while MLH showed no significant effects on the five measured traits. The strongest predictors of fibrinogen distribution in the population sample were smoking (p<0.001), body mass index and albumin (p=0.001). D-dimers and vWF were mainly predicted by age (p<0.001) and albumin (p=0.002); tPA by age, sex, body mass index, triglycerides and uric acid (p<0.001); and CRP by albumin and FEV1 (p<0.001). *Conclusions*. Lack of strong impact of genome-wide heterozygosity and outbreeding on biochemical markers of hemostasis and inflammation, which was observed for some other important biochemical traits, may suggest that their genetic control is not as highly polygenic and that they could therefore represent more promising targets for quantitative trait loci mapping and further genetic association studies.

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COMPLICATIONS OF β-THALASSEMIA INTERMEDIA: A 12-YEAR LEBANESE EXPERIENCE

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Background. Thalassemia is an inherited disease that affects · or ,-chain molecules of hemoglobin. The Middle East has one of the highest rates of incidence worldwide, and the Lebanese population specifically has 3% carrier prevalence approximately with one-third of the population suffering from Thalassemia Intermedia. *Aims*. To demonstrate the complications and survival of Thalassemia Intermedia in Lebanon. *Methods*. Thalassemia Intermedia patients were selected according to the following inclusion criteria: First transfusion at or after 2 years of age and/or absence of regular transfusion dependency. These criteria were used

because some of the patients were originally misdiagnosed in other clinics as thalassemia major and were started early on regular transfusion. *Results*. The overall analysis population was formed of 92 patients out of a total of 109. Females comprised 51.1%. All patients are born in the time period between 1970 and 2004. Average serum ferritin for patients without complications was lower than those with complications (911±771 vs. 1347± 764). In the survival analysis, the most recent birth cohorts (after 1994) had a more extended complication free period (p=0.009) with no reported complications. There are 44 patients born before 1983 and 31 patients born between 1984 and 1993. There is significant difference in the complications of both groups with a far worse disease progression for those born between 1984 and 1993 (p=0.003). Male gender seems to confer worse disease free- survival than female gender (p=0.078). Furthermore, our results have shown that one patient suffered from congestive heart failure, and 25 patients (54.3%) sustained pulmonary hypertension. The other frequent complications are endocrinologic, with hypogonadism affecting 10.9% of patients (n=10) and hypothyroidism present in 9.8% (n=9). Of the 109 patients who had their charts reviewed, 79.8% were splenectomized and 43.1% were on chelation therapy. After crossmatching each complication with splenectomy and chelation separately for any possible relationship, hypothyroidism versus splenectomy was significant with all splenectomized patients developing hypothyroidism as a complication (p=0.049). *Conclusions*. Survival analysis of thalassemic groups is difficult. Traditional Kaplan-Meier survival analysis requires all patients be followed from birth and have similar exposure during follow up. The presence of a single care center has annulled the issue of exposure and follow up. There is significant difference in the complications of both groups with a far worse disease progression for those born between 1984 and 1993 (p=0.003). The most common complications reported among Lebanese thalassemics are attributed to pulmonary hypertension (54.3%). Endocrinologic dysfunction (hypogonadism 10.9% and hypothyroidism 9.8%), infection and thrombotic events are documented. Furthermore, 79.8% of TI patients were splenectomized and 43.1% were on chelation therapy. Crossmatching each complication with splenectomy and chelation separately for any possible relationship, hypothyroidism versus splenectomy was significant with all splenectomized patients developing hypothyroidism as a complication (p=0.049).

Table 1. Complications of TI among 92 patients.

Complication	Number*	Percentage of population
Congestive Heart Failure	1/92	1.1
Pulmonary Hypertension (defined with TR > 25	25/46**	54.3
Hypogonadism	10/92	10.9
Hypothyroidism	9/92	9.8
Diabetes Mellitus	2/92	2.2
Thrombotic event	7/92	7.6
Leg Ulcers	5/92	5.4
Hepatitis C	6/92	6.5
Splenectomy***	87/109	79.8
Chelation Therapy***	47/109	43.1

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RITUXIMAB IN COMBINATION WITH DOSE-DENSE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) AUSTRIAN WORKING PARTY MEDICAL TUMOR THERAPY (AGMT) STUDY NHL-8

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Background. For centuries polychemotherapy with cyclophosphamide, doxorubicin, vincristin, and prednisolon (CHOP) was the standard treatment for DLBCL. Recently the concept of dose density emerged. With dose-dense CEOP/IMVP-Dexa (cyclophosphamide, epirubicin, vincristin, prednisolon, ifosfamide, etoposide, methrotrexate, and dexamethasone) we were able to improve the survival of patients with aggressive lymphomas in comparison to CHOP. However, others have shown a improvement of survival by adding rituxmab to CHOP. Aim of our phase II study was to evaluate the feasibility and results of the combination of dose-dense CEOP/IMVP-Dexa with rituximab in untreated DLBCL. *Methods*. We treated 30 patients under the age of 61 years with untreated DLBCL with R-CEOP/IMVP-Dexa: Rituximab 375 mg/qm i.v. d1+15, cyclophosphamide 750 mg/qm i.v. d1, vincristine 1,5 mg/qm i.v. d1+8, prednisolon 100 mg p.o d1-5, ifosfamide 2000 mg/qm i.v. d15-17, etoposide 80 mg/qm i.v. d15-17, dexamethasone 20 mg p.o. d15-19, and methotrexate 800 mg/qm i.v. d22. Cycles were repeated every 29 days 4 times. The results were compared with an age matched historical control group treated with the same chemotherapy without rituximab and another age matched historical control group treated with 3-weekly CHOP. *Results*. Twenty-five of 29 (86%) and 34 of 37 (91%) patients treated with dose-dense chemotherapy with and without rituximab, respectively achieved a complete remission (CR) or CR undetermined (Cru). Time to treatment failure after 2 years was better for rituximab plus dose-dense therapy (0.94 (95% CI 0.63-0.99) versus 0.72 (95%CI 0.55-0.83)), but this difference did not reach statistical significance (p=0.06). Two year survival was identical in both groups. Compared with 3-weekly CHOP dose-dense therapy with and without rituximab achieved an absolute benefit in overall survival after 2 years of 30% (p=0.025). With the exception of lymphopenia we observed no increase of toxicity by the addition of rituximab to dose-dense therapy. *Summary and Conclusions*. The early results of this trial show that the addition of rituximab to dose-dense therapy is feasible, but it does not improve the results. Longer follow up is necessary for final analyses of the role of rituximab in combination with dose-dense therapy. In comparison to 3-weekly CHOP dose-dense therapy with rituximab improves survival by 30%.

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RITUXIMAB, GEMCITABINE AND OXALIPLATINUM: AN EFFECTIVE REGIMEN IN PATIENTS WITH REFRACTORY AND RELAPSING MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma constitutes one of the poorest prognosis non-Hodgkin lymphomas. The median survival is less than 3 years and at present is incurable with conventional chemotherapy. Although new intensive regimens and high-dose chemotherapy with stem cell rescue give raise a higher response rate, the outcome for patients who relapse is very poor. Moreover, due to the advanced age of presentation, limited effective salvage regimens are available. Thus, the development of effective regimens with acceptable toxicity in this elderly population is warranted. *Aims*. To evaluate the clinical activity and toxicity of a new salvage regimen, which combines gemcitabine, oxaliplatin, and rituximab (GEMOX-R), in patients with relapsing or refractory mantle cell lymphoma. *Patients and Methods*. Herein, 13 patients of an ongoing phase II study for refractory or relapsing mantle cell lymphoma are reported. GEMOX-R consisted of Rituximab (375 mg/m^2) on day 1, gemcitabine 1000mg/m^2 and oxaliplatin 100 mg/m^2. Treatment was given every 15 days if feasible or every 21 days. The median number of cycles was 6. The median age of the patients was 72 years. Seven patients were primary refractory and the other 6 patients received the treatment for relapsing disease. Five patients had received previously the HyperCVAD regimen and 2 patients had undergone an autologous stem cell transplant. At GEMOX-R treatment, 92% percent of the patients presented an Ann Arbor stage III-IV, high LDH in 36% of patients, a higher than 1 ECOG performance status in 30% of patients and IPI>2 was present in 46%. *Results*. Sixty-seven percent of the patients achieved a CR and 17% a PR for a total response rate of 84%. With a median follow up for alive patients of 14 months, OS was 56% and PFS was 46% at 24 months. The median survival was 14.3 months. At present 5 patients remain free of progression. Two patients remain free of disease at 17 and 36 months respectively. The major toxicity was thrombopenia grade III-IV present in 31% of the patients. Neurotoxicity grade I-II was present in 46% of patients. Factors related with overall survival were ECOG performance status and a-IPI at GEMOX-R. *Summary/Conclusions*. These preliminary observations in 13 patients suggest that GEMOX-R displays an outstanding efficacy with an excellent toxicity profile in an elderly population heavily treated with prior intense regimens for this disease. The high response rate with a high CR rate suggests a striking synergism between

these new drugs in the lymphoma drug armamentarium. More experience with this new regimen for Mantle cell lymphoma is needed to confirm this initial appealing experience.

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THE GEMOX-R (GEMCITABINE, OXALIPLATIN, RITUXIMAB) REGIMEN IS A HIGHLY EFFECTIVE SALVAGE REGIMEN IN ELDERLY PATIENTS WITH REFRACTORY-RELAPSING DIFFUSE LARGE B-CELL LYMPHOMA (DLCL) NO CANDIDATES TO AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT). A PHASE II STUDY

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Background. The prognosis of old or immunocompromised patients with refractory or relapsing DLCL is very poor. The current standard of salvage therapy followed by consolidation with ASCT for patients chemosensitive to the salvage regimen is not feasible for most of these patients. Thus, new active regimens with an acceptable toxicity profile are needed. *Aims*. To report the results of a phase II trial of 33 patients with refractory or relapsing DLCL treated with GEMOX-R. *Methods*. Thirty-three elderly patients with refractory or relapsing DLCL not suitable for ASCT enter into a phase II study with GEMOX-R. Treatment consisted on Rituximab (375 mg/m^2) day 1, Gemcitabine (1000 mg/m^2) day 1 and Oxaliplatin (100 mg/m^2) day 1. Treatment was repeated in 2 weeks intervals if feasible or every 3 weeks for a planned 6-8 courses with staging after the third course. Response was evaluated according to Cheson criteria (Cheson *et al*. J. Clin. Oncol. 1999; 17: 1244). *Results*. Median age of the population was 69 (32-85). Sixty-one% of the patients were males. One patient with severe cardiac disease was treated in first-line and the rest had received CHOP-R (61%), CHOP (21%), EPOCH-R (12%) and CNOP (3%) as first-line therapy. Thirty six percent of the patients were primary refractory and 42% of the patients received GEMOX-R at first relapse. At GEMOX-R, 73% of patients presented with an Ann Arbor stage III-IV, high LDH in 55% of cases, an ECOG>1 was present in 52%, a-IPI>1 was observed in 67% of the cases. The median number of GEMOX-R cycles administered was 4. The response rate to GEMOX-R was 47% with 36% CR. Neutropenia grade III-IV was observed in 39% of the patients and thrombopenia grade III-IV was presented in 36% of the patients. Neurotoxicity grade III-IV was observed in 6% of cases. The median follow up for alive patients is 11 months, and the median survival is 10.8 months. At 12 months the OS is 47% and the PFS is 31%. One HIV patient primarily refractory to CHOP-R achieved a CR with 3 courses of GEMOX-R and received an ASCT as consolidation. This patient remain alive free of disease 26 months after the transplant. Factors related with overall survival were ECOG performance status and a-IPI at GEMOX-R as well as response to GMOX-R. *Summary/conclusions*. GEMOX-R is a new salvage regimen for DLCL with high activity and relatively safe toxicity profile, which can be offered to elderly patients not candidates of ASCT consolidation. The high efficacy of the regimen in this unfavourable population and also in immunocompromised situations warrant further investigation of this regimen in all salvage situations of this type of lymphomas.

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A COMBINATION CHEMOTHERAPY WITH BORTEZOMIB, BENDAMUSTINE AND PREDNISONE FOR PATIENTS WITH REFRACTORY/RELAPSED MULTIPLE MYELOMA: AN UNICENTRIC RETROSPECTIVE TRIAL

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Introduction. Bortezomib is a novel proteasome inhibitor that has shown important clinical efficacy either as a single agent or in combination with other cytostatic agents in relapsed/refractory multiple myeloma (MM). The combinated treatment of bortezomib with bendamustine and prednisone (BPV) was retrospectively assessed to determine the efficacy and toxicity of this regiment in patients with advanced MM. Clinical studies in patients with newly diagnosed and relapsed MM have shown that bendamustine is effective as single agent as well as in combination with prednisone. In a phase III study, overall response rate for bendamustine and prednisone was 75% at first line therapy. *Methods*. Between January 2005 and October 2006, 31 patients (median age 63; range 31-77 years) with relapsed or refractory MM (20 patients stage IIIa, 11 patients IIIb) were treated with bendamustine 60 (-80) mg/m^2 on day 1 and 2, bortezomib 1,3 mg/m^2 on day 1,4,8 and 11, and prednisone 100 mg on day 1,2,4,8 and 11. Cycles were repeated every 21 days until maximum response or progressive disease. The time from first diagnosis ranged from 6 to 183 (median 40) months. The duration of the last remission before beginning the BPV-therapy was 6 (range 0-36) months. Previous therapy lines from 1 to 6 (median 2), and included 13 x thalidomide, 11 x autologous PBSCT, and 7 x allogeneic PBSCT. 11 patients were refractory to the last treatment. The majority of the patients (n=18) had preexistent severe thrombocytopenia or leukocytopenia (WHO grade 3 or 4). Response was assessed using EBMT criteria modified to include near complete remission (nCR) and very good partial remission (VGPR). *Results*. 22 patients responded after at least one cycle of chemotherapy with 5 nCR, 4 VGPR, 10 PR and 3 MR. 4 patients had stable disease and 5 patients had a progress. With a median follow up of 7 months, EFS and OS at twelve months were 37% and 50%, respectively. The median number of the BPV-treatment was 2 (1-6) cycles. 14 of 22 responding patients showed a rapid decrease of the myeloma protein and reached the best response after the first cycle and 6 after the second cycle. The regimen was well-tolerated with few significant side effects reported. New cytopenias occured infrequently (two patients had a thrombocytopenia grade 3, and 1 patient had a grade 4 thrombocytopenia). 3 patients had a mild new polyneuropathy (grade 1). *Summary*. These early results indicate that the combination of bortezomib, bendamustine and prednisone is well tolerated in a heavily pretreated population of patients with relapsed or refractory MM. Because of these encouraging clinical responses, we plan to further evaluate this combination in a larger group of patients with relapsed/refractory myeloma.

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POSITRON EMISSION TOMOGRAPHY USING 18F-FLUORODEOXY GLUCOSE FOR EVALUATION OF RESIDUAL FOLLICULAR NON HODGKIN LYMPHOMA

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Background. Positron emission tomography (PET) using 18F-fluorodeoxyglucose (FDG) has emerged as a useful method for monitoring responses and evaluate residual lesion in a variety of cancers. *Aims*. The present study was performed to evaluate the utility of PET-FDG to determine the persistence of residual disease in follicular non-Hodgkin lymphoma patients resting with residual lesion in computed tomography (CT) after chemotherapy. *Methods*. Twenty two consecutive follicular non-Hodgkin lymphoma patients with a residual lesion in CT documented one month after therapy completion entered the study. A PET-FDG was prospectively performed within one week from the CT scan. Evaluation by means of biopsy with pathological study was proposed to all FDG-PET positive patients. *Results*. FDG-PET was positive in 10 patients. The median uptake value (suv) of these patients was 5,6 (range 1,4-28,3). Among these 10 positive patients, seven agree to perform a biopsy, resulting in six positive residual lymphoma cells and one negative. All three FDG-PET positive patients who did not agree to the pathologic study progressed. FDG-PET was negative in 12 patients. With a median follow up of 15 months from PET execution, no relapse was recorded among 11 patients. Just one patient progressed within three months. In the present study, FDG-PET had sensitivity of 90% and a specifity of 91,7%, a positive predictive value of 90% and a negative predictive value of 91,7 %. *Conclusions*. PET-FDG is a reliable method for the diagnosis of residual tumor in follicular non-Hodgkin lymphoma and may contribute to improve the management of these patients.

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LONG TERM THERAPY AND EFFICACY OF LOW-DOSE THALIDOMIDE IN RELAPSED-REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background and Aim. Actually Thalidomide, often combined with other agents, is the drug of choice in the treatment of relapsed/refractory MM, and recently its role as first line therapy and in maintenance after HDT has been emphasized. Except progression of disease, the most common reason for thalidomide withdrawal is long term toxicity, especially peripheral neuropathy (PN). Thalidomide sensory neurotoxicity was found to be cumulative dose dependent and occurs when the total dose is beyond 20 g (Cavaletti). Therefore the lower effective daily dose

of the drug is crucial to allow long term therapy. We report here a single centre experience with low dose thalidomide in relapsed/refractory patients. *Methods and Results*. From October 1999 to September 2006 we treated 34 patients affected by relapsed/refractory MM with Thalidomide; in the first year we used the dose escalation (from 100 to 800 mg) suggested by Barlogie, but the low compliance of our cohort of patients prompt us to utilize a fixed dose of 100mg/die from start of therapy. Dexamethasone (40 mg i.v. dd 1-4 every 28 days for 4 cycles) was added, in the case of lack of response, intended as less than 25% monoclonal protein (MP) reduction after 2 months of therapy. Thalidomide was maintained at 100 mg daily dose until progression of disease or intolerable toxicity. Informed consent was obtained prior to therapy. Characteristic of patients are shown in Table 1. The total response rate was 82% (28 out of 34 patients) : 1 VGPR (>90% MP reduction), 7 GPR (between 75% and 90%), 15 PR (between 50% and 75%), 5 MR between 25% and 50%). Treatment interruption was necessary for neurotoxicity (grade 3-4) only in 3 patients, whereas 11 patients had progressive disease. The median survival from thalidomide start is 20 months; the median time of therapy is 18 months. Although PN occurred after a variable period of time in more than 70% of patients, low daily dose of Thalidomide allowed us to prolong consistently the treatment phase, with neurologic toxicity < 2 in the majority of patients. *Conclusions*. Although Barlogie found that a cumulative dose of more than 42 g in the first 3 months of treatment was significantly associated with a better response, satisfactory response rates were also observed with low dose thalidomide alone or in association with Dex or cytotoxic regimen. Durie reported in 2000 a dose escalation phase II study where doses between 50 and 400 mg were utilized, with dose escalation based upon a lack of response. A remarkable observation was that in responding patients the magnitude (% of regression) and duration of response did not appear to be influenced by dose. Relationship between Thalidomide doses, response rate and survival in MM has not been investigated with large prospective studies; nevertheless most clinical studies utilize a dose of 200 mg or more, although some spontaneous clinical trials testing Thalidomide at lower dosage have already shown its efficacy. Thalidomide withdrawal is often associated with a shorter time to progression. For this reason the possibility to prolong treatment in relapsed/refractory MM patients as long as possible should be the goal of the salvage therapy. In our experience low-dose Thalidomide without escalation is associated with a better tolerance and a longer treatment opportunity.

Table 1.

FEATURES	N (%)
Patients	34 (100)
Male/female	22/12 (65/35)
Median age, yr (range)	66 (31-80)
Median time from diagnosis, yr (range)	2,2 (0,3-8,75)
Median n° of previous lines of therapy	2 (1-4)
Previous ASCT	11 (32)
Phase of disease relapsed	19
refractory	15
Median max daily dose of thal, mg	100
Days of therapy (median)	525

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SERUM FREE LIGHT-CHAIN MEASUREMENTS FOR MONITORING MINIMAL RESIDUAL DISEASE AFTER STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS

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The quantitative assay for free light chains (FLC) has been reported to be sensitive and specific for detecting and monitoring monoclonal gammopathies. In order to determine the status of disease and the presence of minimal residual disease, we used immunofixation electrophoresis (IFE) in urine and serum and routine laboratory tests for all the patients prior and after the transplantation. After the transplantation, the patients were evaluated by FLC and IFE in serum and urine. 19 patients underwent an autologous peripheral blood stem cell transplantation (SCT) and 6 of them received a second reduced-intensity allogeneic SCT and one receive a second autologous SCT, between may-2001 and june-2006. At the time of diagnosis, 10 patients were IgG, 4 IgA, 4 light chain secretor and 1 nonsecretory. All patients received 6 cycles of VAD as induction chemotherapy and cyclophosphamide (4-7 g/m^2) as mobilization for bone marrow harvesting. Prior the first autologous transplant, 4 patients were in complete remission (CR), 9 partial remission (PR), 3 minimal disease (MR) and 3 in progression disease (PD) and prior the second, 2 were in CR, 1 PR, 1 stable disease (SD) and 3 in PD. After a 1560 median follow up days (742-4100 days), 12 patients were in CR, 2 in PD and 5 showing only positive IFE in urine and/or serum. However, when the patients were evaluated by FLC, 10 patients were in CR, 2 in PD and 7 with only FCL positive in urine or serum. The FLC did not confirm CR in 2 patients evaluated by IFE. In conclusion, it seems that FLC is more sensitive and useful for minimal residual disease evaluation in myeloma patients after SCT.

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A CASE-CONTROL STUDY OF POSSIBLE RISK FACTORS IN PRIMARY CUTANEOUS LYMPHOMAS

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Backgrounds. Epidemiologic studies have indicated some occupational and life style factors, like toxic exposure and smoking, as risk factors for primary cutaneous lymphomas (PCL), especially Mycosis Fungoides. *Aims*. The socio-demographic characteristics, occupational history, life style factors, personal and familiar history of patients with PCL were compared to a control group of healthy subjects, homogeneous for numerosity and matched for sex, age and geographical provenience, in order to evaluate the incidence of possible risk factors in the two populations. *Methods*. All the patients with PCL diagnosed and treated at our Institution from January 1990 to September 2006 were included in the study. Healthy controls were selected from the families of patients referred to our Institutions and matched to the patients for sex, age and geographical provenience. Patients and controls gave their informed consent to fill out a form with data regarding socio-demographic characteristics; occupational history; life style factors (smoking habits and alcohol intake); eye and hair color, type of sun exposure, phototype, and reaction to sun-light; exposure to toxic factors; familiar and personal history for cutaneous disease, burnings, immunologic, hematological, respiratory disorders and neoplasms. Patients' and controls' characteristics and descriptive data were expressed as median and range for continuous variables and by frequency tabulations for categorical variables. All statistical analyses were performed using the SPSS statistical package (SPPS Inc. Chicago, IL). Statistical significance was set at $p<0.05$. *Results*. The present study included 259 patients (170 M, 89 F), with a median age of 62 yrs (range, 15-87), and 259 controls (170 M, 89 F), with a median age of 64 yrs (range 17-95). Patients were affected by T-cell lymphomas in 216 cases, including 191 Mycosis Fungoides, and by B-cell lymphoma in 43. Patients and controls did not differ for hair and eye colour and type of solar exposure, but for a higher incidence for smokers ($p<0.0005$) and easier sensibility to solar exposure ($p=0.005$) observed in patients with PCLs. PCL patients also showed a higher incidence in the familiar history for cutaneous diseases ($p<0.0006$) and neoplasms ($p=0.0113$). In the personal past history, a higher incidence of previous cutaneous diseases ($p=0.0175$) and respiratory diseases ($p=0.0093$) was found in patients compared to controls. *Summary/conclusions*. Our study confirms the previously reported association of smoking with the occurrence of PCL, and seems to indicate the importance in the development of these lymphoproliferative disorders of genetic factors responsible for a familiar predisposition to cutaneous disease and tumors. Personal characteristics, like sensibility to sun-light and previous history of cutaneous and respiratory diseases, seem to be also important.

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ERYTHROPOIETIN, IRON, AND FOLIC ACID IN CHILDREN WITH NEPHROTIC SYNDROME AND NORMAL RENAL FUNCTION

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Backgrounds. Some patients with nephrotic syndrome (NS) and normal kidney function become anemic. To date, the pathogenesis of anemia remains elusive. Massive urinary losses and low serum levels of albumin and other proteins are key findings in NS. Of them, transferrin (Tf) and erythropoietin (Epo) deficiencies have been suggested as factors contributing to the anemia. Urinary excretion of these proteins is believed to be the main mechanism leading to these deficiencies. However, only a few patients with NS develops anemia. *Aims*. To investigate the role of Epo, iron, and folic acid in non-anemic children with NS and normal kidney function. *Methods*. Children with nephrotic syndrome were classified as: Group A: NS without proteinuria, in remission; Group B: NS with proteinuria, at diagnosis or in relapse. Clinical data were recorded, and the following laboratory tests were carried out. Blood samples: complete cells count, urea, creatinine, glucose, cholesterol, total proteins, albumin, Epo, ferritin (Ft), serum iron (SI), Tf, Tf saturation (TS), and folic acid. Urine samples: Epo, proteins, glucose, and ♀2 microglobulin. *Results*. Hemoglobin, serum Epo and folic acid were within normal range in all the patients of both groups. Comparison between groups (table) showed significantly lower levels of SI, Tf, TS and Ft in group B; no significant difference could be demonstrated for urinary Epo, although a trend to reach higher values in group B was evident. Tf and SI levels under normal limits were seen in 100% and 67% of children, respectively, in group B, and none in group A. The percentage of patients with urinary losses of Epo was similar for both groups. A direct correlation between SI and Tf was observed (r: 0.73; p=0.0002); no correlation was found between serum or urinary Epo and other analyzed parameters. *Conclusions*. Urinary losses of Epo were demonstrated in children with NS, whether or not they were in remission: percentage of patients and amount of urinary excretion were similar for both groups. Levels of Tf and SI were invariably normal in group A and subnormal in most patients in group B. Since no patient was anemic, our findings suggest that neither the urinary excretion of Epo nor the low levels of Tf and SI are the main mechanisms leading to anemia. Other associated factors, such as a blunted Epo secretion or a misbalance of soluble Tf receptor, should be present in children with NS developing anemia.

Table 1.

	Group A (n= 7)	Group B (n=15)	p
Mean value:			
Hemoglobin	13.3 g/dl	13.3 g/dl	ns
Serum erythropoietin	9.0 mU/ml	10.5 mU/ml	ns
Urinary erythropoietin	2.1 mU/ml	4.0 mU/ml	0. 10
Serum iron	91.9 ug/dl	48.2 ug/dl	0. 0001
Serum transferrin	349.7 ug/dl	125.3 ug/dl	< 0. 0001
Transferrin saturation	26.4%	38.2%	0. 0183
Serum ferritin	43.6 ng/ml	107.4 ng/ml	0. 0094
Folic acid	9.2 ng/ml	7.6 ng/ml	ns
Urinary Beta-2 microglobulin	648.3 ng/ml	1917.6 ng/ml	ns
Serum albumin	5.1 g/dl	2.7 g/dl	0. 0031
Patients with abnormal levels:			
↓Hemoglobin	0%	0%	ns
↓Serum erythropoietin	0%	0%	ns
Urinary erythropoietin +	57%	73%	ns
↓Serum iron	0%	67%	0. 0034
↓Serum transferrin	0%	100%	<0. 0001
↓Transferrin saturation	0%	7%	ns
↓Serum ferritin	0%	7%	ns
↓Folic acid	0%	0%	ns
↑Urinary Beta-2 microglobulin	29%	57%	ns

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HAEMOSTATIC EVIDENCE OF THROMBOTIC SIGNALLING IN NIGERIAN WOMEN ON INJECTABLE CONTRACEPTIVES

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Background. Oral contraceptives pills (OCP) have been linked inextricably with thrombotic tendencies and hypofibrinolysis in African women while, the role of the long acting injectable contraceptives (IC) has not been fully elucidated. *Aims*. This study was designed to assess the probable effects of IC in thrombogenesis and to compare its use with that of OCP. *Methods*. Twenty five (25) females on IC for up to one year and more (from 3 months) attending the out patient clinic at the Obstetrics and Gynaecology department of Irrua Specialist Teaching Hospital, Ekpoma who consented to the study were investigated together with 25 (age-matched; never had any form of contraceptives) controls. They were grouped into two categories(group A; less than 1 year of use, group B; 1 year and above).Thrombogenic and Rheologic factors such as Haematocrit (HCT), Plasma Fibrinogen Concentration (PFC), Platelet count (PC), Whole blood and Plasma Viscosities (WBV and PV respectively) and Euglobulin lysis time (ELT) were analyzed with standard methods. Student t-test was used for the statistical comparison between controls and the test groups and p<0.05 was considered significant. *Results*. There were statistical significant increases in PFC, PV and ELT (p<0.05 respectively) in both groups A and B compared with the controls, also, they increased significantly when compared with patients on OCP for the same period of usage. However, group B exhibited statistical significant increases in these parameters over group A at pathological ranges showing evidence of time dependency. There were no differences between HCT, PC and WBV in both groups and when compared with OCP users. *Conclusions*. i. Pathological increases in PFC coupled with hypofibrinolysis are possible consequences of injectable contraceptives among users. ii. IC use over a long time may pose serious consequences of abnormal rheology for users. iii. Patients on IC could therefore be proned to thrombotic complications much more readily than OCP via static flow especially in micro vessels.

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MODIFICATION OF PHENOTYPE OF UNSTABLE HEMOGLOBINOPATHY (HEMOGLOBIN HANA α2β2 63 (E7) HIS-ASN)]) BY PARTIAL GLUTATHIONE REDUCTASE DEFICIENCY

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Hemoglobin Hana [α2β2 63 (E7) His-Asn)] was described in a Moravian family from the Czech Republic as an unstable hemoglobin variant with mild Heinz body hemolytic anemia, elevated levels of methemoglobin (12-13%), and variable reticulocytosis (1.8-11%) in the proband and her sister. Their mother was asymptomatic, although she expressed the same aberrant variant of the β-globin gene. No differences in the globin gene expression were detected. Iron metabolism indices were within normal range in all family members. To test whether the erythrocyte antioxidant capacity affects severity of this unstable hemoglobinopathy, key erythrocyte antioxidant parameters, including reduced (GSH) and oxidized glutathione (GSSG), activity of NADH-cytochrome b5 reductase, glucose-6-phosphate dehydrogenase, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were examined. Our results revealed that erythrocytes of both children (in contrast to their mother) exhibited lower GSH content, the ratio of GSSG/GSH was increased 6 to 7 times, and their catalase and glutathione reductase activities were decreased to 71-77% and 40% respectively, when compared to the average activities of normal controls. The decreased glutathione reductase activity could be restored by FAD administration *in vitro*. We suggest that neither Hb Hana hemoglobinopathy nor partial glutathione reductase deficiency would account for hemolytic anemia in the affected subjects. However, the combination of both defects might lead to higher hydroxyl radicals formation and methemoglobinemia. Our findings suggest that partial glutathione reductase deficiency represent modifying factor contributing to the clinical manifestation of Heinz body hemolytic anemia associated with unstable hemoglobin variant.

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COST ANALYSIS OF HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDT/ASCT) IN GREECE

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Background. High-dose therapy with autologous stem cell transplantation (HDT/ASCT) is now routinely used for patients with relapsed Hodgkin's (HL) and non-Hodgkin's (NHL) lymphomas, as well as multiple myeloma (MM). To date there are no available published data

regarding cost analysis of ASCT in Greece. In addition, globally, there are no distinct Disease Related Groups (DRGs) for the allogeneic and autologous stem cell transplantation, neither distinct DRGs per disease. *Aim*. A retrospective cost analysis of all phases of ASCT for HL, NHL and MM was performed in the present study, including 4 phases: phase I: mobilization, phase II: PBSC collection and cryoperservation, phase III: pretransplant testing and phase IV: HDT/ASCT until hospital discharge. *Methods*. Twenty-four patients, hospitalized in the Transplantation Unit of the 1st Department of Internal Medicine, National and Kapodistrian University of Athens during 2005 were retrospectively studied by chart viewing. To determine the use of resources, we mainly followed the micro-costing method. *Results*. The median total cost per patient for all phases of ASCT was 14,722.65€ for MM, 20,788.79€ for HL and 26,318.00€ for NHL. The cost for MM was significantly lower compared to HL and NHL ($p<0.001$). From the 4 phases' analysis, it was shown that the above mentioned cost difference was due to the significantly different costs for phases I and IV and was attributed to the different chemotherapeutic agents in each disease and to the different consumption of resources, depending on the days of hospitalisation. The mean length of hospital stay for phase IV was significantly longer in the lymphomas compared to MM. In addition there was a significant correlation between the length of hospital stay and the cost of high-dose therapy for the lymphomas, not proven for MM. *Conclusions*. Our results indicate that there is a need for splitting DRG 481 (DRG for bone marrow transplantation) into different groups, since significant cost differences exist between the most common diseases for which ASCT is applied. The recognition of these differences will minimize disproportionate charges for Hospital budgets or National Health Systems.

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RITUXIMAB THERAPY FOR PRIMARY CUTANEOUS B-CELL LYMPHOMAS: UNICENTRIC EXPERIENCE

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Background. Primary Cutaneous B-Cell Lymphomas (PCBCLs) are a group of neoplastic diseases heterogeneous for pathogenesis and biological and clinical features. Most of these lymphomas have a good prognosis after local therapy considering the long survival and the unusual occurrence of the disease's spread to other organs. The radiotherapy, alone or after surgical excision, is considered the first choice treatment for these patients. Gamma-Interferon and systemic Doxorubicin-based CHT are also used in a minority of patients. In patients with PCBCL, Rituximab is effective either as intralesional or as systemic therapy and response rate and duration are not yet estabilished. *Patients and Methods*. Between 1999 and 2007, 25 patients were treated (18 males, 7 females), the median age was 49 years (range 28 to 77).Therteen pts presented a single lesion, 12 pts presented multiple and disseminated lesions over the trunk, scalp, and limbs. Two pts presented a progressive disease with lymphonodes involved. Nine patients were not in remission: 8 pts in relapse and 1 pt in progression after different previous treatments :3 pts after chemotherapy, 2 pts after radiotherapy, 2 pts after the bone marrow transplantation and 2 pts after immunotherapy. All patients presented a CD20 positive cutaneous lymphoma. The histological distribution according to WHO classification was: 17 patients presented a DLCL, 7 patients a Marginal Zone Lymphoma (MZL), 1 patient a follicular lymphoma. The Rituximab dose was 375 mg/m^2 administered intravenously once a week for a total of four infusions (days 1, 8, 15 and 22) given as outpatients. None of the patients presented adverse effects during the Rituximab infusion. A severe reduction of circulating B lymphocytes was observed for 7 months, on the average, after the last administration but no patients showed an increased risk of infectious disease. Because of clinical features of the disease, the treatment with Rituximab was associated with chemotherapy in 4 pts and with Interferon in 6 pts. *Results*. All 25 pts were evaluable and 21/25 pts (85%) obtained complete remission (CR); 14 of 15 pts treated with Rituximab alone responded (12 CR; 2 PR) and 9/12 pts are in continous CR (CCR) with a median follow up of 52 months (range 25-88); the two pts in PR remained in PR with Interferon. Five of six pts treated with Retuximab + Interferon obtained CR and they were in CCR with a median follow up of 30 months (range 12-52), while 1 of these pts obtained PR. All four pts treated with Retuximab + chemotherapy obtained CR but 3 /4 pts relapsed at 6, 6, 12 months respectively and one pt is in CCR after 67 months. *Conclusions*. Our experience confirms the efficacy and low toxicity of Rituximab in the treatment of PCBCL , even in elderly pts and in advanced disease. Further studies are necessary to indicate if Rituximab could be considered as a front-line therapy .

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BORTEZOMIB, RITUXIMAB, AND DEXAMETHASON (BORID) INDUCES HIGH RESPONSE RATES AND DURABLE COMPLETE REMISSIONS IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA

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Background. Bortezomib (B) belongs to a new class of anti-cancer agents, the proteasome inhibitors, and has documented activity in multiple myeloma and mantle cell lymphoma (MCL). Preclinical studies suggest that B has synergistic activity with rituximab (R), which provides a rationale for the exploration of treatment combinations. *Aims*. To evaluate the activity and safety of B in combination with R and dexamethasone (BORID) in patients with relapsed and refractory MCL (phase II trial). *Methods*. A treatment cycle consists of B at 1.3 mg/m^2 administered on days 1, 4, 8, and 11, R at 375 mg/m^2 administered on day 1, and dexamethasone 40 mg orally on days 1 to 4. Cycles are repeated every 3 weeks for a total of 6 treatment cycles. Patients (pts) with progressive MCL after at least one prior line of therapy (including CHOP or a CHOP-like regimen) are eligible. *Results*. We have completed enrollment of 16 pts (median age, 67 years; range, 48 to 75 years) after a median of 3 lines of prior therapies (range, 1 to 6, prior rituximab in 88%; thalidomide in 50%; high-dose therapy in 31%; a fludarabine-containing regimen in 31%). Median time between start of frontline therapy and study inclusion was 42 months (range, 11 to 98 months). Severe adverse events (> grade II) included infections (herpes zoster in 2 pts, bacterial pneumonia, mucosal candidiasis), peripheral neuropathy (3 pts), fatigue (2 pts) and vasculitic skin infiltrates in 3 pts. Thrombopenia (<50 G/L) occured in 2 pts. All adverse events were managable by standard means of supportive care and prolongation of the treatment interval between cycles. Of 15 pts evaluable for efficacy, 11 have achieved a response (5 CR, 6 PR), and 2 pts experienced stable disease. Pts in CR were also negative for disease activity by PET scanning (4 pts tested to date). Skin infiltrates (histologically proven T-cell infiltrates) preceded achievement of CR in 2 pts. Remission status appeared to be associated with progression-free survival (PFS): Patients in CR had longer PFS (22+, 17+, 12+, 12, and 4+ months) compared to patients in PR (15, 11, 6+, 6, and 6 months). *Conclusions*. BORID has promising activitiy (73% overall response rate) and managable toxicity in patients with heavily pretreated MCL. Achievement of a CR (33% of patients) appears to be an important factor for sustained disease control, and development of a vasculitic rash may be an early indicator of a favorable response.

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EVALUATION OF THE IMPACT OF 2 WASHING SOLUTIONS ON THE CELL RECOVERY OF CORD BLOOD GRAFTS AFTER THAWING/ FAST AND AUTOMATED PROCESSING WITH SEPAX

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Background. Allogeneic hematopoietic stem cell transplantation is a life saving procedure for hematopoietic malignancies. However, wide application of this procedure is limited by the availability of HLA matched donors. Umbilical cord blood has been increasingly used as an alternative source of stem cells for these patients. As compared to bone marrow transplantation, advantages of cord blood transplantation include ease and safety of the collection, prompt availability and reduced incidence and severity of graft versus host disease. Transplant centers use different methods to infuse cord blood after the conditionning regimen. Indeed, cord blood are either thawed at patient's bed side and directly injected, or washed to remove the cryoprotectant before infusion. *Aims*. The aim of this study was to compare the impact of 2 washing solutions on the recovery and viability of total nucleated cells (TNC), CD34 positive stem cells and granulocyte macrophage colony forming unit of cordon blood units. *Methods*. Six cord bloods cryopreserved in two bags were thawed and processed for washing with either 50% human albumin 4%, 40% NaCl 0.9%, 10% anticoagulant citrate dextrose (ACD) or Voluven (Hydroxyethylstarch 6 g /100 mL) on the automated device Sepax from Biosafe. Viability, and absolute count of recovered cells were evaluated by flow cytometry and colony-forming units (CFU) were evaluated with a clonogenic assay immediately after washing as well as at different times after washing and compared with the parameters meas-

ured before cryopreservation. *Results*. The mean total nucleated cells, viable CD34+ cells, and CFU colonies recovery was 47%, 72% and 55% respectively for the Voluven washing solution. The mean total nucleated cells viable CD34+ cells, and CFU colonies recovery was 37%, 54% and 60% respectively for the albumin, NaCl and ACD washing solution. The comparison of the total nucleated cells viability and the CD34 viability between the Voluven solution and the albumin, NaCl and ACD solution showed respectively 55% and 45% viable TNC, and respectively 78.8% and 68% viable CD34+ cells. *Conclusions* . The washing of cord blood with a solution containing Voluven improves significantly TNC and CD34+ cells recovery and viability. No significant difference was observed on CFU colonies recovery.

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NEW CHROMOSOME ABERRATIONS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (CLL) is a common leukemia that more frequently affects elderly people. The clinical course of CLL is variable and in early stages a prediction of disease progression is difficult. Specific karyotype deviations are important prognostic factors, but within the group of patients with a normal karyotype that is in general associated with a good prognosis a number of patients show an aggressive clinical course. The aim of our study is the detection and characterization of new chromosome aberrations by classical and molecular cytogenetics. In comparison to fluorescence *in situ* hybridization (FISH) conventional cytogenetic analysis by classical chromosome banding plays a minor prognostic role in CLL due to the low proliferation rate of malignant B-cells *in vitro*. As traditionally used sets of mitogens result in poor proliferation response of leukemic B-cells, new cultivation techniques using optimal mitogen combinations (OMC: TNFα + IL-2, SAC + IL-2, TNFα+ TPA10) and immunostimulatory CpG-Oligodinucleotide DSP 30 (COD) plus IL-2 were established in order to enhance the yield of detectable chromosome aberrations in CLL cells. Successful metaphase stimulation by culturing the cells with OMC and/or COD plus IL-2 was observed in 45 CLL cases. Most of these karyotypes showed the same aberrations as obtained by parallel performed interphase FISH. In addition six novel chromosomal aberrations were identified and characterized. Detailed depiction of novel aberrations was possible by FISH using whole chromosome painting probes, centromere-specific, single copy probes and/or multicolor FISH. The relevance of these novel chromosome anomalies in CLL detected by using refined culture techniques and the combination of classical chromosome banding and molecular cytogenetic analyses with respect to other prognostic parameters and to the clinical course will be examined.

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HEMOSTASIS EVALUATION IN PATIENT WITH ACUTE RENAL FAILURE (ARF) DURING HEMODIALYSIS

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Background. Critically ill patients often undergo ARF, for which hemodialysis (HD) is indicated; in about 20% of cases coagulation of the dialytic filter occurs despite anticoagulant therapy and the procedure must be stopped. So far, no prognostic factor has been identified to recognize patients at risk of dialyser clotting. *Aims*. We analysed the role of three different anticoagulation strategies on the occurrence of filter clotting, and we tried to identify a number of hemostatic parameters predictors for dialyser coagulation. *Methods*. 22 critically ill patients were studied before and during HD; 7 were anticoagulated with unfractionated heparin (UFH), 4 dermatan-sulfate (DS), 11 received no anticoagulation because at bleeding risk. Factor VIII:C (VIII:C), von Willebrand factor (vWF), P-selectin, Protein C (P:C), antithrombin III and thromboelastography (TEG) were assayed at T0 (before start of filtration), T1 (4 hours after start), T2 (7 hours after start or at filter clotting). We performed qualitative analysis of vWF by agarose gel electrophoresis, aimed to identify the presence of abnormal multimeric forms (HMWM), at all times. Platelet function was also analysed with PFA-100 instrumentation, in order to find out if this methodology could supply information on platelet (hyper)reactivity. PFA-100 analysis was performed at all times. Anti-heparin/PF4 antibodies (HIT-Ab) were assayed at T0 in all patients. *Results*. 4 patients out of 22 underwent filter coagulation before end of procedure; of these, 3 were treated with UFH, 0 with DS and 1 was not anticoagulated; 2 of these were the only ones positive for HIT Ab. VIII:C level was significantly increased in all patients at baseline and remained unvaried during HD. Mean VIII:C level was 3.03 U/mL± 1.30 (vs 1.00± 0.40 normal range). vWF was also increased (mean 5.72 U/mL± 2.75 vs 1.00 U/mL± 0.40 normal range) and interestingly in 11 out of 22 patients qualitative analysis revealed circulating HMWM. P-selectin was raised in all patients and did not vary throughout HD (3920 ng/mL± 34 at T0, 1702 ng/mL±1926 at T1, 2108 ng/mL±1012 at T2 vs 137±18 ng/mL n.v.). In most patients (14/22, 64%) P:C was heavily reduced, often below 30% (0.28 U/mL±0.9). The PFA-100 instrumentation showed to be unable to detect cell hyperreactivity, and closure times did not correlate with filter coagulation. *Conclusions*. Dialyser clotting in critical patients undergoing HD seems to be a polyfactorial event: indeed pre-term filter coagulation occurred in patients with multiple hemostasis pro-thrombotic derangements and was more frequent among those with the presence of HMW multimers in plasma. Assay of plasma coagulation inhibitors and promotors may be a useful tool to asses the hemostatic balance of such patients and to identify those at higher risk of filter preterm coagulation. A scoring system is proposed for this purpose.

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INTRATHECAL RITUXIMAB IN COMPLEX TREATMENT OF REFRACTORY CD20-POSITIVE PRIMARY CNS LYMPHOMA

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Background. Rituximab binds specifically to the antigen CD20 which is expressed on more than 90% of B-cell NHL and primary CNS lymphoma (PCNSL) but is not expressed by normal neurons and glia in the brain. P*atients and methods*. Three patients with recurrent CD20+ PCNSL, IELSG score 1-2, were treated. High-dose methotrexate (MTX) was the basic systemic chemotherapy in one patient while others received 6 cycles of R-CHOP plus intrathecal therapy (MTX, AraC and hydrocortisone) and 40 Gy whole brain irradiation. All patients showed out MRI evidence of residual brain parenchymal disease. Nine planned intrathecal injections of rituximab in a dose of 20 mg each were given over 5-week period. Rituximab was administered in 2 mL of normal saline during 2 minutes. Tumor response was assessed by weekly CSF cytology and immunocytochemistry, neurological examination twice weekly, and MRI scanning at 5th week compared with baseline. MRI scan, physical and neurological examination were repeated at 9th week (4 week after final injection). *Results*. Intrathecal administration of rituximab was well tolerated and all patients exhibited cytological and biochemical response without CD20+ B cells detectable in CSF and no MRI evidence of PCNSL. Consolidation therapy included 12,5 mg MTX and 100 mg hydrocortisone intrathecaly weekly for 6 weeks. All patients are still in complete remission without CD+B cells in CSF, and normal brain MRI. One patient with IELSG prognostic score 1 is still receiving intensive MTX and Cytarabine treatment which will be followed by high-dose chemotherapy (BEAM) and ASCT. *Conclusions*. Overall survival of patients ranged from 15 to 30 months (15, 18, and 30 months, respectively) and there is no progression of disease after intrathecal rituximab therapy. These suggest that intrathecal application of rituximab may have a role in the treatment of refractory PCNSL.

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A MODIFIED BEAC-CONDITIONING PROTOCOL: A SAFE AND EFFECTIVE THERAPY FOR HIGH RISK ALL

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Stemcelltransplantation is an established strategy for high-risk ALL. Conventional conditioning schedules (eg.TBI+cyclophosphamide and others) do have a substantial toxicity which has to to be regarded seriously. Therefore we used a 7 days lasting chemotherapy conditioning regimen consisting of BCNU (300 mg/m^2), Etoposide (800 mg/m^2), ARA-C (3 g/m^2), Cyclophosphamide (120 mg/kg) and 2-Chlorodeoxyadenosine (24 mg/m^2) for these patients. For unrelated donor-transplantations (UD-Tx) ATG was added for two days. *Patients*. So far, 12 adult patients (6 male, 6 female) with a Ph+ pos. ALL (8), Pre- T-ALL (2), Pro-B ALL (1) in first CR and 1 relapsed cALL, with a median age of 41 years (19-53) were treated. In 6 patients a HLA ID Allo-PBPCT was performed, 1 HLA

ID Allo-BMT, 1 family one mismatched-PBPCT and 3 fully matched UD Allo-PBPCT. One 51 year old woman received an autologous transplant. All Allo-Tx patients received a standard GVHD prophylaxis with cyclosporine and short course MTX. G-CSF was administered in 10/12 patients from day +5 to PMN >1,0 G/L. *Results*. All patients reconstituted with a donor granulopoiesis within 11 days (PMN > 0,5 G/L, range 10-20), and thrombopoiesis within 15 days (plt>50 G/L, range 12-29). Chimerism at day 28 was over 95% donor hematopoiesis by quantitative PCR in all Allo-transplanted patients. Non hematologic side effects were usually mild (mucositis I-II in 10 pat., nausea/emesis grade I-II: 11, enteritis grade II in 2 pat.), severe side effects rare (mucositis grade IV: 1 pat., 1 emesis grade III). All infectious complications were treated successfully (3 FUO, 3 urogenital inf., 1 gastro.intest.inf., 1 pneumonia, 1 invasive aspergillosis and 1 septicaemia). One patient with transient renal failure during acute GVHD-phase (day 38) required passager dialysis. Within the first 100 days 2 patients were treated for a CMV reactivation. Acute GVHD grade 0-I developed in 4 pat. and GVHD grade II-IV in 6 pat. No early TRM occurred. With a median observation time of 19 months (range 9-89) 6/12 patients are alive (5 complete haematologic remissions, 1 Ph^+ALL with relapsed disease). 4 patients died of relapse (4, 10, 10, 17 months after Tx) and two late deaths occurred- 8 months after Tx (cerebral toxoplasmosis) and 10 months after Tx (extensive cGVHD+CMV enteritis). *Conclusions*. According to these first experiences, the modified BEAC conditioning protocol is safe (no TRM before day 100, 2 late deaths) and similarly efficient to other commonly used conditioning regimen in high risk ALL-patients.

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INCIDENCE AND RISK FACTORS ASSOCIATED WITH CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD) AFTER DONOR LYMPHOCYTE INFUSION (DLI): A SIX-YEAR ANALYSIS IN A SINGLE CENTER IN BRAZIL

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Background. DLI is largely used to treat relapse after hematopoietic stem cell transplants (HSCT) for malignant diseases. However this strategy is limited by the occurrence of acute or chronic GVHD, a well-known cause of transplant-related mortality. *Aims*. To evaluate the incidence of cGVHD and associated risk factors (thrombocytopenia, extensive skin disease, progressive onset) among 45 patients who received DLI to treat relapse after HSCT at the BMT center in INCA, between 2001 and 2006. *Methods*. 25 males, 20 females; median age=37 years (13-55). Diagnoses: 19 CML, 11 AML, 5 HL, 4 NHL, 6 others. Conditioning regimen was myeloablative (BuCy) in 33 and non-myeloblative in 12 (10 FluCy, 2 FluMel). All patients received DLI from HLA-matched sibling donors. The median number of DLI per patient was 2 (1-8) and of CD3+ cells was 0.74×10^8/kg (0.05-5.4). *Results*. Fourteen (31%) patients developed acute GVHD, four of them grade IV. The diagnosis of cGVHD was made in 15 patients (33%), with extensive disease in 8 and limited in 7. Pulmonary cGVHD occurred in 3 patients. Progressive onset in 6; extensive skin involvement in 7 and thrombocytopenia in 7. Among 15 patients with cGVHD, 7 (46,5%) had 2 to 3 risk factors. The mortality in this group was 71.4% (5 of 7), while in patients with 0-1 risk factor the mortality was 12.5% (1 of 8) (p=0.04). *Conclusions*. Our results showed a higher mortality when 2 or more of the risk factors described above were present at the onset of cGVHD. Taking into consideration the reduced and heterogeneous sample, the present study suggests the need for close attention to signs of cGVHD after DLI and more aggressive treatment, especially in patients with extensive disease plus thrombocytopenia, who achieved complete remission after immunotherapy.

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METHYLENETETRAHYDROFOLATE REDUCTASE MUTATION AND VENOUS THROMBOSIS IN CHILDREN

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Background. The venous thromboembolism is a serious and complex pathological condition especially for its potential complications. It is related to both congenital and acquired factors. Among the congenital factors, different mutations have been identified leading to a reduced activity of enzymes (even higher than 50%) involved in the homocysteine-methyonine methabolic pathway, such as the methylenetetrahydrofolate reductase enzyme (MTHFR). The homozygotic genotype is found in approximately 10-12% of the population and is associated with a 25% higher homocysteine level in comparison to those without this mutation. The heterozygotic genotype is found in 40% of the population. *Aims*. The aim of this study is to evidentiate the possible correlation between venous thrombosis and MTHFR mutations in children. *Methods and Results*. Between december 2002 and april 2006 nine children aged between 1-132 months were admitted in the Department of Hematology of Children Hospital Bambino Gesù in Rome. They were affected by deep venous thrombosis and were submitted to thrombophilic screening and vascular imaging. The MTHFR polymorphism C677T was found in all the patients (four in homozygosis and five in heterozygosis) and also in their parents. Four heterozygous and three homozygous patients presented increased plasmatic and urinary levels of homocysteine. Since venous thrombosis was only referred in the clinical history, two patients did not received treatments. Seven were treated with low molecular weight heparin (enoxaparin 100 U/kg s.c. x 2 daily), then they all continued the treatment with oral anticoagulant for 6-12 months and all of them performed long term profilaxis with folic acid and B group vitamins. Only one patient presented further thrombotic event and restarded the oral anticoagulant therapy. *Conclusions*. To perform anticoagulant prophylaxis in all patients with MTHFR mutation with previous thrombotic events could be useful, either in homozygosis or heterozygosis, with or without hyperhomocysteinaemia, in case of other thrombotic risk factors (pregnancy, oral contracceptives, sepsis and immobilization). These data suggest that the MTHFR evaluation can be useful in all patient affected by thrombosis. In addition, in presence of hyperhomocysteinemia, it might be reccomended to perform therapy with folic acid and B6 vitamin.

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AUTOLOGUS STEM CELL TRANSPLANTATION (ASCT) CONDITIONING THERAPY INTERMEDIATE DOSE MELPHALAN, BORTEZOMIB, THALIDOMIDE, DEXAMETHASONE (MVTD) IN RELAPSED MULTIPLE MYELOMA (MM)

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Background. ASCT with intermediate or high doses of Melphalan is the most effective therapy in patients with MM being < 65 years old, although this procedure is limited by an high percentage of relapses. Recent advances knowledge of the molecular mechanisms, allowed the development of novel targeted therapies. 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, an enhancement of global responses and an improvement of Progression Free Survival (PFS). *Aims*. The present study is aimed at the evaluation of effectiveness and toxicity of MVTD conditioning therapy with stem cell support in MM relapsed patients. *Patients and Methods*. From January 2005 to December 2006, 11 patients have been subjected to Autologous Transplantation therapy with MVTD conditioning therapy (Melphalan 100 mg/sqm total, day - 6 and - 3, Bortezomib 1.3 mg/sqm day - 6 e - 3, Thalidomide 200 mg from day - 6 to day - 2, Dexamethasone from day - 6 to - 3, CSP infusion at day 0) and SC support. 6 males and 5 females: median age 62 yrs (46-70); all patients in CS III A; seven patients had β2-microglobulin at diagnosis < 3, two > 5, two < 3 and < 5; 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: 1 patient (9%) obtained CR, 4 (36%) a nCR, 4 (36%) a VGPR and 2 (18%) 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 5 months (1-17), 3 (27%) are alive, 6 (55%) still keep the obtained response with a median of 5 months (1-17), 3 (27%) after progression are in rescue therapy, 2 patients (18%) died because of progression. PFS 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. 54% of patients had neutropenia with fever with a median of 2 days (0-8). 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, from the clinical state, and β2- microglobulin. None neurotoxicity has been observed, whether the haematological toxicity was overlapping to that due to conventional Autologous Transplant. Further studies, in larger series of non pluritreated patients are required.

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SICKLE CELL RETINOPATHY (SCR)

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Sickle Cell Anemia (SCA) is caused by a point mutation in the hemoglobin gene which results in hemoglobin polimerization when desoxygenated and in a sickled like shape erythrocyte that becomes less deformable.As the luminal diameter of retinal capillaries is less than the RBC diameter, they have difficulty traversing them and become trapped. The ocular manifestations of SCR, range from transient flashes and floaters to a sudden and profound decrease in vision. The abnormalities result from vaso-occlusion at the arteriolar bifurcations predominantly in the peripheral retina. Based on funduscopic observations and fluorescein angiography SCR is classified as Nonproliferative (NPSR) or proliferative (PSR). Main NPSR abnormlities: comma-shaped vessels, Black Sunburst, venous tortuosity, Salmon-Patch hemorrhages, silver wiring of retinal arterioles, glistening refractile spots, angoid streaks etc PSR is classified in 5 stages (Goldberg): peripheral arteriolar occlusion, peripheral arteriolar-venular anastomoses, neovascularization, vitreous hemorrhage and retinal detachment. Hyphemas have also been described. *Aims*. The purpose of this study was to find the incidence and type of ocular lesions that more frequently occur in SCA patients (pts) from our hospital. A total of 25 pts older than 13 years(17 female,8 males) were enrolled. Pts with diabetes and arterial hypertension were excluded. Correlation with age, sex, severity of anemia or Hb F levels was not done. *Methods*. Measurement of visual acuity, pupillary reactivity, evaluation of the anterior structures of the eye (slit-lamp biomicroscope) and the posterior and peripheral retina through a dilated pupil using direct and indirect ophthalmoscopy, including fluorescein angiography and fundus photographs (only in those with abnormal findings). Refractive defects and Color Vision tests complemented the study. *Results*. 36% of the patients referred visual symptoms: phosphens (100%) and impairment of visual acuity (33%). Fundus abnormalities in 23 out 25 pts (92%), in 87.5% of those asymptomatic and in all who presented the above symptoms. NPSR in all 23 pts: comma sign 72%, venous tortuosity 78.26%, retinal pigmentary changes 47.8% (Black Sunburst 17.4%) Intraretinal and choroid hemorrhages were others abnormalities found. 8 pts (34.78%) with PSR had: arteriolar occlusion, peripheral arteriolar-venular anastomoses and neovascularization (Sea-Fans) each in 50%, retinal detachment in 1 case (12.5%). Refractive errors in 92%, hypermetropia with or without astigmatism 20 cases (86%), no myopia or abnormal color vision found. *Conclusions*. phosphens was found in all symptomatic patients, this could probably alert the physician to fundus lesions in SS pts. Venous tortuosity an early and common characteristic of SCR was found in a higher incidence that is usually reported (78.3% vs 47%) although Black Sunburst was lower, others pigmented lesions made a total of 65.2% of cases. PSR in more than a third of our cases was higher than usually reported (34.7% vs 3%) This incidence needs to be confirmed with a higher number of patients. Because of the high rate of asymptomatic pts, early stages of eye disease go undetected unless a frequent eye exam beginning early in life is performed by a specialist

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HIGHLIGHTING THE USEFULNESS OF FLOW CYTOMETRY AS A DIAGNOSTIC TOOL IN A RARE CASE OF BERNARD-SOULIER SYNDROME

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Background. Bernard-Soulier syndrome (BSS) or giant platelet syndrome is a severe but infrequent congenital platelet disorder caused by qualitative or quantitative abnormalities in the platelet membrane von Willebrand factor (vWF) receptor complex. The vWF factor receptor, also known as platelet glycoprotein (GP) Ib/IX/V complex mediates platelet adhesion to the subendothelial matrix after vascular wall lesion in order to accomplish haemostasis averting any possibly baneful haemorrhage. BSS as a member of the heterogeneous group of inherited giant platelet disorders (IGPDs) is characterized by thrombocytopenia, large dysfunctional platelets and incapability in vWF factor induced nummulation. BSS's clinical features include prolonged bleeding time, mucosal bleeding, purpuric skin bleeding, epistaxis, ecchymoses, and menorrhagia. *Aim*. In this study we delineate the glycoprotein expression profile of a rare case of BSS in a young asymptomatic female using *ex vivo* phenotyping technology by means of flow cytometry. In this report we. This method has the advantage of being a rapid and sensitive tool for the study of platelet disorders assessing the size of platelets and their surface antigenic profile by qualitation and quantitation the expression of various receptors. *Methods*. A young Greek woman presented in our outpatients' department of Haematology for investigation of her low platelet count (50.000/μL) which was identified after a routine laboratory check-up. Levels of vWF were within the normal range and turbidimetric platelet aggregation showed no response after stimulation via several concentrations of ristocetin. BSS was suspected and flow cytometric analysis was performed to confirm the diagnosis.We have used flow cytometry analysis, in a Becton-Dickinson FACScan flow cytometer, to study the binding efficacy of murine monoclonal antibodies to platelets. Anti-GPIIb-IIIa, anti-GPIV, anti-GPIX, and anti-GPIb constituted the monoclonal fluorescent quartet we have used to asses the BSS glycoprotein expression status. The analysis was performed in one age/sex matched control and in one patient suffering from the syndrome. Our analysis failed to identify variations on the expression level of the glycoprotein IV. However, great protein quantity differences revealed when we compared the expression of the glycoproteins IX and Ib between the control and the BSS patient (≥ 35-fold and 143-fold decrease, respectively. *Results*. With a view to distinguish the red blood cell subpoplulations, CD41 immunostaining was performed for an accurate platelet determination as the very large platelets in BSS are often mistaken as lymphocytes and might overlay to leukocyte region. After the CD41-electronically gating of the platelet subpopulation, extended research was carried out, measuring the surface expression of platelet glycoproteins. CD36 flow cytometric analysis showed no difference in the expression of glycoprotein IV among the patient and the control. Meanwhile CD42a and CD42b immunostaining revealed great differences in the GPIX and GPIb expression profile. In the patient the GPIX was expressed at lower levels (2,6%) than in the normal control (90,46%) and the expression level of GPIb was also, markedly reduced (0.61% compared to 87,33%). *Conclusions*. In the cytomic era the establishment of new technologies capable to unravel the pathogenetic mechanisms and to diagnose rare and difficult to distinguish diseases such as the Bernard-Soulier syndrome becomes a necessity. Flow cytometry gives the answer against this challenge, being a many-valued, rapid and precise method in phenotyping the glycoprotein profile of the platelets.

1199

IDENTICAL PROGNOSIS FOR TRANSPLANTED AND NON-TRANSPLANTED PATIENTS WITH HEMATOLOGICAL MALIGNANCY ADMITTED TO THE INTENSIVE CARE UNIT

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Background and aim. There is scarce information on the influence of stem cell transplantation (SCT) on the prognosis of patients with hematological malignancies admitted to an intensive care unit (ICU). The objective of this study was to compare the outcome of transplanted and non-transplanted patients transferred to the ICU for a life-threatening complication. *Patients and Methods*. The mortality during ICU admission, long-term survival and the prognostic factors for survival were analyzed and compared in transplanted vs. non-transplanted patients. *Results*. 116 critically-ill patients with a hematological malignancy transferred to the ICU in a single institution from January 2000 to February 2007. Thirty patients had received SCT prior to ICU admission (17 autologous and 13 allogeneic).Transplanted and non-transplanted patients were comparable for demographic variables except age and disease status. No differences were found in overall survival or survival after discharge from ICU between transplanted and non-transplanted patients. The prognostic factors for survival in transplanted patients were the need for mechanical ventilation (p<0.040), liver function impairment (p=0.004) and fungal infection (p=0.050). The need of mechanical ventilation (p<0.001) or cardiovascular vasoactive drugs (p<0.001) and the presence of renal failure (p=0.003) predicted the outcome in the non-transplanted patients. *Conclusions*. A significant proportion of patients

admitted to ICU were discharged despite previous SCT. These patients did not have a worse prognosis than those transferred to the ICU with a hematologic malignancy, although the prognostic factors for survival were different in the two groups of patients

Table 1.

Overall actuarial probability of survival

	Overall (IC 95%)	No SCT	SCT
6 months	32%(23-41)	35%(25-45)	26%(10-42)
12 months	26%(17-35)	27%(17-37)	26%(10-42)
20 months	23%(15-31)	25%(15-35)	17%(0-34)
48 months	22%(14-30)	23%(13-33)	17%(0-34)

Actuarial probability of survival for discharged patients

	Overall (IC 95%)	No SCT	SCT
6 months	74%(61-87)	73%(59-94)	72%(37-100)
12 months	60%(45-75)	56%(39-73)	72%(37-100)
20 months	53%(37-69)	53%(36-70)	48%(3-93)
48 months	50%(34-66)	49%(30-66)	48%(3-93)

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SEQUENTIAL STUDY OF CYTOKINE AND LYMPHOCYTE PATTERNS IN PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Chronic GVHD (cGVHD) is becoming a more and more frequent complication of allogeneic stem cell transplantation (SCT), because of increasing use of matched unrelated donors (MUD), peripheral blood stem cells (PBSC) and reduced intensity conditioning (RIC) regimens. Defects of both thymic function and peripheral immune regulation seem to play a key role in its pathogenesis by inducing hyperactivation of immune-system with cytokine dysregulation. *Aims*. We tried to identify a possible correlation between patterns of cytokines and of lymphocytes and cGVHD. *Methods*. We prospectively evaluated cytokine levels and lymphocyte subsets in the late period after transplantation (from 3rd month) in 12 patients who underwent RIC-SCT. *Results*. Nine patients developed cGVHD at a median time of 6 months (range, 6-10) from transplant. Before the onset of cGVHD, the patients had an overall imbalance toward a Th1-TNF-α-response, as shown by: higher levels of TNF-α from 3rd to 6th month, higher TNF-α/sTNF-RI ratio at 3rd, 5th and 6th month, higher TNF-α/sTNF-RII ratio at 3rd and 5th month, higher TNF-α/IL-4 ratio at 3rd and 4th month, higher TNF-α/IL-6 ratio at 5th and 6th month, higher TNF-α/IFN-gamma ratio from 3rd to 6th month. Th-2 cytokines were higher only at 4th month. Patients with cGVHD showed lower levels of circulating NK cells and lower $CD3^+CD152^+/CD3^+CD28^+$ ratio at 3rd month. *Conclusion*. We observed increased levels of both Th1 and Th2 cytokines with different kinetics after RIC-SCT but with an overall prevalence of a TNF-α oriented response before the onset of cGVHD. Defects of immunoregulatory cells could be related to these fluctuating and unbalanced cytokine patterns. Defining such patterns could be an important tool for cGVHD modulation and for targeted therapies. However, further studies with more patients are required to support these preliminary results.

1201

PHARMACOLOGICAL CURRENT TREATMENT OF CHRONIC MYELOID LEUKAEMIA IN SPAIN. TRANSVERSAL AND OBSERVATIONAL STUDY IN 330 PATIENTS OF THE SPANISH REGISTRY OF CHRONIC MYELOID LEUKEMIA (RELMC)

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Introduction. Little is known about the actual situation of the current pharmacological treatment of Chronic Myeloid Leukaemia (CML) in Spain since the introduction of imatinib and other TK inhibitors and its repercussion in the change of treatment and outcome of these patients in our country. *Methods*. Inclusion criteria: CML patients treated only with drugs. The study collected patients treated at any time with Imatinib, and patients treated after 31st Dec 2002, with any drug.The information has been introduced by *in situ* monitoring by a data manager and was web based. The study is observational and population based, and comprises of complete information in pharmacological treatment of the first 330 patients with CML of diferent hospitals around different Spanish regions. *Results*. 330 patients, 61% men, 39% women. All of them were in the first chronic phase in the moment the study started(Sokal Index risk group low 55%, intermediate 31% high 14%) (Hasford risk group low 54%, intermediate 42% high 4% . The median of age was 53 years (ratio 20-91 years). 24% of the patients had received IFN as first line treatment. In the moment of analysis, 71% of the patients received Imatinib 400 mg, 1% imatinib 500, 12% imatinib 600 mg, 6% imatinib 800 mg, 1% dasatinib 100, 1% dasatinib 140 and 8% different combinations of interferon α and hydroxiurea. The Table 1 summarizes the best response of those patients treated with Imatinib, at any dose. *Conclusion*. This study is the first population based study which describes the pharmacological treatment of CML in Spain, outside clinical trials. We show the great importance of imatinib as current treatment in the CML.The results are fairly similar to those reported in clinical trials, and it would be a useful base for new longitudinal studies.

Table 1.

	After IFN failure	IMATINIB De novo
Hematological response		
Complete response	81%	97%
Partial response	13%	1%
No response	2%	1%
Accelerated phase	0%	1%
Cytogenetic response		
Complete	81%	76%
Partial	14%	9%
Minor	3%	1%
Minimal	1%	7%
No response	3%	7%
Molecular response	N=37	N=100
Complete	42%	35%

1202

UTILITY OF THE PFA-100TM FOR SCREENING OF PAEDIATRIC PATIENTS WITH PRIMARY HAEMOSTATIC DIATHESIS

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Background. The PFA-100TM is a platelet function analyzer, which uses high shear stress blood flow to simulate primary haemostasis and assess platelet function. The experience of PFA-100TM use in the paediatric setting is to date still relatively limited. *Aim*. Given the large number of children managed and screened for haemostatic disorders in our unit we decided to determine the performance of PFA-100TM instrument as a global test of primary haemostasis. *Patients and Methods*. We evaluated the performance of both PFA-100TM cartridges (collagen/ADP and collagen/Epi) in 365 consecutive children referred with potential primary haemostatic diathesis. The mean age was 5.7 years, with a range from one month to 18.2 years (218 boys, 179 girls). At the end of the study, the PFA-100TM results were compared with the confirmed diagnosis and analysed for the specificity and the sensitivity. Receiver operating characteristic curves were used to compare the relationship between sensitivity and specificity for the two different PFA-100TM cartridges for all the samples and for each of the patients groups. *Results*. Von Willebrand's disease (VWD) was the commonest haemostatic disorder (14.3%). Forty-eight children had VWD type 1, one VWD type 2B and three VWD type 3. Mild platelet defects were diagnosed in 12.6%; 18 had Hermansky Pudlak syndrome, four storage platelet defect and 24 platelet release defect. Severe platelet defects was found in 4.1% of the children; 14 with Glanzmann's thrombasthenia and one with Bernard Soulier's disease. Thrombocytopenia of various causes was the diagnosis in 8.2% of the population studied, and treatment with non-steroid anti-inflammatory drugs in 5.5%. Normal platelet function was found in 25.8% of the children and a definite diagnosis was not confirmed in 29.5% for several reasons; did not appear for follow-up, were severely ill or deceased. The specificity of the device was found to be 83%. The sensitivity was found to be 100% for severe haemostatic disorders such as VWD type 2B and 3, Glanzmann's thrombasthenia and Bernard-Soulier's disease. A high sensitivity (95.8%) was yielded for VWD type 1, but the PFA-100TM performed worse (82.6%) for mild storage and release platelet defects. The ability of the device to predict the absence of a certain disorder (negative predictive value) was 100% for the severe disorders, 97.5% for VWD type 1 and 90.7% for mild platelet function disorders. The positive predictive value for a specific defect was low (20-74.2%). *Conclusions*: The PFA-100TM, if used appropriately, can play an important role as a simple and rapid first-line screening test for primary haemostasis in children. Because of its high negative predictive value in at least one of the cartridges, it should increase the efficiency of the conventional diagnostic assessment of potential platelet defects. Future improvement of the device's cartridges may help to increase its sensitivity to mild VWD and platelet function defects.

1203

DEFERIPRONE (DFO) IS AN EFFECTIVE ORAL IRON CHELATOR IN PATIENTS WITH HEREDITARY HAEMOCHROMATOSIS (HH) FAILING, OR INTOLERANT TO, VENESECTION OR DESFERRIOXAMINE (DFO) THERAPY

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Background. Hereditary Haemochromatosis (HH) is an inherited iron loading disorder associated with excessive iron accumulation and damage to organs and tissues such as the heart, pancreas, liver, skin and joints. The commonest method employed for removing iron stores in patients with HH is by venesection. However, some patients are unable to tolerate the intensive venesection required to achieve optimal reduction in iron stores. Those failing venesection are most commonly offered second-line therapy with desferrioxamine (DFO). This has several toxicities and requires considerable compliance and lifestyle modifications which many patients find unacceptable. Deferiprone (DFP) is an oral iron chelator approved by the European Regulatory Authority in 1999 as second line therapy for transfusional haemochromatosis in patients with thalasaemia unable to tolerate DFO. It is generally well tolerated but its major side effect is the idiosyncratic development of agranulocytosis. There is no literature on the off-license use of DFP to lower iron stores in patients with HH despite there being a good pharmacobiological reason to suggest this may be beneficial *Aims*. To assess the efficacy and tolerability of DFP in lowering iron stores in patients with HH failing, or intolerant to, venesection and DFO at our institution. *Methods*. We retrospectively reviewed clinical outcomes in all patients with HH at our institution who were treated with DFP to lower iron stores following failure, or intolerance to, venesection or DFO therapy. *Results*. The results are summarised in Table 1. Seven patients with HH (M=6, F=1) were eligible for inclusion in the study which covered a period from October 2004 to February 2007. Their average age was 60 years (range 42-70 years). All but one were homozygous for the C282Y mutation. The remaining individual (patient 2) was an H63D heterozygote. The average serum ferritin on commencement of deferiprone was 1718 micrograms/litre (range 633-4929 micrograms/litre). DFP was commenced up to the recommended dose of 75 milligrams per kilogram per day, which equated to total daily doses of 0.5 grams-6.0 grams. Doses were modified according to clinical response and side effects. Serum ferritin levels were measured every 3 months for up to 12 months. The average serum ferritin level at 3 months, 6 months, 9 months and 12 months was 1485 (n=6 evaluable patients), 1242 (n=5), 1166 (n=5) and 1067 (n=4) micrograms/litre. This represented an approximate 38% reduction in ferritin levels with DFP at 12 months. Two patients developed reversible grade 2 neutropenia which necessitated dose reduction (patient 3) and discontinuation (patient 7). No infections were noted during this period. Patient 6 developed abdominal pains and diarrhoea, opting to discontinue therapy after less than 4 weeks. *Conclusions*. DFP appears efficacious at lowering iron stores in patients with HH who fail, or are intolerant to, venesection or DFO therapy, with low toxicity. Our observations merit longer follow up and support further large scale studies to evaluate the safety, efficacy, cost effectiveness and patient quality of life of using oral DFP as standard second line therapy, following venesection, in patients with HH.

Table 1.

1204

THE ROLE OF HYPERCOAGULABLE STATE IN PATHOLOGY – STUDY ON A GROUP OF 7500 CASES

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The pathogenesis of hypercoagulable state is particularly complex: from factors concerning the vascular wall up to genetic changes characteristic of inherited thrombotic disorders. The study we have carried out attempts to establish the significance of the various entities for the clinic. Investigating a group of 7500 patients hospitalized in the clinics of the institute and surveying the pathology induced by hypercoagulable states, we insisted only on the deep thromboses of the peripheral veins, thromboses of the central retina vein, the thromboses of the cerebral vein, renal vein, Budd-Chiari syndrome, disseminated intravascular coagulation (DIC), small vessels thromboses, the factors occurring in the arterial pathology being more complex. The conclusions of our study point out to a 36,26/1000 hospitalised subjects the frequency of the pathology induced by hypercoagulable states, of which: -Non Hematologic Malignancy that induced 22,79% of the thrombotic events -Collagen diseases (SLE, RA, SS): that induced 10,29% of the thrombotic

events -Postoperative states: 9,19% -Chronic myeloproliferative disorders(ET, PV, CML): 9,92% -Dislipidemias, diabetes mellitus: 5,14% -Inherited thrombophilia (Antithrombin III deficiency, Protein C deficiency, Protein S deficiency): 4,41% -Anticardiolipin Syndrome: 5,14% -Lymphoproliferative Disorders (Non-Hodgkin's Lymphomas, IgA Multiple myeloma, Hodgkin's Disease): 9,55% -Secondary polycythaemia: 3,67%, -Cryoglobulinemia: 2,94%, -Infectious diseases: 2,94%, -Paroxysmal Nocturnal Haemoglobinuria (PNH): 1,83%, -Nephrotic syndrome: 1,83%, -Congestive heart failure: 1,83%, -Horton arteritis:1,47%, -Behcet disease:0,73%, -Other causes (oral contraceptives, obesity, artificial surface, ankylosing spondilitis, MDS): 6,25%. The 6,25 ratio (230,61/36,26) between arterial and venous pathology point out the major role of the vascular wall in the hypercoagulable state. The prevalent features of the pathology induced by the inherited thrombophilia are the recurrence of the thromboses, the early age, and its resistance to therapy.

1205

MUTATIONAL SCREENING IN 10 PATIENTS WITH TYPES 2 VON WILLEBRANDS DISEASE

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Introduction. von Willebrand disease (VWD) type 2 is caused by von Willebrand factor (vWF) functional and structural defects. VWD type 2N, an autossomic recessive disorder characterized by a defective binding of vWF to factor VIII and low FVIII/FvWAg ratio, is associated with mutations in the FVIII-binding domain (D'-D3). VWD subtypes 2A, 2B and 2M, with an autossomic dominant inheritance, are characterized by a discrepancy between the level of vWF antigen and the vWF activity or collagen-binding capacity. Subtypes 2A and 2B have no high molecular weight (HMW) vWF multimers. VWD 2A shows an increased susceptibility to proteolysis and prominent subbands in the triplet structure due to, in most of cases, mutations in the A2 domain, close to ADAMTS13 cleavage site - Y1605 and M1606. Subtype 2B is often associated to a moderate-mild thrombocytopenia, probably due an increased binding of HMW multimers to platelets, caused by gain-function mutations within A1 domain. Subtype 2M, defined by a low platelet-dependent function with normal HMW multimers, is also associated with mutations in the A1domain. *Aim*. In order to provide the correct diagnose and treatment we studied 10 unrelated patients with vWD subtype 2 attending our Haemophilia Centre. *Methods*. VWD types and subtypes differentiation was done according to ISTH Scientific Subcommittee on VWD criteria. The vWF function was studied by ELISA tests; ristocetin induced platelet agglutination (RIPA) and multimeric structure of VWF SDS-agarose electrophoresis with automatic densitometry. vWF gene exons 18-25 (D'-D3 domain), exon 28 (A1-A2 domain) and respective intron/exon boundaries were studied by direct sequence of PCR fragments.

Table 1. vWD 2N- mutation analyse data.

Patient (n)	vWF:Ag/ VIII:C ratio	VIII:C (%)	vWF:FVIIIB (%)	Multimers	Exon	Mutation	AA	status
1	0.08	5	0	Normal	19	nt 2446, C > T	R816W	Htz
3	0.41±0.05	27.0 ± 6.2	5.7 ± 3.3	Normal	20	nt 2561, G> A	R854Q	Hm

Table 2. vWD 2A, 2B and 2M- mutation analyse data.

Patient (n)	vWD type	vWF:Ag/ vWF:Co ratio	vWF:Ag/ vWF:CB ratio	RIPA	Multimers	Mutation	AA	Domain
1	2A	0.21	0.32	dimin	IIE	nt 3815, G > T	C1272F	A1
2	2B	0.46±0.22	0.56±0.19	incr.	IIB	nt 3916, C> T	R1306W	A1
1	2M	0.68	0.64	dimin	Abnormal	nt 4105, T>A	F1369I	
						nt 4135, C>T	R1379C	A1
1	2A	0.57	0.53	dimin	IIA	nt 4517, C>T	S1506L	A2
1	2A	0.45	0.50	dimin	IIA	nt 4883, T>C	I1628T	A2

(n)- number of patients; AA- aminoacid substitution; Htz-Heterozygous; Hm-Homozygous- incr.-increased; dimin-diminished

Results. Four patients vWD 2N have FVIII:C binding site mutations: three are homozygous R854Q and one is heterozygous R816W (Table 1). Among the 6 patients with a VWF:RCo/VWF:Ag ratio < 0.7 and absence of HMW multimers, 5 different mutations were identified in exon 28, in the heterozygous state, (Table 2): 2A (n=3) S1506L, I1628T and C1272F (not previously be described); 2B (n=2) R1306W; 2M (n=1) F1369I and R1379C. Mutation R1379C is usually associated with type. *Discussion*. Our 3 homozygous R854Q 2N patients have a moderate phenotype, as usually described. One of these patients was originally diagnosed as mild haemophilia A. In the fourth type 2 N patient the R816W heterozygous state does not explain the markedly reduced FVIII levels and the absence of vWF:VIIIB. The absence of other mutations within the FVIII binding domain suggests a null allele as described by several authors. In only one of the type 2B patients has thrombocytopenia ($54\pm20\times10^9$). The new 2A C1272F mutation, located in the first cysteine of the C1272-C1458 loop, is expected to disrupt an important interaction region between vWF and GPIbα. Patients with mutations S1506L, I1628T at A2 domain have the expected multimer pattern according to the vicinity of ADAMTS13 Y1605/M1606. In 2M subtype patient the abnormal multimer pattern is most probably due to mutation R1379C as described (ISTH SSC vWF database). In our experience, multimer analysis is very useful to draw the strategy for the vWF molecular studies in order to differentiate the VWD subtypes.

1206

RAISING AWARENESS OF ANEMIA IN ONCOLOGY CENTERS USING A COMPUTERIZED EVIDENCE-BASED DECISION SUPPORT SYSTEM FOR ERYTHROPOIETIC PROTEIN USE IN CANCER-RELATED ANEMIA

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Background. There is evidence to suggest that the publication of evidence-based practice guidelines (EBPGs) does not assure their application in patient care. Computerized clinical decision support systems (CDSSs) incorporating EBPGs may promote evidence-based decision-making with regard to individual patients. Most CDSSs are knowledge-based and not evidence-based and the most effective CDSSs are those integrated into workflow, providing point of care guidance, and offering actionable recommendations. However, few CDSSs are validated in terms of algorithmic operationalization. *Aims*. RESPOND is a CDSS based on the 2006 update of the European Organisation for Research and Treatment of Cancer (EORTC) EBPGs for erythropoietic proteins in cancer-related anemia. Using the RESPOND CDSS, the objective was to attain an intraclass correlation coefficient (ICC) >0.90 for each operationalization, employing a maximum of three iterations. *Methods*. Descriptive study. A panel of five experts (three physicians, two nurses) rated the 27 algorithms derived from the EORTC EBPGs in terms of accuracy (binary: yes/no). If the algorithms were judged inaccurate, the experts were requested to explain and propose the suitable correction.

Table 1. Example of operationalization of guideline

Guideline	Criteria – if met ≥ recommendation generated
In patients with cancer-related anemia not undergoing chemotherapy and/or radiotherapy, treatment with erythropoietic proteins should be initiated at an Hb level of 9–11 g/dL based on anemia-related symptoms	((if chemo = no) AND (if radio = no)) AND (if auto transplant = no) AND (if allo transplant = no) AND ((if Hb >8.9 g/dL) AND (if Hb <11.1 g/dL)) AND (if symptoms = yes) AND (if epo = no)

Hb, hemoglobin

Results. In iteration 1, three of the experts agreed with all 27 sets; one disagreed with one set and one disagreed with four sets; therefore the ICC=1.00 for the remaining 22 sets, and was ≥0.90 for five sets. Three sets concerned additional output to be generated, and one a substantive inaccuracy; all were corrected. One set concerned a suggestion not supported by evidence, hence this was not incorporated. In iteration 2, all five experts agreed with all sets, thus the ICC=1.00 for 27 sets. *Conclusions*. The RESPOND CDSS is currently being developed and validated. This CDSS is based on algorithms that accurately specify, in the form of

conditional dependency rules, the EORTC EBPGs for erythropoietic proteins.

1207

DEFIBROTIDE IN THE PREVENTION AND TREATMENT OF VENO OCCLUSIVE DISEASE IN AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN.

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Background. Veno-occlusive disease (VOD) is a common (10-50%) and serious complication of stem cell transplantation. *Aims*. To investigate the effectiveness of defibrotide as prophylaxis and treatment against VOD in children which has been shown to effective in adults. *Methods*. Defibrotide prophylaxis (20 mg/kg/day) was given to 47 successive patients who underwent transplantation between April 2004 and December 2005. Incidence of VOD was compared with 56 historical controls transplanted between November 2001 and April 2004. High risk patients in the control group (busulphan conditioning and deranged liver function) received ursodeoxycholic acid, tinzaparin and glutamine as VOD prophylaxis. The groups were matched for sex, age, type of transplant (unrelated, related allogeneic or autologous transplant) and risk. *Results*. The mean maximum bilirubin was the same in both groups (32 µmol/L). In the defibrotide group, 2 patients (4%) developed VOD (Seattle criteria). 2 further patients were also treated for presumed VOD but this was not confirmed on liver biopsy. Defibrotide dose was increased in all 4 patients to 40-60 mg/kg/day. All patients' symptoms resolved within 14 days and are currently alive 30- 330 days post transplant. No serious side effects were reported. Of the control group 4 patients (7%) had VOD. 2 of these patients had reversed hepatic vein flow. 3 of these patients received Busulphan conditioning and 2 died in ITU 30 days post transplant, partly due to VOD. VOD was associated with busulphan conditioning (p= 0.001) and not with age, sex, type of transplant, Graft vs Host Disease, deranged liver function prior to transplant or type of antifungal prophylaxis. Although our study demonstrates a small overall incidence of VOD this was reduced in the defibrotide group where there was also no VOD related mortality. Our study suggests that defibrotide can be effectively used as prophylaxis and treatment in high risk patients but further larger multi centre studies should be carried out.

1208

VALUE OF THE MORPHOLOGIC CHARACTERISTICS OF THE BONE MARROW IN THE DIAGNOSISI OF ESSENTIAL THROMBOCYTHEMIA: STUDY OF 22 CASES.

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Background. The criteria diagnoses of the WHO for essential throboocythemia (ET) confer value to the histopathology and facilitate more precise diagnoses in the differentiation of ET of other chronic myeloproliferative disorders, in special initial stages of myelofibrosis (MF) with a significant unfavorable prognosis. *Aims*. Histopathologic revision of cases initially diagnosed ET and correlation with the clinical and analytical evolution of them. METHODS Hystopathologic revision of 22 cases of patients initially diagnosed of ET throughout 14 years was done. Morphologic criteria are used according to the WHO and the described ones according to Thiele *Et al*. (Histol Histopathol. 2005; 20: 633-644). We valued: cellular density in relation to the age, number of megakaryocytes, presencet of clusters dense, clusters lax, number of megakaryocytes of great, intermediate and small size, nuclear lobulation, defects of maturation (nucleus of bulbous and naked aspect). Also it is made the evaluation of cellular density and reticulynic fibrosis according to the European consensus (Thiele et t al. Haematologica 2005; 90:1128 - 32) and gradation of the MF is made in four degrees. The cellular percentage has been obtained from digital images by means of the software of analysis of image Image-Pro Plus® 5,0 (Average Cybernetics, U.S.A.). *Results*. The hystopathologic study of the 22 samples of bone marrow reviewed shows that, 4 of them - cases 10.11.13 and 18 - (18.18%) present a degree of increased cellularity according to the age of the patient and to corresponding by the criterion the initial diagnosis ET. In reference to the myelofibrosis degree, 2 - cases 18 and 20 - of the 22 reviewed bone biopsies (9.1%), present a degree of reticulynic fibrosis superior to hoped for the initial diagnosis of ET. During the pursuit of the patients thrombotic complications take place in 3 cases (1 cerbrovascular disease, 1 pulmonary embolism (PE) and 1 venous thrombosis/PE). *Conclusions*. Applying recent consensus of standardized criteria for the hystophatologic study of the biopsies of bony marrow, 5 analyzed samples of a total of 22, (22.72%) corresponding ones to biopsies initially diagnosed of ET, they could be redefined like pertaining to precocious stages of myelofibrosis. Even though which in only one of these five cases (case 13) presence of thrombotic and/or hemorragic complications is observed and a worse control of the numbers of platelets (in relation to the line number required of treatment) this redefinition diagnoses can be of utility facing the evolution foretells end of the process.

1209

THE EFFECT OF RADIOLOGICAL IMAGING STUDIES ON THE RISK OF SECONDARY MALIGNANCY DEVELOPMENT IN PATIENTS WITH HODGKINS LYMPHOMA

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Background. Recently, reports suggesting that diagnostic radiological imaging studies could play a role on the risk of secondary malignancies have been published. Nowadays, a guideline about the use radiological techniques in patients with multiple myeloma have been reported by UK Myeloma Forum Guidelines Working Group and Nordic Myeloma Study Group which emphasized unnecessary risk due to X-ray exposure originating from frequent imaging investigations. *Aims*. The aim of our study is to calculate the average amount of accumulated radiation by means of radiological imaging studies performed intensively in diagnosis and follow-up of patients with Hodgkin's lymphoma and to evaluate whether this amount of accumulation account for a real risk for secondary malignancies. *Methods*. This study consists of 15 male patients, whose mean age was 23,67±4,24 years. All radiological imaging studies performed in Hodgkin's lymphoma patients were noted in detail and average radiation dose accumulation was calculated according to data of National Radiological Protection Board (NRPB) and Biological Effects of Ionizing Radiation (BEIR) VII report. *Results*. Median radiation dose which patients subjected during median 14,5 months of disease duration was 85,19 miliSievert (mSv) and 161,08 mSv according to data of NRPB and BEIR VII report, respectively. The cumulative radiation dose because of radiological imaging studies is 8,5-16 times greater than that of the described dose having 1 in 1.000 chance of cancer development according to BEIR VII report. Approximately, this amount is equivalent to the dose of natural background radiation received during 35-70 years. *Conclusions*. Our study demonstrated that radiation dose accumulation because of radiological imaging studies used in diagnosis, staging and follow-up of patients with Hodgkin's lymphoma was high enough to cause development of secondary malignancies. Finally, it is obvious that the radiological imaging study policies used in follow-up of these patients should be overviewed.

1210

ENDOGLIN AS A NEW BIO INDICATOR OF HAEMORHEOPHERESIS/IMMUNOAPHERESIS PROCEDURE EFFICIENCY

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Background. An ideal tool for immediate evaluation of extracorporeal elimination therapy would be a serum marker, levels of which would correlate with disease activity and reflect an improvement after elimination therapy. Still, there are no suitable markers for extracorporeal elimination in familial hypercholesterolemia (FH) which would reliably determine the therapy intensity immediately after the procedure and would be able to show activity of atherosclerosis. *Aims*. In a previous study, we found that the levels of P-selectin, MCP-1, hsCRP and CD40L were reduced after LDL-apheresis. Although the post-elimination decreases in all four markers reached statistical significance, we speculated that endoglin (sCD105) levels could represent an even more effective tool for evaluation of treatment efficacy. *Patients and Methods*. Altogether 40 examinations of endoglin level in 11 patients with severe FH and long-term treatment (4.5±2.8 years) by extracorporeal elimination (LDL-apheresis and haemorheopheresis) were done. Informed consent was obtained. We measured sCD105 levels immediately before and after two consecutive elimination procedures. *Results*. Baseline serum sCD105 levels were significantly higher in the patients than in the control group. The decrease in sCD105 after both LDL-elimination series was also statistically significant. Endoglin level normalized after procedures in all of 40 except one measurements. *Conclusions*. We conclude that endoglin can serve as a useful marker for evaluation of the treatment

efficacy and decreased atherosclerosis activity in patients with familial hyperlipoproteinemia treated by extracorporeal LDL-cholesterol elimination. sCD105 levels are increased in patients with severe FH and decrease after extracorporeal elimination what is the first observation.

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1211

A NEW VARIANT MLL-SEPT2 FUSION TRANSCRIPT IN THERAPY-RELATED ACUTE MYELOID LEUKEMIA WITH T(2;11)(Q37;Q23)

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Background. 11q23 MLL gene rearrangements are found in acute leukemias and are associated with a poor prognosis. To date, 87 different chromosome bands have been described with 11q23 MLL rearrangements and about 51 fusion genes have been identified. 5-10% of MLL gene rearrangements are found in patients with therapy-related leukemia after treatment with topoisomerase II inhibitors, or high-dose radiotherapy. T(2;11)(q37;q23) is a rare recurrent abnormality in (therapy-related) acute myeloid leukemia (t-AML). *Methods and Results*. A 66-year-old male was diagnosed with t-AML (FAB AML-M2) after previous chemotherapy for gastric carcinoma. Cytogenetics showed a 46,XY,t(2;11)(q37;q23)[4]/51,sl,+8,+17,+21,+22,+mar[10]/46,XY[5] karyotype. FISH analysis demonstrated an 11q23 MLL rearrangement. In the process to identify the MLL-partner gene on chromosome 2 band q37 a paper was published describing the characterization of a MLL-SEPT2 fusion transcript in a t(2;11)(q37;q23)-positive t-AML (FAB AML-M4). To investigate the presence of a MLL-SEPT2 fusion mRNA in our case, RT-PCR and sequencing analysis was performed, revealing an in-frame MLL-SEPT2 fusion of exon 6 of MLL to exon 3 of SEPT2. Therefore, the MLL moiety lacks exon 7 that is included in the previously reported fusion transcript. *Conclusions*. We report on a novel t(2;11)(q37;q23) positive t-AML resulting in a new variant MLL-SEPT2 fusion transcript (type II). A review of the literature demonstrated that t(2;11)(q37;q23) is a rare recurrent chromosomal aberration thus far reported in two cases of *de novo* AML, and two cases of t-AML, not linked to any specific AML FAB-subtype. It has been founded as a sole abnormality or in addition to other aberrancies. In our case the t(2;11)(q37;q23) was found as a sole aberration with clonal evolution towards additional numerical and structural changes.

1212

DIVERSE ANTIOXIDANT EFFECTS ON PRENEOPLASIC LESIONS INDUCTION IN PROMOTION STAGE IN A CHEMICAL HEPATOCARCINOGENESIS MODEL

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Background. The Transcriptional factor NF-κB is involved in oncogenesis process due to cellular proliferation regulatory proteins. NF-κB is a transcriptional element activated by several stimuli, including oxidative stress. After several inductors, NF-κB inhibitors IκB's are rapidly phosphorylated by IKK kinase and ubiquitinated by E3 ubiquitin-ligase to subsequent degradation by 26S proteasome. Then, NF-κB free translocates to nucleus. Current evidence involves oxidative stress to inflammation and degenerative diseases, such as rheumatoid arthritis, Alzheimer and carcinogenesis. One of major approach to inhibit or diminish the redox transcription factors activation has been the antioxidant therapy. The antioxidants S-Adenosyl-methionine (SAM), N-acetyl-cysteine and Quercetin, have been able to protect in degenerative disorders. *Aims*. To determine the role of antioxidants in preneoplasic lesions induction and the modulation of NF-κB signaling pathway. *Methods*. Fisher rats 344 were subjected to carcinogenic treatment. Rats were initiated with Diethyl-nitrosamine (DEN) (200 mg/kg i.p.). At 7 day after initiation, 2-Acetyl-amino-fluorene (2-AAF) was administered by gavage during 3 days (25 mg/kg). On day 10, rats were subjected to Partial Hepatectomy (PH). The antioxidants were administered separately during the carcinogenic treatment (TC), since 24 hr after DEN until 2 hr before PH. In order to evaluate the antioxidant effects, the caffeic acid phenethyl ester, a selective NF-κB inhibitor was administered 2 hr before PH. All groups were sacrificed 30 min after PH. The preneoplasic lesions induction was determined by tumor markers Gamma-glutamyl-transpeptidase (GGT) and Glutathione-S-transferase placental (GST-p). The nuclear levels of NF-κB and the signaling pathway activation were determined by western blot analysis. The glutathione levels were measured by Ellman's method. *Results*. The carcinogenic treatment induced preneoplasic lesions, Rel A/p65 nuclear levels increase, IKKα/IKKβ, and IκB-α phosphorylation. The SAM treatment diminished 64% the GGT preneoplasic foci and 68% in GGT+ area. However, GST-p tumor marker was not diminished. SAM exerted an antioxidant effect incremented 104.7% glutathione levels above normal liver. Also, SAM caused a significant reduction of 86.8% in Rel A/p65 nuclear levels in comparison to TC, a 47.3% IKKα/IKKβ and 54.6% IkB-α phosphorylation decrease. NAC caused a significant GGT foci number decrease (69.1%), and area (63.95%). The GST-p number foci and area were significant diminished in 57% and 73.9%, respectively. However, glutathione levels were not altered. Unexpectedly, NAC did not diminish the Rel A/p65 nuclear levels, although reduced 54% IKK·/IKK, and 65.1% IκB-α phosphorylation. Quercetin flavonoid only diminished 83.2% the GGT+ area, although reduced the GST-p number foci and area, 45.8% and 86.4% respectively. Quercetin not exerted an antioxidant effect in glutathione levels, while Rel A/p65 nuclear levels were considerably reduced. IKKα/IKKβ and IkB-α phosphorylation diminution were not observed.

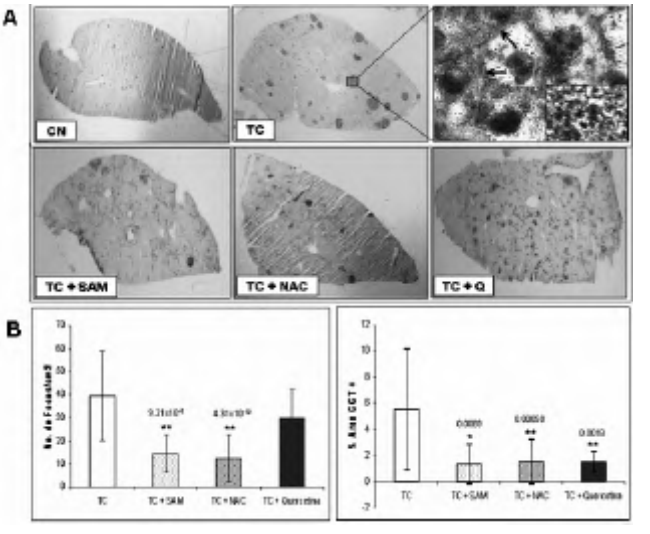

Figure 1. Diminution of preneoplasic lesion by antioxidant treatment. A. Histological determination of GGT activity in TC and antioxidant treatments. B. Quantification of preneoplasic nodules and area. Aroows indicate the GGT cellular localization.

Conclusions. The NF-κB pathway is a key component of the redox signaling in cancer process. The reactive oxygen species are involved in preneoplasic lesion induction on liver cancer. The glutathione precursors SAM and NAC exerted a NF-κB diminution by IKK and IkB-α phosphorylation inhibition. The preneoplasic lesion diminution of quercetin is an independent mechanism of NF-κB.

1213

INCIDENCE OF FLT-3 MUTATIONS IN IRANIAN ADULT PATIENTS WITH DIFFERENT SUBTYPES OF ACUTE MYELOID LEUKAEMIA

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Background. Fms-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family along with KIT and FMS. Two clusters of activating FLT3 mutations are known: FLT3-internal tandem duplications (FLT3-ITD) in the juxtamembrane (JM) domain in 20-25% and FLT3-point mutations within the activation loop of the tyrosin kinase domain (TKD), which mostly affects asparate 835 (D835) in 7-10% of patients respectively.These abnormalities considered as the most frequent molecular abnormalities in AML, which predict unfavorable outcome. However, the data concerning the incidence and associations with patients characteristics vary in different studies. *Aims*. The aim of study was to analyze the impact of FLT3 mutations in cohort of 202 newly diagnosed Iranian patients with differents subtypes of AML and to correlate FLT3 positive status with some clinical and biological features. *Methods*. All adult patients diagnosed in main haematology centers. Peripheral blood or bone marrow from 202 patients were screened. Genomic DNA polymerase chain reaction (PCR) assay was performed to detect FLT3-ITDs located from exon 11 to exon 12 and interon 11 (PCR band(s)>329bp). Asp 835 point mutations in exon 20 of the FLT3

gene were detected with PCR followed by digestion with EcoRV of the 114 bp PCR product (PCR band(s) 68 and 46bp for wild type). Non fully digested products were considered as mutated. *Results*. The median age of onset was 47±12 (range from18-75) years, (116 males and 86 females).FLT3-ITD were detected in 36/202 patients (18%) and D835 mutations in 12/202 (6%). In the study group of 202 patients according to FAB classification, rate of FLT3 mutations were found higher in M3 with 40/176 (22.7%). The distribution of FLT3 mutations was as follows: FLT3-ITD was detected in M2 2/8 (25%) patients, in M3 32/176 (18.2%), in M4 2/8 (25%). D835 mutation was found in 8 patients with M3 and 4 patients with M2 and M5 type of AML. The majority cases were acute promyelocyte leukaemia and characterized by the T-15-17. Evidence to date suggest that PML/RARA is insufficient for leukomogenesis. Potential candidate include mutations of FLT3, which could confer a proliferative advantage thereby complementing the differentiation block induced by PML-RARA. There was no correlation between patients with ITD status and gender and age. A positive correlation with high presenting WBC > 20000/micl (58%) and high percentage of circulating blast cells was demonstrated in ITD positive patients (p<0.05). Differently, D835 mutations were not associated with high white blood cells count and blast cells percentage.The high frequency of FLT3-ITD positive mutations in patients with leukocytosis provides a rationale for evaluation of FLT3 inhibitors as a component of induction therapy. *Conclusion*. In this study we demonstrated that the FLT3 mutations is a frequent molecular lesions in Iranian AML patients, the incidence of ITD and D835 was 18% and 6% respectively.The presence of ITD were associated significantly with M3 morphology and with high WBC and blast cells.

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1214

ACTIVATED GAMMA-DELTA T CD 56+ LYMPHOCYTES (GDT CD56+) PERCENTAGE IN PERIPHERAL BLOOD AS A MARKER OF NORMAL IMMUNOLOGICAL RECONSTRUCTION AFTER TRANSPLANTATION AND GOOD PROGNOSIS IN MULTIPLE MYELOMA (MM) AND LYMPHOMA MALIGNUM (NHL) PATIENTS TREATED MEGACHEMOTHERAPY AND PERIPHERAL BLOOD STEM CELL T

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Background. Gamma-delta T lymphocytes reveal an immune response to acute lymphoblastic leukemia following allogenic BMT, resultating in improved relapse-free survival for those patients, who survive 100 days from transplant. Activated gdT cells, expressed antigen CD 56 (gd T CD56+), demonstrate cytolytic antitumour activity against myeloma and lymphoma cells. *Aim*. gd T CD56+cells mean percentage (%) in peripheral blood in multiple myeloma (MM) and lymphoma malignum (NHL) patients, treated megachemotherapy (mega-chmt) and peripheral blood stem cell transplantation (PBSCT) was investigated. Material and *Methods*. 10 patients (pts): 6 MM and 4 NHL diagnosed and treated in Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of WrocПaw Medical University, Poland and 14 healthy controls were included into analysis. Among 6 MM pts 2 were in II, 3 in III stages, 1 pt was MM non-secretorius, according to Durie-Salomon classification. 4 pts were classifieled to A and 2 to B groups. Among 4 NHL pts 3 were in IVA and 1 pt in IVB stages according to Ann- Arbor classification. 1 of them had histopatological diagnosis of B- lymphoblastic, 1 - diffuse large B cell, 1- follicular and 1 pt B-cell T rich lymphomas. Blood samples of MM and NHL pts were taken before and after mega-chmt + PBSCT. gd T cells were estimated by flow-cytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies (MoAbs:Ab-anti TCRgamma1-FITC (Becton-Dickinson), Ab-anti CD14-RPE, Ab-anti CD-45-FITC, CD56-RPE (DAKO) and Ab IgG1 kappa - FITC (Becton-Dickinson), and Ab IgG1- RPE (DAKO) as negative controls.) *Results*. Mean% of total gd T lymphocytes in peripheral blood before mega-chmt+PBSCT was 1,72 of all MM and 3,63 of all NHL pts. It was lower than in control group (1,72 and 3,63 vs 5,63). In MM pts, after mega-chmt+PBSCT, gd T cells mean % increased ,compared before treatment: 3,73 vs 1,72, but was also lower than in health volunteers: 3,73 vs 5,63. Similarly, after mega-chmt+PBSCT in MM pts activated gd T CD56+cells mean % was higher than before transplantation: 0,37 vs 0,18, p= 0,04 (statisticaly significant). Instead, in NHL pts after mega-chmt+PBSCT, gd T cells mean % decreased, compared before treatment: 1,13 vs 3,63 and was lower than in health volunteers: 1,13 vs 5,63. After mega-chmt+PBSCT in NHL pts activated gd T CD56+ cells mean % were lower than before transplantation: 0,12 vs 0,32, p=NS. All MM and NHL pts (100%) stayed in complete remission (RC) 6 months after mega-chmt+PBSCT. *Conclusions*. This study shows, that in MM pts the increase and in NHL pts the decrease of all gdT lymphocytes and activated gd T CD56+ cells in peripheral blood after mega-chmt+ PBSCT may be attributed to a normal immunological reconstruction after transplantation and may imply better prognosis, because all of studied pts stayed in complete remission half a year after transplantation. Our studied patients group is small, so it needs a further research.

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ACQUIRED FACTOR V INHIBITOR IN A PATIENT WITH SEVERE HAEMATURIA

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Background. Factor V (FV) inhibitors are rare, developing as spontaneous autoantibodies; alloantibodies after surgical exposure to bovine thrombin; or in patients with FV congenital deficiency submitted to transfusion with fresh frozen plasma. Autoantibodies are frequently idiopathic, but have been associated to surgeries, antibiotics, autoimmune diseases, infections, malignancies. Patients are mostly asymptomatic and inhibitors are mostly transitory, which is why FV inhibitors are rarely detected and the need for treatment is questionable. *Case report*. A 52-year old female was admitted for sudden macroscopic haematuria. No history of bleeding episodes. History of tonsillitis medicated with penicillin a month before admission. Laboratory data revealed pyuria and bacteriuria; normal platelet count; and prolonged prothrombin time (PT) (79sec) and activated partial thromboplastin time (aPTT) (133.6sec). A circulating FV inhibitor was found in high titer (114U Bethesda). The investigation for an etiology was inconclusive. Temporary resolution of haematuria was achieved after administration of levofloxacin. Recurrence of haematuria was accompanied by renal colic and anaemia (haemoglobin 8.3 g/dL). Intravenous immunoglobulin (IGIV) (1 mg/Kg/day) was given, which resulted in partial correction of PT and aPTT and resolution of haematuria. Further prolongation of PT and aPTT occurred, which was unresponsive to IGIV (1.5 mg/Kg/day). Administration of oral prednisolone (1 mg/Kg/day) was followed by completely corrected PT and aPTT, normal FV activity and undetectable inhibitor in plasma. *Conclusions*. The etiology of this FV inhibitor was not clarified, although an association to penicillin or infection cannot be excluded. Major bleeding determined the need for treatment, which is mostly empiric, considering the variable success of the therapeutic options, based on antibody binding or elimination and control of bleeding episodes.

1216

FMC±R REGIMENTS EFFICIENCY IN TREATMENT OF PRIMARY EXTRAGASTRIC MALT LYMPHOMAS

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Background. Extragastric mucosa- associated lymphoid tissue lymphomas (EMALTL) are rare tumors. Optimal therapy for these lymphomas is controversial. Although surgery and radiotherapy are traditionally applied management, but the role of chemotherapy is not established. *Aim*. efficacy and safety assessment of the FMC (fludarabine, cyclophosphamid, mitoxantrone) ±R (rituximab) chemotherapy scheme in treatment EMALTL. *Methods*. Between august 2003 and December 2006 10 patients EMALTL with Stage IE received FMC ±R chemotherapy. There were 7 women and 3 men. The mean age at diagnosis was 52 years (35 -67 years), 4 patients were older than 60. Presenting sites included periorbital soft tissue (n=7), duodenum (n=1), lung (n=1), spinal cord dura (n=1). The type of lymphoma was classified according to the WHO classification system. The FMC scheme (25 mg/m^2 per day fludarabine on days 1-3; 200 mg/m^2 per day cyclophosphamide on days 1-3; 10 mg/m^2 mitoxantrone on day 1 only) was used for 6 pts (3-6 cycles) and FMCR (as FMC + rituximab 375 mg/m^2 0 day) was used for 4 pts (4-6 cycles). *Results*. All patients achieved complete responses. The mean follow-up is 10 month (range 1-41). There were no differences between FMC and FMCR in ability to induce remissions and its duration. No relapses have been registered so far. Radiotherapy was not used. Two patients received palliative operative treatment before chemotherapy. *Summary*. The fludarabine-containing FMC or FMCR regimens are highly effective frontline treatment for patients with EMALTL. The optimal number of cycles is not established, may be less than 6 cycles, especially in cases with rapid response achievement. By our data, additional value of surgery and radiotherapy is not evident. Efficacy of adding rituximab to FNC therapy in EMALTL must be further investigated.

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INCIDENCE OF EARLY STAGE IDIOPATHIC MYELOFIBROSIS: SINGLE INSTITUTION EXPERIENCE

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Background. Early stage idiopathic myelofibrosis with associated thrombocytosis (i.e. prefibrotic myelofibrosis and early idiopathic myelofibrosis) are with difficulty distinguished from true essential thrombocytemia because of the high platelet count-sometimes greater than 1.000×10^9/L- as well as the lack of specific diagnostic markers (i.e. evident splenomegaly; high serum levels of LDH; circulating immature myeloid cells, erythroblasts and dacryocytes). In these select circumstances, only bone marrow features unequivocally help distinguish true essential thrombocytemia from early stage idiopathic myelofibrosis with associated thrombocytosis. In essential thrombocytemia megakaryocytes are mostly giant and contain an enlarged nucleus with deep lobulations, but lack the cytological abnormalities. In prefibrotic or early idiopathic myelofibrosis megakaryocytes present bizarre forms with marked nuclear-cytoplasmic anomalies. These evident dysplastic features exert a significant impact on prognosis and life expectancy; in fact, life expectancy is normal in true essential thrombocytemia but significantly shortened in prefibrotic myelofibrosis as well as in the various fibrotic stages of myelofibrosis. In addition, prefibrotic stages account for about 20-25% of all idiopathic myelofibrosis. *Methods and Results*. We have evaluated the incidence of early stages in our adult patients affected by idiopathic myelofibrosis. The data of 32 patients (19 males, 13 females; median age 71 years [range 35-84]) were retrospectively analysed; 7 patients (22%) were affected by early stage idiopathic myelofibrosis (4 prefibrotic myelofibrosis, 3 early idiopathic myelofibrosis). Analysis was focused on the discriminating impact of bone marrow and peripheral blood morphology. Our patients with early stage idiopathic myelofibrosis showed at diagnosis pronounced thrombocythemia (median value 879×10^9/L [range 427'1.654]). The number of WBC was not significantly elevated (median value 13×10^9/L [range 6-15]). Morphological examination of peripheral blood did not show evidence of immature myeloid cells or erythroblasts; the red blood cells were normocromic and normocytic in all patients however minimal anisocytosis was observed during the course of disease; in addition, peripheral blood smear showed frequently macro-platelets, agranular platelets and small aggregates. The bone marrow biopsies were hypercellular and dominated by atypical immature megakaryocytes, conspicuously large due to an increase of nuclear and cellular size, and always grouped in clusters of four/six elements; reticulin fibrosis was minimal (MF1) or absent (MF0). At diagnosis and during overall clinical follow-up all patients were asymptomatic: in fact they did not present fatigue, pruritus, fever, weight loss, bone pain, night sweats, spleen pain. Physical exam and abdominal echography detected occasionally very small splenomegaly. Cytogenetic analysis revealed a normal kariotype. LDH serum levels were normal. *Conclusion.* 1) In our Institution the incidence of early stage of idiopathic myelofibrosis is similar to that of international reports; 2) prefibrotic or early idiopathic myelofibrosis are characterized by a relatively indolent clinical course; 3) in view of the lack of specific diagnostic markers a detailed evaluation of bone marrow findings, particularly megakaryopoiesis, is recommended for a differential diagnosis between true essential thrombocytemia and prefibrotic or early idiopathic myelofibrosis with associated thrombocytosis; abnormal megakaryopoiesis offers the possibility of identifying early stages idiopathic myelofibrosis apart from the platelet count, laboratory parameters and clinical symptoms.

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HIGH DOSE SEQUENTIAL CHEMOTHERAPY AS SALVAGE TREATMENT FOR RELAPSED AND REFRACTORY HODGKINS LYMPHOMA

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Background. The optimal approach for patients with Hodgkin's lymphoma (HD) who do not achieve a complete remission (CR) or relapse after induction therapy is still not definite. High-dose chemotherapy with autologous stem cell rescue proved to be more effective than conventional schemes considering the number of second CR and the duration of response. The German Hodgkin Study Group obtained good results with high-dose sequential chemotherapy (HDST) and autologous stem cell transplantation (ASCT) in this subset of patients. *Aims*. Evaluate the impact of HDST in relapsed and refractory HD patients. *Methods*. Since March 2002 to July 2006 16 patients were enrolled in this study in our institution. The schedule was constituted by a first phase (2 cycles of DHAP), a second phase (cyclophosphamide 4g/sqm, methotrexate 8 g/sqm, etoposide 2 g/sqm every 14 days) and an ASCT with BEAM conditioning. All phases but the ASCT one were delivered on outpatient basis. After the first phase a CT evaluation was performed to assess the chemosensibility: patients 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. *Results*. Patients status at the enrolment were: 7 relapsed within an year after the obtainment of CR;7 refractory to the induction therapy (ABVD); 2 refractory to conventional salvage treatment for relapse occurred after 9 years. Characteristics of the patients at diagnosis were: median age 28 years (range 19-42); 8 patients were female; 7 had B symptoms; ECOG performance status were 0 in 10 patients, 1 in five, 2 in one. All patients received the two cycles of DHAP without delays. After this phase 7 patients were excluded, 5 for stable/progressive disease, 2 for not achieving a PR (the late relapsed patients, who are still alive with disease). Nine patients completed the therapy according to the schedule, and after the ASCT all of them were in CR with negative PET scans. A CR patient developed a myelodisplastic syndrome 13 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 patients are still alive and maintain the CR. Median time to treatment failure for the nine patients who completed the HDST was 31 months (range 139 days-52 months). *Conclusions*. HDST is an effective therapy for treatment of relapsed and refractory Hodgkin's lymphoma. We observed a better outcome in the relapsed patients than in the primary refractory ones. After a median observation of more than 30 months we observed only a case of myelotoxicity, but a longer follow-up could better evaluate the real toxicity of this treatment.

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INHIBITORS IN HEMOPHILIA A AND B IN SOUTEAST OF TURKEY

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Background. The most important treatment related complication in hemophiliacs is inhibitor development. We evaluate the inhibitor developing rate of our patients, and discuss the possible reasons of low inhibitor development rates of them. *Materials and Methods*. We performed inhibitor screening of 99 hemophiliacs. Patients with < 1% of clotting factor activities were classified as severe, 1-5% moderate, and > 5-25% mild degree. Standart Bethesda procedure was performed for inhibitor screening, and used of 0.6 units as cut-off level. *Results*. Out of 99 patients; 84 were hemophilia A (84.8%), and 15 were hemophilia B (15.2%). Out of 84 hemophilia A patients; 53 (63.1%) were severe, 24 (28.5%) were moderate, and 7 (8.4%) were mild hemophilia A. Out of 15 hemophilia B patients; 8 (53.3%) were severe, 5 (33.4%) were moderate, and 2 (13.3) were mild hemophilia B. Only one hemophilia A patient was inhibitor positive (1.2% in severe hemophilia A), followed in our service with severe intraabdominal bleeding. None of hemophilia B patients have inhibitor. *Conclusions*. Expected inhibitor development incidence in hemophilia A and B approaches 33% and 3% respectively. Risk factors for inhibitory development may be patient and/or treatment-related. Our patients inhibitor prevalence was lower than previous studies. Factors that may responsible from this results; non of our patients were administered prophylactic factor concentrates, using fresh frozen plasma instead of factor concentrates. Inhibitor development become an important problem in our region with increasing availability of factor concentrates in prophylaxis and bleeding episodes.

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EFFECT OF PALIFERMIN ON THE INCIDENCE OF MUCOSITIS, HOSPITAL STAY, ACUTE GVHD AND TOTAL PARENTERAL NUTRITION IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION

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Kepivance is a keratinocyte growth factor (KGF) which stimulates the growth of cells in skin, mouth, stomach and colon. It has been suggested that Kepivance may reduce the incidence and severity of oral mucositis in patients receiving total body irradiation (TBI) conditioning regimen followed by autologous stem cell rescue. Very few studies have shown the effect of Kepivance on chemotherapy induced mucositis in patients

receiving allogeneic/autologous stem cell transplantation. We analyzed retrospectively the charts of all patients who underwent stem cell transplant in 2005-2006 at Texas Tech University Health Science Center. The charts of 36 patients were reviewed of which 21 received kepivance. Decision to receive kepivance was judged by the treating physician. Patients with high risk of developing mucositis from intensive chemotherapy, older patients, type of conditioning regimen, and type of transplant dictated which patient should receive kepivance. Conditioning regimens in the kepivance group were as follows: high dose melphalan (10); TBI, VP-16 and cyclophosphamide (CTX) (1); BCNU, VP-16 and CTX (2); fludarabine and melphalan (1); melphalan and VP-16 (1); busulfan and fludarabine (6). Conditioning regimens in the non-kepivance group were as follows: melphalan and busulfan (1); melphalan and fludarabine (2); BCNU, VP-16 and CTX (4); high dose melphalan (8). Out of 21 patients who received kepivance, 12 patients received autologous transplant and 9 patients received allogeneic transplants. Of the 21 patients who received kepivance, 8 experienced grade II-III mucositis compared to 3 of 15 patients in the non- kepivance group. No patients in both groups experienced grade IV mucositis. In both groups 50% of the patients required total parenteral nutrition (TPN). The average length of stay in patients who received kepivance was 31 days vs. 36 days in patients who did not receive kepivance. Day 100 mortality, in the kepivance group was 5, all of whom received allogeneic transplant, whereas there was only 1 death (who received allogeneic transplantation) in the non-kepivance group. We concluded that kepivance reduces the hospital stay in patients undergoing stem cell transplantation. However, there were no noted benefits of kepivance in TPN requirement or the incidence of mucositis. This may be due to physician bias in selecting patients who should receive kepivance. High risk patients received kepivance. All the 9 patients in the kepivance group who received allogeneic transplant did not experience acute graft versus host disease. A prospective randomized clinical trial is needed to confirm these results.

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ACUTE CORONARY SYNDROME AND THROMBOPHILIC POLYMORPHISM

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Background. The thrombophilic polymorphisms, Factor V G1691A (Leiden), Prothrombin G20210A Mutation (PT G20210A) and C46T of Factor XII gene, are genetic variants in relation to venous thrombotic desease; in the arterial thrombosis and the acute coronary syndrome his mean had not been established, because currently the dates in relation to thrombophilic risk factor are contradictory. *Aims*. The objective of this study is to define the prevalence in own people of the Phrotrombin 20210 Mutation, the Factor V Leiden and C46T polymorphism in the Factor XII gene in health people and patients with acute coronary syndrome and to establish the thrombophilic risk factor respectively. *Methods*. A prospective study case/control we were included 483 subjects (76 patients and 407 controls).

Table 1. Prevalence of thrombophilic polymorphism in acute coronary syndrome and controls.

	FACTOR XII C46T			LEIDEN G1691A			PT G20210A		
	TT	CT	CC	AA	GA	GG	AA	GA	GG
PATIENTS	1 1.4%	22 31.9%	46 66.7%	0 0%	5 6.6%	71 93.4%	0 0%	7 9.2%	69 90.8%
CONTROLS	10 2.5%	121 30.3%	269 67.3%	0 0%	13 3.2%	394 96.8%	0 0%	14 3.5%	390 96.5%

The patients are recruitment enter the survivals with acute coronary syndrome, at lest three month after the event. The controls are healthy persons, blood donors, they were included in the study voluntarily. In the group of patients, 61 (80.3%) subjects are men and 15 (19.7%) are women; in the controls, 271 (66.6%) subjects are men and 136 (33.4%) are women. The median of age of the patients is 49.9 years (24-74) with typical deviation 12.8 at the moment of the event, 48 (63.2%) patients are 55 years old or less, because this study is planning in young people; in the group of the controls the median is 38.4 years (21-72) typical deviation 10.7. The detection of the Prothrombin 20210 Mutation, the Factor V Leiden and the polymorphism 46C/T of Factor XII gene, by PCR in real time, in liquid phase, in a LightCycler (Roche diagnostics) thermal cycler was made. Stadistical methodology, the descriptive was made by groups in patients and controls; to estimate the risk by square-chi proof. *Results*. The results of the prevalence of the polymorphisms, Factor V Leiden, Prothrombin G20210A Mutation and C46T of Factor XII gene, in patients and controls in the Table 1 are showed. The estimate risk to have got an acute coronary syndrome in relation to genotype GA of the Prothrombin G20210A Mutation is 2.8 (1.1-7.2) IC (95%) p=0.025. *Conclusion*. The Prothrombin G20210A Mutation is a risk factor for acute coronary syndrome

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PROGNOSTIC AND CLINICAL SIGNIFICANCE OF CELL CYCLE PROTEINS IN LEUKEMIC CELLS IN CHILDREN WITH ACUTE LEUKEMIA

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Background. The investigation of the cell cycle proteins may occur very useful in understanding the mechanisms of cell resistance to chemotherapy the biological basis for the therapy of leukemia. *Aims*. to estimate the expression of several proteins involved in the cell cycle and the regulation of cell proliferation in leukemic cells in cases in childhood with acute leukemia. *Methods*. The material of the study-bone marrow from 79 children with initial diagnosed ALL, 37 children with initial diagnosed AML and 7 controls (leukocytes peripheral blood from healthy donors). We studied the quantities of several proteins cyclin-dependent kinases (cdk2 and cdk4) and cyclins (cyclin D, cyclin E), E2F1 and pRb by Western blotting. The rate of proliferating cells was analyzed by flow cytometry, using nuclear cell proliferation-associated antigen (Ki67). *Results*. The levels of expression of cyclin D, cyclin E, cdk2 and cdk4 was much higher of initial B-ALL in children in comparison with T-ALL, pro-B-ALL and controls(p<0.05). The positive rates of proliferation activity cell (Ki67) was much higher of initial T-ALL in children in comparison with B-ALL. The levels of expression of cdk2 and cdk4 in complete remission ALL was higher than in relapse samples (p<0.05). The level of Ki67 in cell in relapse was higher than in complete remission ALL samples (p<0.05). The probability of 5-year DFS in patients with high levels of cyclin D, cyclin E, cdk2 and cdk4 was 99% versus 77%, 76%, 82% and 81% in patients with low levels of these proteins (p<0.05). The probability of 5-year DFS in patients with low levels of Ki67 was 93% versus 61% in patients with high levels of these proteins (p<0.05). *Conclusions*. Expression rates of cyclin D, cyclin E, cdk2, cdk4 and Ki67 have clinical and prognostic significance in childhood leukemia. The initial high levels of cyclin D, E, cdk2 and cdk4 in leukemic cells are associated with good prognosis of 5 years disease-free survival. The initial low levels of Ki67 in leukemic cells are associated with good prognosis of 5 years disease-free survival.

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PREVALENCE OF FACTOR V LEIDEN (G1691A) AND PROTHROMBIN (G20210A) AMONG HEALTHY POPULATION FROM WESTERN IRAN

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Background. The mutation in factor V (FV) G1691A, known as factor V Leiden, and in prothrombin (FII) gene G20210A are the two most prevalent causes of inherited thrombophilia. The prevalence of these mutations varies among different populations. *Aims*. To find the prevalence of factor V Leiden and the prothrombin G20210A gene mutations among healthy individuals from Western Iran. *Methods*. Two hundred and ninety six healthy unrelated individuals including 142 males and 154 females with the mean age of 29.1±16.7 from the Kermanshah Province of Iran were studied. DNA was extracted by phenol chloroform method. The G1691A in factor V was detected using amplification a fragment with 267 bp in exon 10 of factor V gene by polymerase chain reaction (PCR) followed by digestion with Mnl I. A fragment of 245 bp of the 3'-untranslated region of the prothrombin gene was amplified by PCR and digested with Hind III. *Results*. Prothrombin G20210A was found in 5 individuals (1.7%) giving an allele frequency of 0.85%. There were 1 male and 4 females carrier for this mutation. We were able to detect FV Leiden in 268 individuals which among them there were 6 heterozgous

1691GA and 1 homozygous 1691AA. The prevalence of FV 1691 mutation was found to be 2.6% with an allele frequency of 1.49%. Factor V leiden was found in 3 males and 3 females as heterozygous and in a male as homozygous. *Summary/Conclusions*. For the first time the allele frequencies for prothrombin G20210A and FV Leiden for one of ethnic groups (Kurds) living in Iran is reported. The frequency was found for FV 1691A allele is in the range of those reported for Caucasoid subpopulations (1-8.5%) and is similar to some of Asian countries including North India and Saudi Arabia. However, the frequency of prothrombin 20210A allele in our population is higher than those reported for some Middle East countries. Our study indicates that factor V Leiden, and prothrombin gene 20210A are not rare in Iranian population and need to be considered in patients with venous thrombosis.

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CHANGES OF MDM2 AND P53 PROTEINS EXPRESSION IN RELATION TO EARLY TREATMENT RESPONSE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Several pieces of evidence suggest that ineffective apoptosis can be one of the main causes of treatment failure in childhood acute lymphoblastic leukemia (ALL). P53 is the pivotal gene in controlling apoptosis of human cells. MDM2, the endogenous inhibitor of apoptosis is an oncoprotein which downregulates the functional activity of p53 protein. Thus, increased activation of MDM2 can be among factors responsible for treatment failure in childhood ALL. This study aimed to evaluate changes in MDM2 and p53 expression in peripheral blood mononuclear cells collected prior to and after 6 and 12 hours of prednisone administration, and the relation between them and early treatment response. *Patients*. The study was comprised of 35 children (aged 6-192 months, median 95 months) with newly diagnosed ALL. The diagnosis was based on morphologic examination of bone marrow smears stained with MGG and by results of flow cytometric immunophenotyping of bone marrow leukemic cells. All children were treated according to the ALL BFM 90 protocol. Patients with absolute leukemic cell counts of less than 1000/mL of peripheral blood after 7 days of prednisone treatment (plus one intrathecal injection of Mtx) and with leukemic cells below 5% in bone marrow smears aspirated on day 15 were classified as early good responders (n=24), whereas the remaining patients were classified as early poor responders (n=11). The follow up time was 1-42 months (median 21 months). *Methods*. Peripheral blood mononuclear cells were collected prior to and after 6 and 12 hours from prednisone administration. Cytospin preparations of these cells were stained with mouse monoclonal anti-MDM2 antibodies (DakoCytomation) followed by goat anti-mouse antibodies conjugated with APC (Molecular Probes) and mouse monoclonal anti-p53 antibodies conjugated with FITC (DakoCytomation) respectively. Nuclear DNA was stained with propidium iodide (PI). MDM2 - associated long red fluorescence and p53-associated green fluorescence and were measured by laser scanning cytometer (LSC, CompuCyte, USA). In order to assess the rates of apoptotic cells respective slides were stained with polyclonal rabbit anti-PARP p85 fragment followed by FITC conjugated swine anti-rabbit antibody. Cell expressing p89 fragment of PARP were considered apoptotic. Red fluorescence of DNA-bound PI was used as a contouring parameter. Values of long red integrated fluorescence and green integrated fluorescence were recorded as .FCS 3.0 files by WinCyte 3.4 software. *Results*. The mean pretreatment values of p53 expression in both groups did not differ, whereas mean pretreatment values of MDM2 expression were significantly higher in the group of early poor responders (p=0,03).After 12 hours from prednisone administration mean values of p53 expression were significantly higher in the group of good early responders (p=0,01). Higher expression of p53 in these patients was associated with significantly higher rates of apoptotic cells as compared with poor early treatment responders (p=0,03). Moreover, p-EFS in the group of early treatment responders was significantly higher than for the remaining patients (1,0 vs 0,635; p=0,002). *Conclusions*. These data seem to indicate that pretreatment overexpression of MDM2 protein may contribute to poor early treatment response, thus influencing the outcome for these patients.

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EARLY AND LATE COMPLICATIONS OF CHEMOTHERAPY IN ACUTE LYMPHOBLASTIC LEUKAEMIA. A SINGLE PAEDIATRIC INSTITUTION EXPERIENCE

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Background. Improvement in survival rates after acute lymphoblastic leukaemia (ALL) have sharpened the need for research on adverse events after treatment. These sequelae can have negative effects on quality of life of survivors and when severe, they are cause of morbidity. The current study assesses the incidence of early and late effects by a single paediatric institution. *Patients and Methods*. Between December 2005 and January 2007, one hundred and eighty-eight patients, with off-therapy ALL, were followed-up by a specialized multidisciplinary team. The median age of children at diagnosis was 4 years and 6 months (range 3 months-15 years and 6 months), 96 males and 92 female. The median time of follow-up was 51 months (range 3 months-19 years and 6 months). One hundred and eighty-three were treated with ALL-AIEOP protocols, three with infant-ALL protocol and two patients with relapsed-ALL ones. According to immunophenotype, 155 (82,5%) were B-ALL and 16 (8.5%) T-ALL, 1 biclonal (0.5%) and 16 (8.5%) were unclassified. The cranial irradiation was performed on 33 (17.5%) patients and only one child received testis radiotherapy. All patients underwent a periodic clinical, haematological, endocrine, viral, cardiac and radiological checks. A careful long-term follow-up was performed for a rapid identification of organ-specific sequelae or secondary malignancy. *Results*. Seventy-two (38.3%) patients presented at least one early complication and 20 (27.7%) two or more effects. The earliest event were infections (40.6%), followed by neurological sequelae (30.8%). Long-term complications were observed in 121 (64,4%) patients, prevalently endocrine sequelae (51.9%) (especially obesity) and dental complications (14.5%). Few data were available on fertility or sexual dysfunctions, due to young age of patients; only three pregnancies were observed in two female patients. In a median follow-up of 51 months after treatment, only one secondary malignancy was observed, an abdominal ganglioneuroma, completely surgically removed. During routine controls, two relapses (testis and optic nerve) were precociously diagnosed. *Conclusions*. These results suggest that new therapeutic approaches in acute lymphoblastic leukaemia have significantly improved survival rate but it is burdened by high incidence of late effects that sometimes affect negatively the quality of life of survivors. Then, we believe that the patient and family have to be well informed about all possible complications related to treatment. Duty of clinicians is to perform a careful, prolonged follow-up for a precocious identification and treatment of long-term effects. Future studies should focus on strategies aimed at reducing late sequelae, probably using treatment regimen at reduced toxicity.

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HODGKIN LYMPHOMA IN MUTATED ZAP-70 NEGATIVE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: A CASE REPORT

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Background. 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. *Case Report*. 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's clinical conditions suddenly worsened. Indeed, he presented general malaise, emaciation, mild splenomegaly without palpable lymph nodes and severe hypocromic anemia. 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). The patient was unsuitable for any treatment and died two weeks after the HL diagnosis.

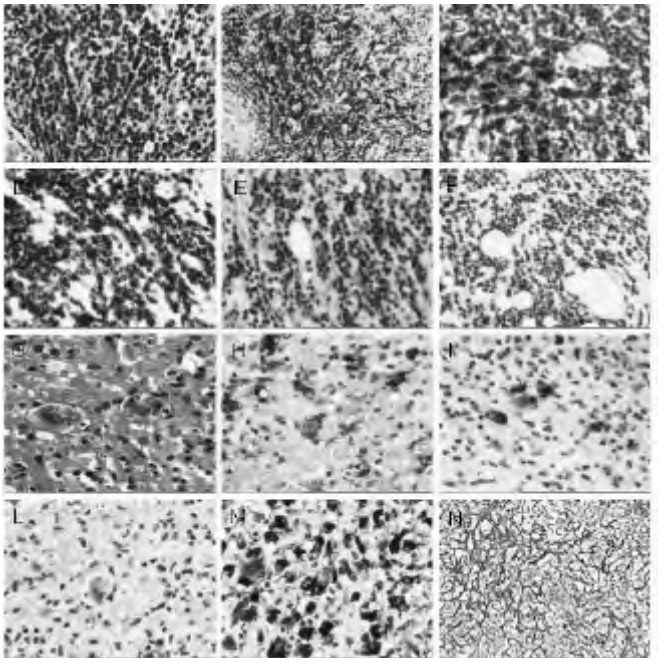

Figure 1. Hodgkin lymphoma in CLL: histological features.

Discussion and Conclusions. Richter syndrome with HL features is an uncommon event, occurring in 0.4% CLL patients and portraying a very poor clinical course with a reported median overall survival (OS) and a progression-free survival (PFS) of 0.8 and 0.4 years, respectively. Most HL transformations of CLL developed in patients who have received previous treatments, mainly fludarabine or other purine analogs and rituximab (Tsimberidou 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 Pricipe *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 outlook 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 and not mandatory interventions, taking into account the potential occurrence of this rare but devastating complication.

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WHICH PARAMETERS OF NUTRITIONAL STATUS SHOULD WE CHOOSE FOR NUTRITIONAL ASSESSMENT DURING HEMATOPOIETIC STEM CELL TRANSPLANTATIONS?- SINGLE CENTRE EXPERIENCE

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Aim. Haematopoietic stem cell transplantation (HSCT) is being used increasingly in attempt to cure many hematological disorders, solid tumours and autoimmune diseases. One of the major challenges in the posttransplant period is nutrition Because nutritional changes after HSCT have not been well studied there is no common knowledge if and how changes of biochemical indices` levels can be useful. We probably can use them as the risk factor of the development of malnutrition, for establishing optimal candidates and also for determining the most appropriate time to start total parenteral nutrition. The best indices have to be inexpensive for estimation, easy for evaluation and they should be independent from parameters of inflammation like increased levels of acute phase proteins. *Methods*. The purpose of this investigation was an assessment of changes in parameters of nutritional status, acute phase proteins' levels and usefulness of investigated parameters for qualifications for total parenteral nutrition. Patients' informed consent was obtained. Nutritional status was assessed in 54 patients during autologous (30 cases) and allogeneic (24 cases) transplantations. Fifteen patients had to be treated with total parenteral nutrition (TPN), eight of them needed prolonged hospitalization. Biochemical (prealbumin, transferrin, retinol binding protein and albumin) and anthropometric indices (body weight, triceps skinfold thickness, midarm circumference) of nutritional status as well as body fat and resting energy expenditure were assessed. The levels of acute phase proteins (C- reactive protein, α1- antitrypsin, α2- macroglobulin and serum precursor of amyloid A) were estimated at the same time. All nutritional indices and acute phase proteins` levels were evaluated during the day before the beginning of conditioning regimen, after chemotherapy completion, and every 7 days until engraftment, at least three times after stem cells infusion. Wilcoxon test and canonical analysis served statistical analyses. *Results and Conclusions*. The measurement of body weight, midarm circumference can be useful for nutritional assessment during autologous and allogeneic HSCT from sibling donors. In patients treated with autologous HSCT high negative correlation was not observed only between retinol binding protein level and acute phase proteins. Thus, estimation of retinol binding protein can be useful for nutritional assessment during autologous haematopoietic stem cell transplantations. Similar observation was done during allogeneic haematopoietic stem cell transplantations from sibling donors. It applied for transferrin (TRF). We found that the estimation of TRF levels can be useful for nutritional assessment during this kind of treatment. Prealbumin level, being measured eight days after conditioning regimen showed in the best way the difference between patients who required or not the prolonged hospitalization and it can helpful to make a decision for TPN treatment.

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HEREDITARY SYSTEMIC AMYLOIDOSIS CAUSED BY A NEW VARIANT LYSOZYME (D67G) IN A ROMANIAN FAMILY

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Lysozyme- related amyloidosis (ALys) has been associated with mutations in the second exon encoding the amyloidogenic precursor protein. To date, four different variants have been identified (I56T, D67H, W64R, F57I), all of which were deemed capable of reducing protein stability and enhancing fibrilogenesis. We now report another case of ALys in a 52-year-old male who had predominant hepatic involvement (as well as a family history indicating that other members had the same disorder) and in whom we found a hitherto unreported mutation in the lysozyme gene. Examination under polarized light of Congo red-stained sections of liver biopsies obtained from the proband (and 2 brothers) revealed extensive green birefringent interstitial deposits, characteristic for amyloid. This material was extracted from 4mm-thick sections cut from formalin-fixed paraffin embedded blocks, purified by reverse phase HPLC and subjected, after trypsin digestion, to chemical analyses by tandem mass spectrometry (MS/MS). These studies identified 109 of the 130 amino acids comprising wild-type lysozyme (peptides encompassing residues 11-15, 63-69, and 114 - 122 were not found). To obtain the complete primary structure of this protein, genomic DNA was isolated from the proband's peripheral blood leukocytes and the PCR products of the 3 functional exons were synthesized. Nucleotide sequence analysis revealed that exon 2 contained (in addition to the unmutated gene) a GAT to GGT transition in codon 85, which would result in the substitution of glycine for aspartic acid at position 67. Based on X-ray crystallographic data, we posit that the resultant profound modification in tertiary structure included by the D67G mutation would render the molecule unstable and thus amyloidogenic. Our findings add to the known variants of lysozyme involved in familial systemic ALys amyloidosis.

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ASSOCIATION OF HEPARANASE GENE (HPSE) SINGLE NUCLEOTIDE POLYMORPHISMS WITH HEMATOLOGICAL MALIGNANCIES

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Heparanase, endo-β-D-glucuronidase, degrades heparan sulfate (HS) glycosaminoglycans-the principal polysaccharide component of the basement membrane and extracellular matrix (ECM). Cleavage of HS disassembles the basement membrane structure and releases HS-bound bioactive angiogenic and growth-promoting mediators. Heparanase activity plays a decisive role in fundamental biological processes associated with remodeling of the ECM, such as cancer metastasis, angiogenesis and inflammation. In the hematopoietic system, heparanase is thought to be associated with normal differentiation and function of myeloid cells and platelets. We investigated heparanase polymorphisms in patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), Hodgkin's disease (HD), and multiple myeloma (MM). Statistically significant correlation was found between rs11099592 and rs6535455 SNPs of the heparanase (HPSE) gene and ALL (χ21df=4.96, p=0.026). Genotype frequency comparisons revealed a significant association with rs4693602 (χ22df=7.276, p=0.026) in MM patients and rs4364254 (χ22df=6.226, p=0.044) in AML patients. Examination of HPSE gene mRNA expression by real-time RT-PCR indicated a significant low expression level of the HPSE gene in ALL patients and a high expression level in MM and AML patients, compared to healthy controls. These data suggest that alterations in HPSE gene are an important determinant in the pathogenesis of these hematological malignancies.

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WITHDRAWN

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ABVD CHEMOTHERAPY EFECTS ON HUMAN GENOME IN HODGKIN DISEASE

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Background. Chemotherapy has an important mutant effect on human genome. Human chromosomal aberration seems to be an important cancer-associate biomarker. ABVD as first line chemotherapy cures most of Hodgkin disease patients. *Material and methods*: We have studied peripheral blood lymphocytes from 18 samples obtained from patients with Hodgkin disease receiving chemotherapy ABVD. To asses the cytogenetic damage rate we looked for chromosomal aberrations on metaphases chromosomes at cumulative doses of chemotherapy. For molecular changes we evaluate the epigenetic effects e.g. hipo/hypermethylation under ABVD regimen of the human genes. *In vitro* analysis were performed on peripheral blood cultures from healthy nonsmoker volunteer donor treated with increasing doses of doxorubicin (0.025×10^{-6}M, 0.05×10^{-6}M, 0.1×10^{-6}M, 0.25×10^{-6}M). *Results*: We had found an evidence for a direct link between chromosomal aberrations and the number of therapy cycles. The most frequent identified aberration were the PCD (premature centromere divisions) and the chromosomal fusions (14.6-26.3% and 10,7-16.9%, respectively). A global imbalance of the methylation pattern was identified with a hypermethylation tendency. Further studies should clarify the epigenetic changes of this chemotherapy regimen.

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OVEREXPRESSION OF HOCT1 AT 6 MONTHS IS RELATED TO MAJOR MOLECULAR RESPONSE TO MESYLATE IMATINIB TREATMENT

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Background. Some studies have shown that human organic cation transporter 1 (hOCT1) is responsible for the influx of imatinib in CML cells, and therefore might determine the intracellular levels and hence the response to imatinib. Although newly diagnosed CML patients have a 90% of event free progression at the end of 5 years of follow-up, there is still a small number of patients that failed to achieve a major cytogenetic response (MCR) at 6 months and/or a complete cytogenetic response at 12 months (CCR). Resistance to imatinib is getting a major concern and some of the mechanisms have already been elucidate as the presence of mutations in the ABL kinase domain, overexpression due BCR-ABL gene amplification, and the presence the ABC transporters proteins involved in drug efflux across the cell membrane. *Methods*. We decided to study the expression of hOCT1 in a cohort of 44 CML (35 CP; 7 AP; 2 BC) patients treated with imatinib as a front line therapy. Mononuclear cells collected at different time as diagnosis, 3, and 6 months (mo) after initiated treatment with imatinib were assayed for hOCT1 expression by quantitative real-time polymerase reaction (PCR) and normalized for Abl expression. *Results*. Patients were defined as responder if they achieved a Major Molecular Response (3 log reduction of BCR-ABL from the standardised baseline) at 6 mo of treatment with imatinib. Among the 44 patients that enter in the study 34 patients were eligible for the analysis at 6 mo, 6 patients have been treated for less than 6 mo, 2 were withdraw from the study because hepatic toxicity grade 3, and 2 died with progressive disease. There was no statistical significance among the median levels of hOCT1 expression at diagnosis, 3 and 6 months. Overexpression was considered when the levels of hOCT1 were higher than the median value of the patients at diagnosis. The presence of hOCT1 overexpression at diagnosis did not interfere with the molecular response at 3 and 6 mo, but when hOCT1 expression at 6 mo was compared with the molecular response at the same period, a statistical difference was noted between the 21/34 responders and 13/34 nonresponders (Kruskal-Wallis test, p=0.016). *Conclusion*. Besides others had published before (Crossman *et als*, 2005) that low levels of expression of hOCT1 at diagnosis may be related to fail in achieving a cytogenetic response, in the present study, we could not conclude that either the presence or absence of overexpression of hOCT1 at diagnosis is a good predictor of molecular to imatinib response after 3 and 6 mo of treatment. On the other hand, the presence of overexpression of hOCT1 is related to a MMR at 6 months of treatment, although some responders (5/21) did not present overexpression of hOCT1, and 4/13 no responders expressed levels of hOCT1 above the median. We can postulate that at the end of 12 mo of treatment those 4 patients that show expression of hOCT1 could achieve a MMR.

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ANAGRELIDE CORRECTS THE ENDOTHELIAL FUNCTION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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The essential thrombocythemia (ET) may be complicated by micro- and macrovascular thrombosis. The microthrombosis is platelet-associated and it is reversed by antiplatelets whereas the macrothrombosis is probably platelet coagulant-associated and it is antiplatelet orphan. Anagrelide (ANA) is a platelet lowering drug that inhibits platelet aggregation. Therefore, we evaluated platelet factor 4 (PF4), marker of platelet activation, prothrombin fragment 1+2 (F1+2) and thrombin antithrombin complex (TAT), markers of coagulant activation, TFPI (tissue factor pathway inhibitor), tissue factor (TF) and von Willebrand factor (vWF), indicators of micro- and macrovascular activation, respectively, in 19 ET patients (12 males and 7 females, mean age 51 years) who fulfilled PVSG. All patients were on antiplatelets either aspirin (10 patients) or indobufen (7 patients) and ticlopidine (2 patients). Their mean duration of disease was 7 years. ANA was administered in dose of 0.5 mg/day, with increases of 0.5 mg/day every 7 days until the platelets decreased below 500×10^9/L and with a average maintenance dosage of 2.1 mg/day. Platelets, PF4, F1+2, TAT, TFPI, TF and vWF were measured before cytoreduction and to complete response defined as platelets < 500×10^9/L. Platelets were measured by automated analyser. PF4, F1+2, TAT, TFPI, TF and vWF were assayed by ELISA. Before ANA all patients had marked platelets ($1057\pm349\times10^9$/L) and high PF4 (121±43 IU/mL vs 5.5±2.6 IU/mL) (p< .0001), TFPI (159±68 ng/mL vs 100±14 ng/mL) (p 0.001) and TF (247±184 pg/mL vs 4.8±2.5 pg/mL) (p<.0001) and low vWF (22±8% vs 92±31%) (p<.0001). After ANA all patients had platelets <500×10^9/L ($392\pm66\times10^9$/L) and normal PF4 (8.4±3.2 IU/mL) as well as TFPI, TF and vWF (96±51 ng/mL and 8.3±0.4 pg/mL and 96±37%, respectively). We found a correlation between PF4 and F1+2 and TAT (p<.0001 and p=0.004, respectively) and between PF4 and TFPI and TF and vWF and TF and TFPI (p=0.003 and p<.0001 and p=0.001, respectively). Additionally, a correlation there was between F1+2 and TF and vWF (p<.0001 and p<.0001, respectively) and between TAT and TF and vWF (p=0.023 and p=0.001, respectively), whereas no correlation there was

between F1+2 and TFPI and between TAT and TFPI. These data suggest that macrovascular activation is platelet coagulant-associated and confirm that microvascular activation is platelet-associated. ANA may have antiplatelet and anticoagulant properties and hence may globally prevent the thrombotic risk in ET.

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IMPACT OF JAK 2 MUTATION STATUS ON RESPONSE TO THERAPY IN PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA

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Background. The human myeloproliferative disorders (MPDs) are clonal diseases originating from transformation of multipotent haemopoietic stem cells. Until 2005, the molecular pathogenesis was obscure. The recent discovery of the JAK2 V617F mutation in these disorders may explain the clonal expansion of MPD cells. Current concept is that there are 2 separate diseases, JAK2 mutated (+) and JAK2 wild type (-). Essential Thrombocythaemia (ET), Polycythaemia Rubra Vera (PRV) and Myelofibrosis are entities within a spectrum of mutated disorders where duplication of mutant JAK2 allele with loss of the normal allele may cause disease progression. Thus, the JAK2 mutation has brought a new approach to the diagnosis, classification and potentially treatment of MPDs. *Aim*. In the present study, the impact of JAK2 status on response to the therapy, in patients with ET is evaluated. *Methods*. A group of 32 patients with Essential Thrombocythaemia ($JAK2^+$ n=14: JAK2- n=18) were studied. Response to therapy was retrospectively assessed and compared with $JAK2^+$ /- status. Other variables studied included platelet count at the time of presentation, time required to achieve appreciable response and the number of times that therapy was changed to achieve that response. A platelet count of $< 500\times10^9$/L was taken as an appreciable response to therapy. *Results*. out of 14 $JAK2^+$ patients, two responded poorly to first line therapy compared to 5 out of 18 JAK2' patients. Interestingly most JAK2- poor responders were either resistant to hydroxycarbamide or required higher doses of hydroxycarbamide to control the counts. This led to the development of side effects and requirement for change over of therapies. However, from the $JAK2^-$ subgroup 8 patients responded well to anagrelide, 2 patients to busalphan, 3 to hydroxycarbamide and 1 patient to interferon alfa. From JAK2+ subgroup 1 patient responded well to anagrelide, 6 to hydroxycarbamide, 4 to busalphan and 1 to interferon alfa. On an average, more patients from JAK2- subgroup required change in therapies to achieve the appreciable response compared to patients from $JAK2^+$ subgroup. In patients with good response, the time required to achieve an appreciable response by a particular therapy was not different in $JAK2^-$ and $JAK2^+$ patients. *Conclusions*. On the basis of this small pilot study, we conclude that ET patients with the JAK2 mutation are more responsive to current first line therapies compared to JAK2- patients. Hence, JAK2 status may inform therapeutic choice in myeloproliferative disorders in the future. A large multi-centre prospective study is required to confirm the above findings.

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HEMOPHAGOCYTIC SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE STUDY

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Background. Hemophagocytic syndrome is a histiocytic disorder characterised by the activation and proliferation of benign macrophages with hemophagocytosis throughout the reticuloendothelial system. Hemophagocytic syndrome may be categorised into primary (or familial) and secondary forms. This syndrome may be secondary to malignancy, infection or autoimmune disease, and mechanisms involved are poorly understood. Hemophagocytic syndrome is rarely reported after hematopoietic stem cell transplantation. *Aims*. This prospective study was conducted to evaluate the incidence of hemophagocytic syndrome after hematopoietic stem cell transplantation (HSCT). *Methods*. Between August 2006 and February 2007, all patients who received a hematopoietic stem cell transplantation, were included in this prospective study, at the National Centre for Bone Marrow Transplantation (Tunisia). All the following criteria were needed for the diagnosis of hemophagocytic syndrome: 1) sustained fever over 7 days 2) cytopenia (neutropenia and/or thrombocytopenia) 3) presence of more than 3% mature macrophages in bone marrow 4) hyperferritinaemia (> 1000 ng/mL) *Results*. During this study, 79 patients (42 male, 37 female) received a hematopoietic stem cell transplantation (34 allogeneic, 45 autologous). In allogeneic HSCT recipients, we observed 4 cases of hemophagocytic syndrome (4/34; 11.7%): 1 case of EBV-related hemophagocytic syndrome, and 3 cases with no evidence of bacterial, fungal or viral infections. Two patients died (including EBV-related hemophagocytic syndrome) despite aggressive supportive care. In autologous HSCT recipients, we observed only 1 case of CMV-related HS (1/45; 2.2%). This patient is alive 3 months after transplant. *Conclusions.* This prospective study provides a relatively high incidence of hemophagocytic syndrome after allogeneic HSCT. When sustained fever with progressive cytopenia and hyperferritinaemia are observed, hemophagocytic syndrome should be suspected, and bone marrow aspirate considered. The rapid diagnosis of hemophagocytic syndrome and the early initiation of an appropriate treatment is essential for patient management.

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A ROLE FOR HLA-G IN CHRONIC LYMPHOCYTIC LEUKEMIA: FIRST RESULTS OF AN ONGOING STUDY

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Background. Chronic lymphocytic leukemia (CLL), which is the most common form of adult leukemia, is a new focus for researchers. Classically used classification systems Binet and Rai do not supply enough information about the prognosis of the disease. For this reason, researchers started to investigate new prognostic markers for CLL. A number of clinical and biological factors of prognostic relevance have been identified. These include clinical characteristics such as age, gender and performance status, and laboratory parameters like lymphocyte count, bone marrow infiltration or LDH increase. Recently, new prognostic parameters have been identified: serum markers such as, sCD23, ß-2 microglobulin or thymidine kinase and genetic markers; such as, gene abnormalities, the mutation status of IgVH genes or markers for these factors such as CD 38 and ZAP 70. *Aims*. In our study, we investigated the effect of HLA-G - which is first detected in early placental trophoblasts -,ZAP-70, CD38, IL-10 on the prognosis of CLL. HLA-G, molecule has limited tissue distribution and exerts multiple immunoregulatory functions. Recent studies indicate an ectopic up-regulation in tumor cells that may favor their escape from anti-tumor immune responses. *Methods*. These parameters were studied retrospectively in circulating B-CLL cells from 20 patients by flow cytometry and IL-10 was studied by Elisa. *Results*. The proportion of leukemic cells expressing HLA-G varied from 1% to 34%. Patients over 12%, HLA-G positive cells (according to receiver operating characteristics [ROC] analyses) had correlation with progression free survival (p=0,045). We also detected a statistically significant difference between Binet stage A; B and C (p=0,046). We also detected a positive correlation between IL-10 and HLA-G (p<0,044). *Conclusions*. We conclude that HLA-G positivity has an effect on progression - free survival, and on disease stages between Binet A B and C. These are the first results of our ongoing study.

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DEVELOPMENT OF A SIMPLE DISCRIMINANT FUNCTION THAT DIFFERENTIATES ATYPICAL B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA FROM MANTLE CELL LYMPHOMA EVALUATION BY FLOW CYTOMETRY AND MOLECULAR CYTOGENETICS (FISH)

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Background. Atypical B cell chronic lymphocytic leukemia (B-CLL) cannot be effectively discriminated from mantle cell lymphoma (MCL) by the common immunophenotypic scoring system. Aim of this study was to develop a simple discriminant function (DF) based on surface markers in general use, that would better differentiate atypical B-CLL from MCL. *Methods*. We studied 144 CD5 positive cases of B cell chronic lymphoproliferative disorders (CD5>30%), by flow cytometry and FISH. They were assigned as typical or atypical according to the well-known 6 antigen scoring system proposed initially by D. Catovsky *et al.* (1994) and later improved by the addition of CD79b (1997). The following cytogenetic anomalies were sought in all patients: t(11;14), 17p-, 11q-, +12, t(14q32), 13q-. *Results*. Scores 0-4, prevailed in MCL, in the diagnostic groups of atypical B-CLL with 17p-, 11q-, +12 and in t(14q32) lymphoproliferative disorder. The novel discriminant function (DF) pro-

posed, was: DF = (CD43%+FMC7%+CD79b% + CD38%) /(CD23%+CD11c%). The percentages refer to CD19 positive B cell gate. DF values greater than 5 were observedin MCL and the t(14q32) disorder but not in the atypical B-CLL diagnostic groups. Its discriminating value is attributed to the increased CD11c% values in atypical B-CLL and the icreased CD38% values in MCL (Table 1). *Conclusions*. The proposed function is simple, is based on commonly used antigens and It can be combined with the common Catovsky score to discriminate B-CLL from MCL. The newly recognized CD5+ chronic lymphoproliferative disorder t(14q32) however, cannot be discriminated from MCL by both methods.

Table 1.

Diagnosis n=144	n	Score 0-4	DF	DF>5	CD11c%	CD38%
MCL t(11;14)	29	29 100%	10,8 (8,5-18)	26 89,6%	11,9 (6,9-16,5)	63,3 (25,7-9)
B-CLL 17p-	8	3 37,5%	2,6 (1,8-3,8)	0 0%	29 (21-42,5)	28 (6-43,9)
B-CLL 11q-	10	3 30%	2,3 (1,7-3,5)	0 0%	27 (14-37,5)	7 (1,3-50,5)
B-CLL +12	19	10 52,6%	2,1 (1,8-2,9)	0 0%	35,9 (25,7-64)	18,8 (3,6-57)
t(14q32)	9	6 66,6%	4 (2,6-11)	4 44,4%	15 (6-30,5)	12 (8-54)
B-CLL(-)	37	5 13,5%	1,7 (1,4-2,3)	0 0%	34 (19,6-45,2)	6 (1-29,5)
B-CLL 13q-	32	6 18,7%	2 (1,4-2,5)	2 6,2%	28,3 (16,7-41)	1,3 (1-19,8)

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GEMCITABINE IN THE TREATMENT OF RELAPSED AND REFRACTORY HODGKIN'S DISEASE

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Background. Patients with refractory Hodgkin's disease or relapsing after high-dose therapy and autografting have a poor prognosis. Here, we present our experiences with gemcitabine in this setting. *Aims*. This study's objective was to determine the efficacy and safety of gemcitabine in patients with relapsed or chemotherapy-refractory Hodgkin's lymphoma (HL). *Patients and Methods*. We treated 10 patients /70% patients were male/ with relapsed or refractory Hodgkin's disease with gemcitabine. The median age was 43 years (range, 20-76). 4 patient had stage IIB disease, 2 patients had stage IIIB disease and 4 patients had stage IVB disease. 6 patients had mediastinal lokalisation of disease. 6 patients received BEACOPP regimen in first line of therapy, while 4 patients received ABVD. 7 patients had received radiotherapy. 80% of patients had early relapse of disease. 5 patients had an Eastern Cooperative Oncology Group performance status (PS) of 0, 4 patients had a PS of 1. Gemcitabine was administered at a starting dose of 1250mg m2 on days 1,8 and 15 every 3 weeks in combination with steroids. All patients had received at least 2 cycles of Gemcitabine (range, 2-6). *Results*. The median follow-up period was 6 months. Hematological toxicity grade 3-4 occurred in 7 patients leading to dose reductions. No other non-hematological toxicities were observed. The response rate was 30% with 1 patients achieving complete remission (CR) and 2 patients partial remission (PR). 7 patients have discontinued treatment because of disease progression. The median time to treatment failure was 4.5 months, and survival was 11 months. Responses were seen in patients refractory to previous treatment. The so far longest responder has been in CR for over 40 months. *Conclusions*. Gemcitabine is potentially effective treatment for Hodgkin's disease. According to our results and results of other investigators, studies on great number of patients is needed. Heavily pretreated patients often require dose reductions. Diagnostic groups were defined according to immunophenotypic, FISH, histological and clinical findings as follows: MCL (n=29), B-CLL (n=106), 9 cases with IgH rearrangement, excluding those with t(14;18) ♀ t(11;14). Whenever cytogenetic anomalies coexisted, hierarchical diagnostic criteria were set as following t(11;14)>t(14q32)>17p->11q->+12>13q-. B-CLL without positive FISH findings was assigned as B-CLL (-).

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LOW DOSE THALIDOMIDE PLUS VALPROIC ACID COMBINATION THERAPY SHOWS HIGHER HEMATOLOGIC IMPROVEMENT THAN SINGLE AGENT THERAPY IN MYELODYSPLASTIC SYNDROME CANNOT BE CANDIDATE TO INTENSIVE TREATMENT

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Background. Thalidomide is a potentially useful drug for myelodysplastic syndrome (MDS) because of its immune-modulatory effect with anti-cytokine and anti-angiogenic effects. Valproic acid (VPA) inhibits histone deacetylase activity and can induce differentiation as well as apoptosis in leukemic cell lines *in vitro*. *Aims*. This study is investigated whether thalidomide plus VPA combination therapy has more potent therapeutic effect than single agent therapy. *Methods*. Twenty-six patients with MDS and chronic myelomonocytic leukemia (CMML) were treated with thalidomide combined with VPA therapy. Thalidomide was administered orally at a dose of 50 mg fixed dose per day and VPA was administered to reach serum concentrations between 50 and 100 ug/mL in two or three divided doses per day. Bone marrow biopsy and aspirates specimens were taken at diagnosis and at the end of the study (three months later). Weekly or biweekly complete blood counts with differentials were obtained, and upon completion of 12 weeks of therapy. In patient of any evidence of response, stable disease or hematologic improvements, thalidomide and VPA were continued until response disappeared. *Results*. 10 patients have completed at least 12 weeks of therapy and five patients discontinued therapy within 12 weeks due to intolerable side effects and death of disease aggravation. Of the 9 male and 6 female (median age, 68 years), 3 had refractory anemia (RA), 2 had RA with ringed sideroblasts, 5 had RA with excess blasts-1, 4 had hypoplastic MDS, and one had CMML. No complete response was seen, but one patients who had hypoplastic MDS showed partial response. 73.3% of patients (11 out of 15) showed hematologic improvement (9 patients with erythroid improvement, 6 with platelet improvement, and 4 with neutrophil improvement). The median response duration was 8.5 weeks (range, 2-23 weeks). *Conclusions*. Thalidomide combined with valproic acid therapy is more effective than thalidomide single agent in improving cytopenias of MDS patients.

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UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES IN A SINGLE CENTER IN BRAZIL

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Background. A number of hematological malignancies can be cured by allogeneic stem cell transplantation but only approximately 35% of Brazilians have a suitable histocompatible related donor. For those patients in need of an allogeneic transplant, but for whom a suitable matched related or unrelated adult donor cannot be found, the use of banked unrelated umbilical cord blood has emerged as a potential option. *Aims*. The aim of this study was to define the feasibility of allogeneic stem cell transplantation using single unrelated cord blood units in a cohort of adults and children with poor prognosis leukaemia in a single institution in Brazil. *Methods*.Twenty patients, 13 children and 7 adults, with hematological malignancies: 9 with acute lymphoblastic leukaemia, 8 with acute myeloid leukemia, 3 with chronic myeloid leukemia received transplants of cryopreserved cord blood. Seven patients had active disease at time of the transplant. The conditioning therapy were high-dose cyclophosphamide and total body irradiation; Bulssufan with high-dose cyclophosphamide or Mephalan and antithymocyte globulin. Patients were given post-transplant immunosuppression with cyclosporin and methylprednisolone. *Results*. Patients had a median age of 8 years (range: 8m to 51y). Cord blood units contained a median $3{,}83\times10^7$ nucleated cells/kg recipient body weight and were matched for four (8 cases);five (9 cases) or 6 (2 cases) major histocompatibility complex class 1 and 2 antigens. Two patients did not engraft and three recovered with disease. In 15 evaluable patients, the neutrophil recovery to 0.5×10^9/L was seen by median day 20 after transplant and platelet recovery to 20×10^9/L occurred by median day 57. With a median follow-up of 24 months (1,5-130 months), 8 patients were alive, five of them without evidence of relapse. The causes of death were infection in 6 patients,

underlying disease in 3, graft versus host disease (GVHD) in two and multiple organ failure in one. Infection was the most common complication (90%). The agents of infection in the patients who died were: S. aureus and acinetobacter; aspergillus; candida sp, cytomegalovirus and adenovirus. Acute GVHD grades II-IV was seen in 7 of 15 (46,6%) who survived more than 30 days and limited chronic GVHD was seen 1 in 9 patients who survived more than 100 days post-transplant. *Conclusions*. Unrelated cord blood transplantation is feasible in patients with high-risk malignancy in Brazil with infection relating to immunosuppression being the major limitation.

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SIGNIFICANCE OF PLATELET-DERIVED MICROPARTICLES IN PATIENTS WITH RECURRENT FETAL LOSSES

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Background. Platelet-derived microparticles (PMP) have procoagulant activity and are produced by platelet activation or physiological stimulation. PMP are associated with various prothrombotic states. One of the proposed causes of recurrent fetal losses is uteroplacental thrombosis and PMP may be associated with it. The aim of this study was to investigate the significance of PMP in women with recurrent fetal losses. *Methods*. The platelet CD62P as a platelet activation marker and $CD42b^+$ microparticles as PMP were measured by flow cytometry. Measurements by flow cytometry were done in whole blood of 13 women with recurrent fetal losses and 11 age-matched healthy women controls with no previous history of fetal loss. *Results*. PMP levels in patients group were higher than in normal subjects (4.13±1.09% vs 2.54±1.47%, $p<0.006$). CD62P levels were not different between in patients group (14.48±10.41%) and in control group (10.37±10.55%, $p>0.05$). *Conclusions*. Our findings, increased levels of PMP in patients group, suggest that PMP might have a role of pathogenesis of recurrent fetal losses.

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EFFICACY OF MICAFUNGIN IN PROPHYLAXIS AGAINST INVASIVE FUNGAL INFECTIONS IN PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Allogeneic hematopoietic stem cell transplantation (HSCT) recipients usually receive fluconazole (FCZ) or amphotericin B (AMPH-B) for standard antifungal prophylaxis. However, invasive fungal infections (IFI) have become an important problem in patients undergoing allogeneic HSCT, especially in aged or severely immunosuppressed recipients. Because of fungal resistance and lack of anti-Aspergillus activity with FCZ and renal toxicity with AMPH-B, alternative prophylactic regimens have become necessary. Since many antifungal drugs have recently become clinically available, the optimal strategy for prophylactic antifungal therapy has not yet to be determined. *Aims*. To resolve this issue, we therefore conducted a prospective study to compare the effectiveness and safety of recently available micafungin (MCFG) with those of FCZ in preventing IFI during the first 100 days after allogeneic HSCT. *Methods*. Patients undergoing allogeneic marrow, peripheral blood stem cell, or cord blood stem cell transplantation were eligible for the study. Patients undergoing allogeneic HSCT received MCFG at 100 mg/day intravenously beginning 14 days prior to HSCT and continuing until engraftment. To evaluate the efficacy and safety of MCFG, data for patients undergoing allogeneic HSCT who received FCZ at 400 mg/day orally or intravenously were analyzed retrospectively. After obtaining engraftment, patients in both groups received FCZ at 200 mg/day orally until cessation of administration of immunosuppressants for prophylaxis or treatment of GVHD. IFI were diagnosed by using criteria published by the European Organization for Research and Treatment of Cancer. Treatment of high-dose AMPH-B (0.5-1.0 mg/kg/day intravenously), MCFG (150-300 mg/day intravenously), or voriconazole (400 mg/day intravenously or orally) was started for patients with suspected or proven IFI. Drug-associated toxicity was evaluated, and proven, probable, or possible IFI were analyzed. Mortality from fungal infection and overall mortality were also determined. *Results*. From December 2003 to June 2006, a total of 31 patients were enrolled in the trial. Two patients were excluded because of discontinuation of MCFG treatment due to drug eruption and because of early death due to capillary leak syndrome. Data for 29 patients who had undergone allogeneic HSCT with FCZ for prophylactic IFI from May 2000 to October 2003 were also retrospectively analyzed for comparison. The incidences of proven IFI in the two groups were not significantly different (0% in the MCFG group and 7% in the FCZ group, $p=0.491$). The total incidences of proven, probable or possible IFI were also not significantly different (34% in the MCFG group and 45% in the FCZ group, $p=0.592$). The survival rates at 100 days post-transplantation were similar in the two groups (86% in the MCFG group and 76% in the FCZ group, $p=0.504$). Rates of death attributable to IFI were similar in the two groups (0% in the MCFG group and 7% in the FCZ group, $p=0.491$). MCFG was well tolerated. *Conclusions*. We conclude that MCFG is as effective as FCZ in prophylaxis against IFI during immunosuppression in patients undergoing allogeneic HSCT.

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CHILDHOOD NON HODGKIN LYMPHOMA IN DEVELOPING COUNTRIES; CAN INTENSIVE CHEMOTHERAPEUTIC REGIMENS BE APPLIED?

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Background. Non Hodgkin Lymphoma (NHL) is the third most common childhood cancer. It is divided further into subtypes that differ in their clinical, histopathological and management aspects. Using multi agent intensive chemotherapy significantly improved the outcome of high-risk patients. Decreasing the treatment intensity in low risk patients maintained their superior outcome while reducing the long-term toxicity of the treatment. *Aims*. To retrospectively evaluate the outcome of children diagnosed with NHL and treated at King Hussein Cancer Center (KHCC) using the multi-agent intensive protocols developed by the European and North American cooperative groups and to compare the treatment outcome at KHCC to the treatment outcome in Europe and North America using the same protocols. *Methods*. Between January 2004 and December 2006, 54 children were treated at KHCC for NHL. Median age was 8.6 years (range 11 months-18 years). Male to female ratio was 4.4:1. Of those, 30 patients (55.5%) had Burkitt's Lymphoma (BL), 8 patients (14.8%) had Diffuse Large B cell Lymphoma (DLBL) with 5 of them having primary Mediastinal disease, 11 patients (20%) had Lymphoblastic Lymphoma (LL), 4 patients (7.4%) had Anaplastic Large Cell Lymphoma (ALCL) and 1 patient (1.8%) had Large T Cell Lymphoma. BL and DLBL patients were treated on LMB 96 protocol; LL patients were treated on BFM 95 protocol and ALCL with 2 different protocols (POG and BFM). *Results*. 30 patients had BL. With a median follow up of 23.5 months (2-37 months), 26 patients (86.6%) were alive in complete response (CR). 2 patients died in CR secondary to treatment related complications, one died with progressive disease and one alive patient progressed while on therapy. 8 patients had DLBL, of those 5 (62.5%) are alive in remission with a median follow up of 27 months (2-36 months), 3 patients with primary mediastinal disease died due to disease progression. 6 patients had T-Cell LL, 3 patients had precursor B LL and 2 patients had LL with unknown phenotype. All LL patients are alive in CR with a median follow up of 24 months (2-37 months). Two patients with ALCL were treated according to BFM 90 protocol and two patients with ALCL were treated on POG 8615 protocol (standard arm). All ALCL patients are alive in CR with a median follow up of 18 months (7-28 months). *Conclusions*. Adopting intensive treatment protocols such as LMB, POG and BFM is feasible in countries with limited resources like Jordan if adequate supportive care can be provided. The outcome of our patients is comparable to the outcome reported in Western countries. The poor outcome of DLBL patients with mediastinal disease makes it necessary to explore more novel therapies.

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CONSECUTIVE DETECTION OF MLL REARRANGEMENTS IN INFANTS ACUTE LYMPHOBLASTIC LEUKEMIA DURING TREATMENT WITH ALL-TRANS RETINOIC ACID IN COMBINATION WITH CONVENTIONAL CHEMOTHERAPY

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Background. Despite of many attempts worldwide, treatment results and prognosis of infant leukemia with MLL rearrangements are rather dismal. Recently new treatment approach with all-trans retinoic acid (ATRA) for such patients has been applied in our clinic. It is based on combination of conventional chemotherapy and simultaneous pulses of ATRA. ATRA is a natural provitamin A. It has been approved for the

treatment of acute promyelocityc leukemia and some other hematological malignancies. *Aim*. To estimate the elimination speed of MLL rearrangements in patients enrolled in phase I/II of single institutional study. *Methods*. Since September 2003 4 patients from our clinic with MLL rearrangements have been enrolled in this study. All of them have had pro-pro-B (BI) immunophenotype. Leucocytes from bone marrow were obtained. Nested PCR for MLL/AF4 (A. Borkhardt *et al.*, 1994) and MLL/ENL fusion genes (FG) were performed. Design of primers, probe and real-time quantitative PCR (qRT-PCR) conditions for MLL/AF4 FG detection was previously described (J. Gabert *et al*., 2003). β2-microglobulin was used as control gene. Normalized copy number (NCN) of MLL/AF4 was calculated. This value was multiplied by 10 000 000 and Log10 was taken. In accordance with treatment design patient with MLL/ENL has been treated by intermediate risk arm, while 3 others infants with MLL/AF4 have been enrolled to the high risk (HR) schedule. Treatment and diagnostics has been approved by Institutional Ethics committee. Parents' informed consent were obtained in all cases. *Results*. Qualitative nested PCR revealed that 3 patients had MLL/AF4 and 1 had MLL/ENL FG transcripts. In 2 patients with MLL-AF4 and 1 patient with MLL/ENL total elimination of FG were detected after first course of ATRA administration on day 43. FG were not detected by means of nested PCR with sensitivity of 10-5. In 1 patient elimination of MLL/AF4 was found on the first day of protocol II after 7-th ATRA pulse. In this patient qRT-PCR was performed on days 1, 15, 36 as well as first days of HR blocks 1(1), 3(3), first day of protocol II. There was not available material for qRT-PCR on first days of HR blocks 2(2), 1(4), 2(5), 3(6). Although in all above mentioned time points nested PCR of MLL/AF4 FG transcripts was performed. On the day 1 Log10 NCN of MLL/AF4 was 4.77. Within induction therapy Log10 NCN values were almost equal-2.36 on day 15 and 2.33 day 36, respectively. Significant reduction was found after first course of ATRA, NCN was 1.95. After that NCN was going down by degrees and on the first day of HR blocks 3(3) it was 0.39. Since the first day of protocol II MLL/AF4 FG transcript has been detected neither in qRT-PCR nor in nested PCR. All subsequent PCR examinations have not been detected the MLL/AF4 FG. *Conclusions*. It has been proved the effectiveness of ATRA-content regimen for infants with MLL/AF4 and MLL/ENL FG transcripts. Administration of ATRA led to significant reduction of MLL/AF4 and MLL/ENL transcripts down to undetectable level.

1246

DIFFERENT TYPES OF BONE MARROW INVOLVEMENT BY DIFFUSE LARGE B-CELL LYMPHOMA: SPECIAL FEATURES AND RELATIVE BM CHANGES

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Background. The aim of the study was to evaluate the special features of bone marrow involvement by Diffuse Large B-cell Lymphoma (DLBCL), relative BM changes and possible immunomorphological discordance with other involved sites. *Methods*. We studied bone marrow (BM) samples (24 aspirates and 23 trephine biopsies) from 24 patients consecutively diagnosed with DLBCL in a single institution during a 3-year period (2003-2006). The main including criteria was prominent lymphoid infiltration in BM trephine biopsy's samples and atypical lymphoid (blasts) cells presence or lymphocytosis > 28% in BM aspirate's slides. For the diagnosis of DLBCL the current WHO criteria was used. 14 (58%) cases were nodal and 10 (42%)-extranodal. Sites of the extranodal disease were: soft tissues-4 cases, Waldeyer's ring (WR)-2, GI tract-2, primary BM/bone involvement-1, and breast involvement '1 case. When available, clinical data at diagnosis, including components of the International Prognostic Index (IPI), were reviewed. Selected group consisted of 16-previously untreated patients (7 cases-low risk, 3-intermediate high and 6 'high IPI groups), 3- currently treated, 2-relapsed and 3-with not knower clinical stage. Immunophenotyping BM study was performed in 8 cases of aspirate (flow cytometry-FC) and in 17 of trephine biopsy simples (immunohistochemistry). Panel of monoclonal antibodies routinely included lineage-associated and immune markers, proliferation and apoptosis markers, and more recently stage-specific markers of B- and T-cell differentiation. *Results*. We observed 3 main types of BM changes in selected group, according to complete trephine biopsy investigation: diffuse blasts cells infiltration-10 cases (42%), T-cell rich like pattern of involvement-5 cases (21%) and clear reactive T-cell patchy infiltration-9 (37%). Diffuse blast cell infiltration was associated with nodal presentation (9/10), low T-cells count (immunohistochemistry detection on BM trephine biopsies) high IPI or relapsed group patients. T-cell rich like BM infiltration pattern in contrast to primary sites composed of non-neoplastic T cells and only <10% large discrete neoplastic B cells were present. The latest pattern was associated both with nodal and extranodal presentation and low IPI score. The patchy T-cell BM infiltration was associated on the whole with extranodal presentation (7/9) and low risk IPI score. *Conclusions*. We conclude that there are existed some differences in BM involvement by DLBCL, especially between nodal and extranodal DLBCL forms. The latest had been shown in association with prominent T- cell reaction. This may be indirect evidence of it immunogenic nature in contrast to nodal form.

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DIAGNOSTIC AND PROGNOSTIC VALUE OF PROCALCITONIN (PCT) SERUM LEVELS IN NEUTROPENIC PATIENTS

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Studies in neutropenic patients have evaluated diverse markers of inflammation as predictors of patient subgroups with different types of infection. The serum concentration of PCT a 116 amino acid propeptide of calcitonin was reported to be markedly increased in severe systemic infection primarily of bacterial origin, and serum levels appeared to correlate with disease severity. The aim of the present study was to assess the potential value of serum PCT compared to other parameters such as: C-reactive protein (CRP)(N<6 mg/L), serum bicarbonate(N:24-26mmol/l), serum lactate (N<2.2 mmol/l) and phosphatemia (N:0,8-1,4mmol/l) to predict infection and outcome in neutropenic patients. Between July 2006 and January 2007, 27 neutropenic patients (21 AML, 6ALL) with 50 neutropenic febrile episodes were included in this study. All patients received empiric antibiotherapy for the first febrile episode with Piperacillin/Tazobactam + PolymyxinB IV. Consecutive sample for PCT (semi-quantitative test, BRAHAMS), CRP, Phosphatemia, serum lactate, serum bicarbonate were taken at different neutropenic febrile episodes. Clinically documented infection (14%)were ;pulmonary infection (4), neutropenic enterocolitis (1), mucositis (1),cutaneous lesion (1). Microbiologically documented infection (30%) were due to: Klebsiella (7), Staphylococcus (6), E.coli (1), Acinetobacter (1). Septic shock occurred in 6 episodes (12%), leading to death in 5 patients 18, 51% (5/27). Negative PCT (<0,5 ng/mL) was noted in 18 episodes, whereas high level(>10 ng/mL) were observed in 11 episodes. Median serum level of CRP was 88,8mg/L (2,2-183 mg/L),median serum lactate was 2,3 mmol/L (0,8-3,3 mmo/L), median serum bicarbonate was 24,3 mmol/L (9,4-32,1 mmol/L) and median phosphatemia was 1,08 mmol/L (0,26-1,75 mmol/L). Association between each biological parameter with infection and outcome was examined and tested with Fisher exact test. Significance was defined as p<0,05. PCT>0,5 ng/mL (p=0,004, Odds ratio=6,6) and CRP>100 mg/L (p=0,008; Odds ratio=6,1) were correlated with documented infection (clinically or microbiologically). CRP>100 mg/L was found to be associated with Gram negative infection (p=0,045). PCT>10 ng/mL (p=0,017; Odds ratio=10,57) and serum lactate >3 mmol/L (p=0,04; Odds ratio=16) are correlated with occurrence of septic shock. Serum level of PCT using semi-quantitative test have equivalent diagnostic and prognostic value for the diagnosis of infection and for outcome as CRP and serum lactate in neutropenic patient.

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ALLOGENEIC HAEMOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS AT HIGH RISK FOR TOXICITY USING TREOSULFAN/CYCLOPHOSPHAMIDE±ALEMTUZUMAB CONDITIONING

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Background. Allogeneic transplantation is associated with well documented toxicity. Treosulfan is an alkylating agent which has been reported as offering reduced toxicity compared with busulfan without compromising engraftment or increasing relapse rates. In patients thought to be at high risk from toxicity associated with standard radiotherapy based myeloablative conditioning, but requiring intensive conditioning, we used Treosulfan (14 g/m^2 D -6 to -4), Cyclophosphamide (60 mg/kg D -3 to -2) and for those with volunteer unrelated or mismatched sibling donors, MabCampath (10 mg bd iv D -6 to -1). *Methods*. We retrospectively evaluated 12 patients (median age 42 range 25 to 50) who received this regimen at our institution between 2001 and 2006 with a median follow up of 398 days (range 64-1922). The patients were all at high risk for relapse and had the following diagnoses AML CR1(3), AML CR2(2),

ALL CR2(2), MDS(1), secondary MDS post AML(2), blast crisis CML(2). Most patients were heavily pre-treated and 5 had previous TBI containing transplants (3 autografts, 2 allografts). Two had significantly low transfer factor pre-transplant (DLCO less than 60% predicted) and two patients had previous confirmed invasive fungal infections. The donors were 4 matched sibling, 1 mismatched sibling and 7 matched unrelated with PBSC used in 9 transplants; median CD34 dose 6.8×10^6 CD34/kg (range 1.17 to 11.48) and BM in 3; median mononuclear dose 3.25×10^8 mononuclear cells/kg (range 2.65 to 4.32). *Results*. Toxicities were limited with maximum Grade II mucositis and no patient required continuous opiate infusion. Renal and hepatic toxicity was minimal and thought to be unrelated to the regimen. There were no cases of VOD. The median length of stay was 42 days (range 23 to 50). 11/12 patients engrafted (1 developed RSV at Day +10 and subsequently died) with median time to platelet recovery $>20\times10^9$/L Day +13, $>50\times10^9$/L Day +17, neutrophils $>0.5\times10^6$/L Day +19, $>1\times10^6$/L Day +21. One patient developed acute graft rejection within 100 days. Two patients had evidence of mixed lymphoid chimerism. One has received DLI with resolution and neither has relapsed. The incidence of acute GVHD was low with only Grade 1 skin involvement in 4 patients. One patient has developed severe extensive chronic GvHD requiring long term immunosuppression. Three patients have relapsed and 2 have died, the third remains alive in remission with extensive chronic GVHD four years after DLI. The non-relapse mortality at D100 was 8% (1/12) with an overall survival at 1 year of 75%. The regimen has been well tolerated with a mean Karnovsky score at Day 100 of 70% and at 1 year 90%. *Conclusions*. We found this conditioning regimen to be well tolerated with acceptable levels of toxicity in high risk patients without compromising engraftment or increasing relapse rates to unacceptable levels. We suggest consideration of this regimen for patients who are at high risk of relapse and for whom a TBI containing myeloablative regimen is unsuitable due to co-morbidity or previous radiotherapy.

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OCCURRENCE OF EBV INDUCED B CELLS LYMPHOMA IN TWO PATIENTS TREATED CONCOMITANTLY WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND ALEMTUZUMAB

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Because of strong immunosuppression, Epstein Barr Virus (EBV) positive lymphoproliferative disorders (LPD) are frequently observed in HIV patients or after transplantation. EBV+ LPD have been reported after treatment of low-grade lymphomas or chronic lymphocytic leukaemia (CLL) with Fludarabine. We report here two cases of an EBV+ LPD occurring after the use of Fludarabine, Cyclophosphamide and Alemtuzumab. A 68 year-old man had a diagnosis of B CLL (Matutes score 4/5) with a del 11 q on conventional cytogenetic analysis of peripheral blood lymphocytes. He was treated with 5 courses of oral Fludarabine (50 mg/day D1-D3) and Cyclophosphamide (400 mg/day D1-D3) from September 2003 to February 2004 with good response. He relapsed in April 2006 with multiple peripheral lymph nodes and splenomegaly. Cytological examination of lymph nodes showed features of CLL with no large cells. Total lymphocytes count was 1090/mm^3. He received 9 weekly subcutaneous injections of Alemtuzumab (30 mg/injection). In June 2006, erythroblastopenia and acute hepatitis occurred and a parvovirus B19 infection was diagnosed. He received intravenous immunoglobulins. Treatment with Alemtuzumab was stopped. In January 2007, high fever, abdominal pain, night sweats and blood analysis consistent with an haemophagocytic syndrome appeared. Computed tomographic scan showed hepatosplenomegaly and multiple abdominal enlarged lymph nodes . The detection of EBV by PCR analysis was positive in the serum. A lymph node biopsy showed features of EBV$^+$ CD20$^+$ Hodgkin-like lymphoproliferative disorder consistent with the diagnosis of EBV induced B cell lymphoma. The patient is now treated with Rituximab and CHVP with a good response. A 68 year-old man had in 2004 a diagnosis of T prolymphocytic leukaemia without clinical symptoms nor tumoral syndrome. He was untreated until 2005 when he was referred to our center with major asthenia, hepato-splenomegaly, pleural effusion and ascitis; total lymphocytes count was 108 000/mm^3. Immunophenotype revealed T lymphocytes CD3$^+$ CD4$^+$ CD52$^+$. A course of CHOP was unsuccessful and treatment with oral Fludarabine (50 mg/d D1-D3), oral Cyclophosphamide (600 mg/d D1-D3) and Alemtuzumab (30 mg/injection) was started in February 2006. He received a total of 8 monthly courses of Fludarabine and Cyclophosphamide and Alemtuzumab by subcutaneous injections at a dose of 30 mg times 3 weekly for 4 months, then once per month for 6 months. In December 2006 he noted an enlarged cervical lymph node. Fever, night sweats, and serum analysis were consistent with an hemophagocytic syndrome. Histological examination of the cervical lymph node showed features of EBV+ diffuse large B cell lymphoma consistent with the diagnosis of EBV induced B cell lymphoma. The patient is currently treated with Rituximab and CHOP. These two cases are different by the initial pathology and the way of administration of Fludarabine, Cyclophosphamide and Alemtuzumab. Nevertheless they emphasize the risk of EBV induced lymphoma in severe immunodeficient patients. The concomitant use of three immunosuppressive agents must be weighted carefully. The monitoring of EBV viral load in serum should be performed routinely as is done the measure of cytomegalovirus viral load .

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FROM STEM CELLS TO CIRCULATING BLOOD: DYNAMIC AND ARCHITECTURAL ASPECTS OF HEMATOPOIESIS

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Background. Circulating blood cells undergo continuous cell turnover and are constantly being produced within the bone marrow in the process of hematopoiesis. At the root of blood cell formation are the hematopoietic stem cells (HSC). Recently, it was shown that the size of the active HSC pool is small (~400 cells in adult humans) and these cells replicate at a very slow rate (~1/year). In contrast, the average marrow output of ~ cells per day in huge. To date, no simple model has been able to capture the essential features of the scaling in time and population that characterize hematopoiesis. *Aims*. To develop a testable model that can account for the amplification and differentiation of blood cell formation over such a wide range of time scales. *Methods*. We consider that hematopoiesis is divided into compartments where cells in any compartment i divide to produce 2 daughter cells. The two cells either differentiate (with probability ?) and move to the next compartment (i+1) or, with probability (1-?), remain in compartment i for amplification of that compartment. Cells lost from compartment i, are replaced by cells from compartment i-1 so that the population within each compartment remains constant under stationary conditions. We utilize data from granulocyte production to calibrate the model. *Results.* Our model predicts that there are at least 31 amplification and differentiation steps between the active HSC pool and circulating blood cells. In any given compartment, meaning that in general, cells in any compartment differentiate and move to the next compartment downstream rather than self-renew. The model predicts the size and the replication rate of cells within any compartment. When tested on the limited data available for PIG-A mutant cells in the circulation, the model accurately predicted the average life duration of these clones in healthy adults. *Summary/Conclusions*. Hematopoiesis can be visualized as a series of cell filled compartments where amplification and differentiation occur. Blood production is due to an exponential increase in the number of cells due to increasingly rapid replication rates as differentiation occurs. The model makes predictions that agree closely with the limited data available in the literature. The model can be utilized to gain insights into various hematopoietic disorders and such studies are underway.

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HIGH SERUM FERRITIN LEVEL IS AN IMPORTANT PREDICTIVE FACTOR FOR POOR STEM CELL MOBILIZATION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Background. Iron overload presented by high level of serum ferritin is often observed in various hematologic diseases. Iron overload has some negative effect on engraftment and survival after hematopoietic stem cell transplantation. *Aims*. We analyzed the effect of transfusional iron overload in patients with hematologic malignancy on mobilization of CD34$^+$ cells. *Methods*. We evaluated 50 patients, 18 (36%) with multiple myeloma, 25 (50%) with malignant lymphoma and 7 (14%) with AML. Median duration from diagnosis to stem cell collection is 166 days (range, 27 - 484 days) and mean number of packed RBC transfusion is 4.8 unit (range, 0 - 20 units). Iron overload is defined that serum ferritin level is >1000 ng/mL. The patients with non-transfusional hyperferritinemia were excluded. *Results*. Fifteen patients (30%) were considered iron over-

loaded group and 35 (70%) were non-iron overloaded group. Mean serum ferritin level was 2,432 ng/mL in iron overloaded group and 678ng/mL in non-iron overloaded group (p<0.0001). Mean number of blood transfusion was 13 units in iron overloaded group and 1.5 units in non-iron overloaded group (p<0.0001). There was no significant difference between two groups in sex, age, burden of previous chemoradiotherapy, disease status at harvest, dosage of G-CSF and mobilizing chemotherapy regimens. However notable statistical difference was found in total number of $CD34^+$ cells and number of $CD34^+$ cells per apheresis (13.1×10^6/kg vs. 5.7×10^6/kg, p=0.01; 7.4×10^6/kg/apheresis vs. 2.1×10^6/kg/apheresis, p<0.0001). These statistical differences in total number of $CD34^+$ cells and number of $CD34^+$ cells per apheresis according to the level of serum ferritin were constantly shown for each disease entities. *Conclusions*. The patients with transfusional iron overload showed inferior outcomes in harvesting autologous hematopoietic stem cells in cases with hematologic malignancies. We suggest that the transfusion-associated iron overload is one of the predictive factors for poor mobilization of autologous hematopoietic stem cells.

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LUPUS ANTICOAGULANT ASSOCIATED WITH LYMPHOID NEOPLASMS

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Background. Data on the prevalence and clinical significance of antiphospholipid antibodies (APLA) in patients with lymphoid neoplasms (LN) are still limited. The link between APLA, especially lupus anticoagulant (LA), and hemorrhagic or thrombotic complications of LN remain unclear. Our aim was to investigate the frequency of LA at diagnosis and its role in clinical feature of the LN. *Patients and Methods*. This study included 48 LN patients (5 pts with chronic lymphocytic leukemia (CLL), 24 - non-Hodgkin's lymphoma (NHL), 3 - Hodgkin's lymphoma (HL), 16 pts with multiple myeloma (**MM**). LA was diagnosed by panel of coagulation tests proposed by the Subcommitte for Standardization of Lupus Anticoagulants/ Phospholipid-Dependent Antibodies. *Results*. LA was found in 8 (16,7%) LN patients (1 pt with CLL, 3 pts with NçL, 4 pts with **MM**). The ratios of coagulation time of the patient's plasma in screening phospholipid-dependent coagulation tests were increased, including dilute Russell's viper venom time (DRVVT). The presence of LA was accepted by mixing tests with normal plasma. The antiphospholipid nature of inhibitors was confirmed by repeating at least one of the abnormal coagulation tests in the presence of an increased concentration of phospholipids and high textarin-ecarin ratio. Platelet activation status was considerably disturbed in patients with LA (33,5±7,3 s) as compared to patients without LA (22,5±5,7 s). There was not a significant difference of the incidence of LA in patients with definite categories of LN, though the higher frequency of LA was found in patients with **MM** (25% pts) than in CLL's and malignant lymphomas (14%). Thrombocytopenia (<50×10^9/L) was diagnosed before treatment in 3 (7,5%) patients without LA and in 3 (37,5%) patients with LA, that was a nearly significant difference (χ^2=3,09; p<0,1). Bleedings were observed in 4 LN patients without thrombocytopenia (>50×10^9/L) including 1 (25%) patient without LA (CLL, intestinal bleeding) and 3 LA patients (1 - NHL, 2-**MM**) (subcutaneous hemorrhages and nasal bleeding), that was a significant difference (χ^2=6,60; p<0,01). We observed 1 case of retina thrombosis in **MM** patient without LA. *Conclusion*. The results suggest the high frequency of LA in patients with LN. The presence of LA may be regarded as a cause of platelet dysfunction and bleedings. LA in LN patients, probably, has autoimmune genesis and its influence on patients' haemostasis require further investigations.

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EVOLUTION OF RESISTANCE TO IMATINIB MESILATE AND MULTIDRUG RESISTANCE IN ACCELERATED PHASE OF CHRONIC MYELOID LEUKEMIA

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The aims of this study were to determine the frequency and the time of occurrence of the resistance to imatinib and multidrug resistance in AP CML patients treated with imatinib. 116 AP CML patients (Ph+/BCR-ABL+) were treated with imatinib for 4 years. Between February 2001 to August 2005 116 patients meeting the criteria of accelerated phase CML were treated at the Hematological Research Cente & Federal Research Center of Pediatric Hematology, Oncology and Immunology Moscow. *Patients and Methods*. among the 116 enrolled patients (53 were males and 63 females). Median age of patients was 43 years (from 18 to 75 years). Preceding chronic phase lasted for 29 months (range 0-151 months). Median time before the therapy in all patients was 36,4 months. Most of the patients received prior therapy for CML (IFN-α, hydroxyurea, cytarabine, e.t.c.). Median duration of treatment with imatinib was 41 months (6,5-51 months). *Results*: Imatinib induced CHR in 95 patients (82%). By 3 months of the treatment 75% of our patients had CHR. After 3 months of imatinib therapy probability of CHR proved to be only 6.8% and after 6 months - 3.5%. Primary hematologic resistance, defined as failure to obtain a complete hematologic response despite imatinib treatment, was found in 21 of 116 AP CML patients (18%). Most of these patients (17 of 29) died of CML progression (median survival after cancellation of imatinib was 4 months). Our data shows that 34% of AP CML patients didn't have any cytogenetic response (within the period of surveillance) while 45% of the patients achieved major cytogenetic response (CCR 36%;+ PCR 9%). Achievement of cytogenetic response greatly influenced survival of the patients. In fact, overall survival of the patients with CCR, PCR and MNCR was 92%, 64% and 56% accordingly. The overall survival of patients without cytogenetic response was 30%. The sensitivity of AP CML patients to imatinib could be predicted by the number of factors found at the time of AP diagnosis. We analyzed whether traditional clinical and hematologic parameters could serve for prognosis of imatinib therapy efficacy or patient's resistance to the drug. Prediction of the response to imatinib would greatly facilitate therapeutic decisions. Our analysis shows that the number of the AP CML signs found in patients at AP diagnosis has prognostic significance (Figure 1A). Among the patients with most favorable prognosis (who had only 1 of unflavored factors) shorter duration of life was registered in patients with splenomegaly and with thrombocytopenia (Figure 1B).

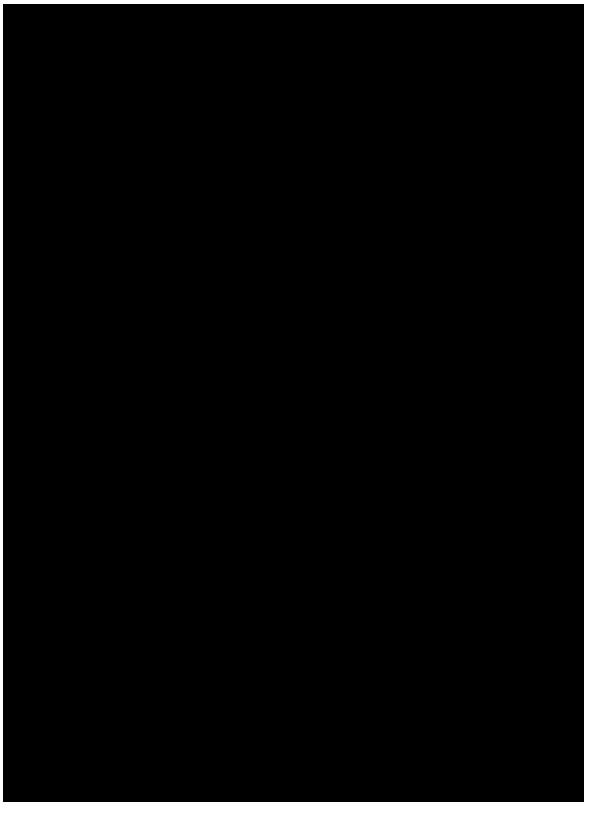

Figure 1.

Thus, both the number of the factors, and also some particular factors could predict therapy efficacy or resistance of the patents to the drug.The second aim of our study was to look at MDR evolution in the course of AP CML treatment with imatinib. We used P-glycoprotein (Pgp) as a marker of MDR. Our data presented here show that almost 40% of AP CML patients express functional Pgp in the PB cells before imatinib treatment. P-glycoprotein (Pgp)-positivity increased in the course of the therapy of AP CML patients with imatinib, more than 80% of the patients became Pgp positive by 6-12 months of imatinib treatment. Within the period between 3-12 months of imatinib treatment there was a good correlation between Pgp-positivity and elevated Rh123 efflux. Patients treat-

ed for a longer time with imatinib demonstrated lack of this correlation. *Conclusions*. Our data show that this phenomenon could be connected with concomitant expression of several ABC transporters by the cells of the patients. We suppose that imatinib selects cell variants expressing several ABC transporters and thus selects primitive leukemic stem cells.

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MYELOABLATIVE VERSUS NON-MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ADVANCED ACUTE MYELOID LEUKEMIA OR MYELODYSPLASTIC SYNDROMES: A RETROSPECTIVE ANALYSIS

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Background. In nonmyeloablative hematopoietic stem cell transplantation (HSCT), graft-versus-leukemia effects have replaced high-dose cytotoxic therapy as the conceptual basis for treating underlying malignancies. Results in patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS) raised the possibility that the advantage obtained with reduced non-relapse mortality might be abrogated by graft failure and disease progression. The advantages and disadvantages of conventional and reduced-intensity conditioning must be analyzed carefully. *Aims*. We analyzed the transplantation outcomes in patients with AML or MDS in advanced status according to the conditioning regimen intensity. *Methods*. Transplantation outcomes were analyzed in patients with AML or MDS. A total of 22 patients receiving nonmyeloablative stem cell transplantation (NST) were compared with eleven patients receiving myeloablative transplantation. All patients had advanced disease at the time of transplantation, and 9 patients had previously received more than one stem cell transplantation. Cyclosporine or FK506 was used for graft-versus-host disease (GVHD) prophylaxis. *Results*. There were no statistical difference in age (median age 37.5 vs 36.0 years, p=0.715) and previous mean number of chemotherapy (2.6 vs 1.6, p=0.086) between the non-myeloablative and myeloablative conditioning group. Patients receiving NST more likely had received previous transplantation (47% vs 22%, p=0.407). The cumulative risk of graft failure (4.5% for NST, 0% for myeloablative; p=0.602) or relapse rate (18.2% for NST, 27.3% for myeloablative; p=0.260) was not different according to conditioning regimen. However, the non-relapse transplantation-related mortality was significantly higher in myeloablative transplantation (30%) compared to NST (11.5%, p=0.015). Overall survival at 2 years was 62% for NST and 22% for myeloablative transplantation (p=0.009). Event-free survival at 2 years was also significantly higher in NST compared to myeloablative transplantation (p=0.014). *Conclusions*. These results suggest that NST is a reasonable alternative for patients with advanced AML or MDS at high risk for relapse and transplantation-related complications.

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IN VITRO EFFECTS OF KETOLIDE TELITHROMYCIN ON SOME HOST CELLS' FUNCTIONS

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Background. A 10-day treatment period of exacerbation of asthma with the semisynthetic macrolide derivative telithromycin causes improvement in FEV1, even in patients who don't meet the criteria for infection with M. pneumoniae and C. pneumoniae (Johnston *et al.*, N Engl J Med 2006; 354: 1589-600). However, the mechanisms by which these effects occur remain unclear. *Aims* . The aim of this study is to evaluate the *in vitro* effects of telithromycin on some functions of neutrophils, monocytes and lymphocytes. *Methods*. On 15 samples of normal heparinized human blood we assessed chemotaxis, phagocytic capacity, oxidative burst of neutrophils and monocytes by flow cytometry; the activation of mitogen stimulated T lymphocytes using flow cytometry analysis of surface markers CD69, CD25, HLA-DR, and ELISA detection of gamma-interferon and IL-4 in the supernatant of stimulated cells culture; the basophils' activation with a cytometric method for the evaluation of CD63 expression after anti-IgE and IL-3 stimulation. Each test was done in triplicate in the absence or presence of 1,2 ng of telithromycin per microliter. *Results*. In the samples preincubated with telithromycin we observed a reduction of 54% in the neutrophils' phagocytosis and a reduction of 70% of basophils' activation as compared with the tests performed in the absence of the antibiotic (p< 0.001); no significant differences were observed in all the other tests performed. *Summary/Conclusions.* These findings demonstrate that telithromycin, like others related macrolide antibiotics, has immunomodulatory effects which may enhance clinical care in some airway disorders.

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MONONUCLEOTIDE MARKERS ARE CONTINUOUSLY STABLE IN GASTRIC MALT LYMPHOMAS AFTER H.PYLORI ERADICATION THERAPY AND CHEMOTHERAPY

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Background. Microsatellite Instability (MSI) has been correlated with chronic infection and neoplasia. In recent years a new term named Elevated Microsatellites at Selected Tetranucleotides (EMAST) has been introduced for selective MSI at tri- or tetranucleotide repeats, pinpointing to the discrete role of the responsible repair mechanisms for those microsatellites. MALT lymphomas represent a paradigm of chronic infection and progression to neoplasia, and studies for the presence of MSI in this disease entity have given conflicting results. Mononucleotide markers seem to be more stable than tri-/tetranucleotides, but it remains unclear if any kind of disease treatment leads to the development of mononucleotide MSI. *Aim*. To investigate the occurrence of mononucleotide MSI in early staged gastric MALT lymphoma patients at diagnosis and after treatment. *Methods*. We studied gastric whole biopsies from 10 patients with diagnosed MALT lymphoma and H. pylori infection at diagnosis using 5 different mononucleotides markers. MSI was defined as a frame shift or appearance of novel bands in at least 2/5 mononucleotide markers. In 4 of the patients we had serial biopsies after completion of eradication therapy and after chemotherapy. We compared our results with similar studies having used either mono- or tri-/tetranucleotide markers. *Results*. All patients received H. pylori eradication therapy. None of the analyzed biopsies of any of the 10 patients at the time of diagnosis or the 4 patients with serial biopsies showed MSI. *Conclusion*. Mononucleotide markers are continuously stable at the course of MALT disease, meaning that the responsible repair mechanisms are fully functional. Further research should be focused on tri-/tetranucleotide MSI (EMAST) to elucidate its role in the evolution in the MALT lymphoma.

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NATURAL KILLER CELL COUNT AND ACTIVITY, PERFORIN EXPRESSION, FAS AND SOLUBLE FAS LIGAND LEVELS IN IMMUNOCOMPETENT CHILDREN WITH VARICELLA ZOSTER VIRUS INFECTION

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Background. Varicella is a common childhood infection and natural killer (NK) cells are involved in direct killing of virally infected cells via apoptosis with perforin-granzyme or Fas-Fas ligand pathways. Since varicella zoster virus (VZV) vaccination is not routinely applied in Turkey, epidemics could be observed in wintertime. *Aim*. The aim of this study is to determine NK cell count and activity, perforin expression, Fas and soluble Fas ligand levels in immunocompetent children with VZV infection and define any possible relations between the levels and development of varicella complications. *Methods*. Forthy immunocompetent children between 1-16 years of age with VZV infection who presented within the first three days of the onset of symtoms were included in the study. Hemogram, peripheral blood smear, NK cell count, NK cell activity, perforin expression, Fas and soluble Fas ligand were examined in admission (day 0) and 15 days later. The same laboratory examinations were made also among 39 healthy controls with no infection. NK cell count, perforin expression and Fas measurements were made by flow cytometric analysis. Soluble Fas ligand was measured by ELISA. NK cell activity was measured by flow cytometry as K562 cell binding of NK cells. *Results*. Hemoglobin, leukocyte and thrombocytes were significantly lower on day 0, when compared to day 15. Thrombocytopenia was present in two patients. Lymphopenia was observed in 32% of the patients. NK cell count, NK cell activity and perforin expression levels were significantly lower on day 0, when compared to day 15 levels. A positive correlation was present between day 0 NK cell count and perforin expression. Fas and soluble Fas ligand levels were found to be higher on day 0, when compared to day 15 levels. The studied parameters of control group were similar to 15th day results of VZV group. *Summary/Conclusions*. The lower hemoglobin, leukocyte and thrombocyte levels on day 0 when compared to day 15 may indicate the myelosuppressive effect of VZV. Lower NK cell count and NK cell activity have been reported previously. In our study, no severe VZV assosciated complication developed and this may indicate that the defect in NK cells may be

mild. Fas and soluble Fas ligand levels were found to be higher on day 0, when compared to day 15 levels and this finding suggests that Fas-Fas ligand apototic pathway is active during the acute phase of the VZV infection and remits after recovery of illness. NK cell count and activity, perforin expression, Fas and soluble Fas ligand levels will be analyzed in future studies in severely complicated VZV infected patients.

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CIRCUMCISION IN PATIENTS WITH HEMOPHILIA: TEN YEARS EXPERIENCE IN ADANA, TURKEY

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Fiftyty-one boys with hemophilia (age range, 6 months to 18 years), 37 (72,5%) with hemophilia A and 14 (27,5%) with hemophilia B were circumcised in Cukurova University, Faculty of Medicine, Department of Pediatric Hematology, between 1997 and 2007. Two of children with hemophilia have inhibitors. 12 (23,5%) cases were mild, 24 (47,0%) cases were moderate and 15 (29,5%) cases were severe hemophilia. After starting systemic prophylaxis including factor substitution and DDAVP (desmopressin acetate) in for reducing factor doses, 5 patients underwent circumcision under local anesthesia and 46 patients underwent circumcision under general anesthesia. All patients were given tranexamic acid. Duration of the hospitalization period was 2 to 7 days according to whether or not complication. Transient minimal bleeding was observed in 23 (45%) patients and easily responded to factor administration. No life treatening hemorrahges were observed. Minimal local edema and hyperemia along the excision line was observed in all patients. The objective of this retrospective study was to reported the experience of circumcision in patients with hemophilia in the Cukurova University, Faculty of Medicine, Department of Pediatric Hematology.

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INTENSIFIED IMMUNOCHEMOTHERAPY WITH HIGH DOSE CONSOLIDATION AND AUTOLOGOUS STEM CELL RESCUE IN MANTLE CELL LYMPHOMA

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Background. Mantel cell lymphoma (MCL) is an aggressive B- cell lymphoma which is characterised by early dissemination and unfavourable clinical course. The implementation of autologous stem cell transplantation (mid 1990s) extended the therapeutic options for this poor- risk subtype. Particularly the intensification of the chemotherapeutic regimen and the combination with the monoclonal antibody Rituximab led to sustained disease control. We evaluated the efficacy of an intensified immunochemotherapy combined with high-dose therapy (HDT) and autologous stem cell rescue (ASCT) in patients with mantle cell lymphoma. *Methods*. Since 1995 14 out of 25 consecutive patients with newly diagnosed or pre-treated MCL of stage III-IV were eligible for HDT followed by autologous stem cell transplantation. Exclusion criteria for the remaining 11 patients were age over 70 (6), resistance to chemotherapy (2), severe comorbidity (apoplexia (1), chronic atrial fibrillation(1)) and recurrent infectious complications (tuberculosis (1)). Induction therapy consisted of Rituximab (375 mg/m^2; d1) plus CHOP-21 for 4-6 cycles followed by intensification (and priming) chemotherapy for 2 to 3 cycles of CLAEG (3 days Cladribine 0,2 mg/kg, ARA- C 1,5 g/mÇ, Etoposid 60 mg/m^2) and on day 1 Daunoxome 80 mg/m^2 or Idarubicine 8 mg/m^2. Autologous stem cell transplantation was performed at a median of 9 (7-13) months from diagnosis with 11 primary patients and 3 patients in relapsed disease status. In addition to high dose conditioning chemotherapy (BEAM) 4 doses of Rituximab (d -9, -1, +48 and +55) were administered. *Results*. Fourteen Patients (6 female, 8 male) at a median age of 57 (49- 66) years were treated. 5 reached CR, 4 VGPR and 5 PR before receiving ASCT. In 4 patients Rituximab at day +48 and +55 was cancelled due to complications such as pneumonia (2), herpes zoster (1) or exanthema (recurrent folliculitis/ erythema like lesions) (1). A median number of 5,64 ×10^6 /kg CD 34 positive cells (1,65-29,5) were reinfused. The engraftment was generally prompt and durable (granulocytes > 0,5 G/l day +10 (9-11), platelets > 50 G/l day +14 (10-17)). Only in one case (male patient, age at TX: 55 years) a delayed regeneration (d +140; granulocytes > 0,5 G/L +11, platelets day +21) was observed. Side effects of transplantation were mucositis grade I- II in 12 patients, 4 suffered from emesis grade II- III and 4 from enteritis grade I- II. Fever of unknown origin occurred in 4, septicaemia in 2 and pneumonia in 2. Grade 4 toxicities and treatment related deaths were not observed. Following transplantation all patients reached clinical and molecular CR. 12 patients are still in CCR with a median observation period of 33 months (5-58). 2 patients relapsed after 17 and 26 months and died 58 and 34 months after transplantation. As late infectious complications 3 patients developed pneumonia and 3 herpes zoster. A propagation of erythema migrans and sarcoidosis was seen in 1 patient each. *Conclusions*. R- CHOP intensified by high dose treatment (CLAEG- D or CLAEG- Ida) including ASCT using BEAM regimen and Rituximab is accompanied by acceptable toxicity and improves outcome of patients with MCL, allowing long-term disease control.

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RESULTS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA TREATMENT USING EORTC 58881 AND EORTC 58951 PROTOCOLS

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Childhood acute lymphoblastic leukemia (ALL) results have improved over time. The current results of the Department of Hematology in treating childhood ALL are being reported. From 1/1995 to 12/2004, 135 children (86 boys and 49 girls) with ALL have been treated with an ALL-EORTC 58881 (N=102 patients) or ALL-EORTC 58951 based protocol (since 1/2002, N=33 patients). Median age of diagnosis was 5,1 years (range, 1-15 years). The median WBC count at diagnosis was 22.3×10^9/L (range 1.2×10^9/L-1420.0×10^9/L). Immunophenotype done in only 60 patients revealed 45 B-cell precursor (40 CD10$^+$, 5 CD10$^-$) and 15 T-cell ALL. Cytogenetic analysis was performed for 105 patients and revealed abnormalities in 47%. Persistent circulating leukemic blasts more than 1.0×10^9/L were present at day 8 in 22 patients (16%). Compared with the *blast-negative* group, these patients had a significantly higher frequency of several adverse clinical features (WBC >50×10^9/L, mediastinal mas, T-cell phenotype) and a significantly poorer prognosis (overall survival at 5 years 30% vs 70% for *blast-negative* group. Relapses were more frequent after EORTC-58881 particularly in *blast-positive* group. We conclude that the EORTC-based protocol as applied in our Department is tolerable with acceptable toxicity and that the modifications made to the protocol by using EORTC 58951 improved the prognosis of patients with poor prognosis particularly T-cell leukaemia and *blast-positive* group. Longer follow-up is necessary to further validate the results and credit of value the modifications made to the protocol.

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POST ASCT RITUXIMAB CONSOLIDATION THERAPY ELIMINATES PERSISTING FDG PET POSITIVITY IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) PATIENTS

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Background. there is evidence in the literature that combination of ASCT and rituximab is beneficial for DLBCL patients but the best treatment plan has not been established yet. *Aims*. to characterize post ASCT minimal residual disease by FDG PET scan and the effect of post ASCT rituximab consolidation therapy on overall survival. *Patients*. between 04/2005-08/2006 16 consecutive DLBCL patients underwent autologous stem cell transplantation in two transplant centers . Transplant indication was relapse after conventional R-CHOP treatment, except for two pts who were transplanted in CR1 because of high risk disease. The median age was 50 years (19-60), female/male ratio was 8/8. All patients received rituximab containing salvage treatment (R-DHAP, RIME, R-GEMP) resulted in complete remission proved by CT scan in 12/16 cases. Conditioning regimen consisted of BEAM, the graft contained median 5.62×10^6 (3.77-11.41) CD34$^+$ cells. One patient died of sepsis. FDG PET scan was performed two months after SCT. 3 months after SCT patients received standard dose (375 mg/m^2) rituximab in four consecutive weeks. *Results*. unfortunately 2 patients relapsed within 2 months following SCT. Rituximab administration had no additive toxicity. 3/13 patients showed FDG PET positivity with low tumor burden indicating minimal residual disease. 3 months post rituximab treatment FDG PET scan turned to negative in all the previously positive (3/3) cases. 13/16 (81%) patients are in complete remission at a median 12 months (6-20) follow up time. *Conclusions*. post SCT rituximab may control low tumor burden without additive toxicity in DLBCL patients. Our data needs further confirmation especially regard-

ing the question weather all or only FDG PET positive patients should receive post-transplant rituximab consolidation.

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ADOPTIVE TRANSFER OF CMV-SPECIFIC T-CELLS SELECTED FOR IFN-GAMMA SECRETION BY MAGNETIC LABELING: A CLOSER LOOK ON THE SELECTED CELLS

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Background. The reactivation of Cytomegalovirus (CMV) and other viruses is one of the major complications after (stem cell) transplantation. In an immuncompromised host recurrent CMV infections occur, due to the lack of antigen-specific T-cells, if T-cell depleted grafts or immunosuppressive therapy is administered. Despite the possibility of treating recurrent CMV infections with antiviral drugs, control of reactivation is not always successful with medication. Repeated treatment may lead to resistant virus strains and high doses of e.g. ganciclovir or foscarnet can lead to severe side effects like graft rejection/loss or renal failure. The adoptive transfer of antigen-specific T-cells has been investigated over the past 12 years, initially using T-cell clones, more recently by transfer of specific T-cell lines. *Aims*. Here we present preclinical data using magnetic selection for IFN-γ producing cells after stimulation with antigen. For enrichment procedures that can be up-scaled for later production of clinical grade samples different media were tested and the stimulation of the T-cells was optimised. The enriched cell fraction can contain different cell types, since enrichment is based solely on the capacity of cells to secret IFN-γ. *Methods*. Peripheral blood mononuclear cells (PBMNC) of healthy, HLA-typed, CMV-seropositive blood donors were stimulated over night using recombinant viral protein pp65 and/or whole viral lysate (strain AD169). For assessment of different media (one standard medium, two GMP-grade media), cell viability, stimulation rates and expansion of IFN-γ secreting fractions were checked. Furthermore, cryopreserved material was retested in two different assays (magnetic selection and intracellular staining). To check the formation of the IFN-γ secreting population, cells were stained for CD3, CD8, CD4, CD14, CD16, CD19, CD20, CD25, CD56 and IFN-γ in different steps of the assay. *Results*. Both GMP-grade media showed similar performance. Retesting of cryopreserved cells revealed high variability in percentage of IFN-γ secreting cells using a secretion assay, while intracellular staining led to results comparable with the secretion assay when using fresh cells. Testing of IFN-γ secreting populations is still ongoing. The recombinant pp65 usually leads to preferential activation of $CD8^+$ cells, while lysate rather activates $CD4^+$ cells. Usage of pp65 as antigene alone will not lead to a feasible stimulation rate in all cases, this may be an HLA-dependant effect. *Conclusions*. Adoptive transfer of CMV-specific T-cells can be the only cure for patients with recurrent drug-resistant CMV-reactivations. Though the benefits of this technique are obvious, there are risks like transfer of alloreactive T-cells. To minimize the risk of this treatment and optimise performance of selected T-cells, knowing the exact formation of the enriched fraction is crucial. FACS-based assays like antibody staining or proliferation testing are suitable for quality testing of cells prior to transfusion. Furthermore stimulation rates and applicability of the assay could be upgraded by usage of a protein cocktail instead of single proteins. Therefore cloning, expression and stimulation testing of several viral proteins has been started in our institution.

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BONE MARROW MICROCASCULAR DENSITY AND FIBROSIS ARE RELATED IN POLYCYTHAEMIA VERA

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The bone marrow microvessel density (MVD) has been shown to be increased in various hematologic malignancies and is correlated with unfavorable prognosis in multiple myeloma. There are evidences of augmented angiogenetic process in polycythaemia vera (PV) either by angiogenic factors measurements or by MVD bone marrow count estimation. The aim of this study was to evaluate the correlation of bone marrow microvascular density and bone marrow fibrosis in patients with PV. A total of 24 patients with PV (mean age 55,6±13,4 years) and of 10 propositus (control group) with no hematologic malignancy were included to the study. The bone marrow slides were prepared from paraffin-embedded blocks. The endothelial antigen of choice was CD34 which has been found superior to other antigens that have been used in literature. The MVD was estimated by visual microvessel grading at 400x (HPF) by two of the authors. The MVD in all patients was found 4,2±0,2 vessels per HPF and there was no correlation with age, time from diagnosis, spleen enlargement and treatment. The MVD in the control group was found statistically significantly lower 1,6±0,2 vessels per HPF (p=0,00002). We divided the group of patients in two subgroups according to the grade of fibrosis (grade 0-1 and grade 1-2) of their bone marrows. In the first subgroup we found 3,4±0,9 vessels per HPF while in the second subgroup we found 5,3±2,1 vessels per HPF. The difference among the two groups is statistically significant (p=0,005) and we found a positive correlation between MVD and reticulin fibrosis grade in patients with PV (r=0,59, p=0,0025). We also measured the angiogenetic factors that are implicated in fibroblast proliferation, bFGF (basic Fibroblast Growth Factor) and VEGF (Vascular Endothelial Growth Factor), in the sera of the two groups of PV patents. We found no statistically significant difference among the two subgroups (p=0,42 and p=0,84) respectively. Even though we found no difference among serum angiogenetic growth factors while similar cytokine mediated effect has been found in myelofibrosis with myeloid metaplasia, the evidences of augmented MVD count in PV patients with higher grade of fibrosis in comparison to PV patients with lower grade of fibrosis, could be explained by the hypothesis that the abnormal angiogenic cytokine milieu acts stimulating fibroblasts enhancing fibrosis.The possible prognostic relevance of these findings deserves further research.

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MANAGEMENT OF GRAM- POSITIVE INFECTIONS WITH LINEZOLID IN IMMUNOCOMPROMIZED PATIENTS

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Infections constitute an important cause of morbidity and mortality in immunocompromized patients. Antibiotic-resistant strains have become a common problem in every-day practice. Linezolid, an oxazolidone, inhibits protein synthesis in bacteria with a unique mechanism of action and is active against resistant strains. We assessed the efficacy and safety of linezolid in the management of 106 documented Gram-positive infections in 87 immunocompromised patients. Forty two women and 45 men were included, aged 8-73 (median 36) years, suffering from: acute leukemia: 58; lymphoma, 14; Hodgkin's disease, 6; aplastic anemia, 2; multiple myeloma, 5; and CML 2 patients. Forty one patients had undergone stem cell transplantation (12 autologous, 29 allogeneic). Seventy three 73 infections occurred during neutropenia. Linezolid was administered at a dose of 600 mg twice daily for 4-26 (median 10) days. Parenteral linezolid was switched to the oral formulation in 36 patients. Most patients had already been treated unsuccessfully with an antibiotic against $Gram^+$ bacteria (64 with teicoplanine, 14 with vancomycin), while in 28 linezolid was administered as first line therapy, based on sample culture. The most common infection was bacteremia (n=45) caused by methicilline-resistant staphylococcus and associated with the presence of a central line catheter in 35 cases. Infections caused by enterococcus were: urinary, 22; soft tissue, 8; respiratory, 6; bacteremia, 6. Twenty two of 42 strains (52%) were resistant to vancomycin/ teicoplanine. The clinical response rate was 78% (83/ 106) and microbiological response rate was 82% (79/96). The most common toxicity was hepatic (increased liver function tests: 8 patients), while there was no discontinuation due to intolerance. On multivariate analysis, the factors predicting for treatment failure where: refractory disease, prior transplantation and co-infection by $Gram^-$ pathogens. In conclusion, linezolid administration was safe and effective in this group of immunocompromised patients. Linezolid played a significant role in sterilizing the central line catheters and in the management of vancomycin resistant enterococci.

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POSACONAZOLE THERAPY IN REFRACTORY INVASIVE CANDIDIASIS IN AN AML PATIENT: A CASE REPORT

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Background. The incidence of systemic mycoses has increased, due to improved survival among patients with impaired immunity. The mortality rates associated with invasive fungal infections is high, in part because, the diagnostics of these infections are difficult which leads to treatment delay. Although there is an increasing range of antimycotic drugs available, there are problems with tolerability and limited knowledge regarding effectivity of combination therapy. *Aim*. We report the case of a 27 year old man with AML t(8;21), thus a favourable risk group of AML, who after final consolidation chemotherapy was diagnosed with invasive candidiasis of the liver, spleen and kidneys. *Case report*. In February 2003 a formerly healthy 27 year old man presented at our clinic with AML t(8;21), which was treated according to local guidelines. Complete remission was obtained after induction therapy. Prophylactic fluconazole was given orally during induction and first consolidation therapy. After final consolidation treatment, the patient developed a severe neutropenia and persistent fever. Candida albicans was isolated from mouth swabs and faeces. CT scans showed multiple, rounded infiltrates in the spleen, liver and kidneys. Collectively, these findings led to the diagnosis of probable fungal infection. The patient was initially treated with liposomal amphotericin B for a month, then switched to voriconazole orally for four months, with the addition of caspofungin during the last month. Despite six months of antifungal treatment, the patient had persistant fever and increased levels of C-reactive protein. Hence a diagnostic splenectomy was performed which confirmed an invasive Candida albicans infection by immunohistology and Candida PCR of the splenic tissue. However, since viable Candida was not isolated, antimycotic resistance determination could not be done. During the coming eleven months, different combinations of voriconazole, caspofungin, itraconazole, fluconazole, liposomal amphotericin B and micafungin had no or minimal effect on the infection; C-reactive protein levels remained high and fever persisted. The patient experienced side effects of the drugs such as neuropathy and severe hypokalemia. After seventeen months of treatment attempts with various antifungals the infection was still not under control. A new azole, posaconazole became available and was introduced. There was a prompt effect on the fever and the levels of C-reactive protein. Control CT scan three months later showed a discrete regression of the infiltrates in the liver. After six months of posaconazole the patient started to work part time. Follow-up CT scans and PET scans showed successive clearing of infiltrates. After nineteen months of posaconazole, the treatment could be discontinued. It is now four years after AML diagnosis, and seven months after fungal treatment was terminated, and the patient works full time. *Summary*. Invasive fungal infection is a diagnostic and therapeutic dilemma for the haematologist. In this case the candida infection was diagnosed by splenectomy. Clinically resistant to several antifungal drugs, finally the new azole posaconazole resolved the infection of this patient.

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CYTOKINETIC STUDIES IN MULTIPLE MYELOMA: PROLIFERATION AND APOPTOSIS IN EARLY RESPONDERS

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Background. Proliferative (propidium-iodide, PC-PI/CD138) and apoptotic (annexin-V, PC-AI/CD138) indices have a very close relation to prognosis of multiple myeloma. High levels of proliferation and/or low values of apoptosis predict groups of patients with poor prognosis. In our study, we examined these cytokinetic parameters also within the group of early responders, that is considered being a prognostically infavourable group itself. *Methods*. Analysed group consists of 125 patients with multiple myeloma, all evaluated at the time of diagnosis, before the start of therapy, devided into two subgroups-late responders (90 patients, 72%) and early responders (35 patients, 28%). Early response was interpreted as objective response within one month of therapy (i.e. CR and PR according to EBMT criteria-decrease of MIG more than 50%, more than 90% decrease of proteinuria). Therapy regimens used included only conventional chemotherapy (regimens MP, VBMCP,VAD, CyVAD and CIDEX), patients treated with HD therapy with the support of autologous stem cell transplantation and patients treated with novel agents (thalidomide, bortezomib) were not included in this group. Within the subgroup of early responders, curves of overall survival were constructed according to different values of proliferative and apoptotic indices. Proliferative activity of plasma cells was measured using propidium iodide index (PC-PI /CD138), rate of apoptosis using annexin-V index (PC-AI/CD138), followed by method of flow-cytometry (DNA-Prep Reagents Kit, Coulter, Software Multicycle fy. Phoenix). For statistical estimation non-parametric Mann-Whitney test, Kaplan-Meier and log rank test were used. *Results*. In the whole group of patients, there was a very small difference in curves of overall survival (OS) in both of the groups (early and late responders), favorising slightly the late responders, however without statistical significance (p=0,291). If we compared the levels of propidium-iodide (proliferative) index in both these groups, there was no difference, either (p=0,733). There was a difference between the values of apoptotic index, with higher levels of apoptosis within the group of early responders, on the border of statistical significance (M 4,8-4,3, p=0,061). In the subgroup of early responders, the OS regarding the levels of both, the proliferative and apoptotic indices was not significantly different. *Conclusions*. Evaluation of cytokinetic parameters (proliferation and apoptosis) is a useful method for determination of prognosis in multiple myeloma patients. High levels of apoptosis itself (but not proliferation) may moreover identify a group of patients with early response. These observations suggest a possible relation of apoptosis levels to the sensitivity of multiple myeloma to therapy. Within the evaluated group, there was no significant difference in the overall survival in late and early responders, and also the evaluation of proliferation and apoptosis within this group only, did not bring any further prognostic information.

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APOPTOSIS RELATED PROTEINS PATTERNS IN MYELODYSPLASTIC SYNDROMES STRATIFIED ACCORDING TO INTERNATIONAL PROGNOSTIC SCORING SYSTEM (IPSS) AND COMPARED TO ACUTE MYELOID LEUKAEMIA

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Introduction. The myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by an increased risk of leukemic transformation. The excessive apoptosis of progenitor cells contributes to the ineffective haematopoiesis in MDS whereas leukemic progression arises through abrogation of apoptotic control, allowing long survival and expansion of neoplastic clones. The outcome and transformation risk of MDS is currently defined by the International Prognostic Scoring System (IPSS). Whether a correlation exists between key apoptosis proteins with IPSS of MDS and leukaemia development is undetermined. The aim of the present study was to evaluate the intracellular quantity of proteins with key role in apoptosis - active Caspase-3, bcl-2 and cleaved PARP, in patients with MDS in correlation with IPSS score and to compare data with acute myeloid leukemia (AML) samples. *Patients and Methods*. Cell lysates from bone marrow were analyzed in 36 patients: 15 patients with MDS [Refractory anemia (n=1), Refractory anemia with ringed sideroblasts (n=4), Refractory cytopenia with/without trilineage dysplasia (n=4), Refractory anemia with excess of blasts, RAEB (n=6)] and 21 patients with AML, diagnosed and subclassified according to WHO criteria. IPSS for MDS patients was determined. The levels of aCaspase-3, bcl-2 and cPARP were measured by flow cytometry using a CBA-based platform (BD, USA). The expression of MLF1 gene was defined by RT-PCR. *Results*. Flow cytometric data showed heterogeneous patterns of intracellular levels of the studied proteins in MDS patients. The mean values for aCaspase-3 (115,74±189,02 U/mL) and bcl-2 (984,88±932.50 U/mL), showed a significant correlation with cPARPs (24,59±29.97 U/mL) (R=0,67, p=0.006 and R=0,53, p=0.04, respectively). The patients were stratified according to IPSS. Four low risk (LR), 5 intermediate risk (IR)-1, 3 IR-2 and 3 high risk (HR) patients were identified. Analysis revealed marked differences in the mean values of the parameters when LR patients were compared to IR/HR. LR patients were characterized with lower levels of aCaspase-3 (38,45±57,69 U/mL); cPARPs (14,20±19,94 U/mL), and bcl-2 (453,62±381,47 U/mL), while in IR/HR patients higher caspase activity (143,85±213,92 U/mL) and cPARPs (28,37±32,84 U/mL), in parallel with the activation of anti-apoptotic mechanisms: bcl-2 (1178,07±1009,85 U/mL), were observed. In comparison, AML patients showed significant increase of bcl-2 levels (3140,94±1998 U/mL), which inversely correlated with lower aCaspase (29,31±48,30 U/mL) (R=-0,42, p=0.01) with comparable levels of cPARPs (30,80±30,65 U/mL). A group of patients with AML/multilineage dysplasia and bone marrow blast cells <33% were identified, who showed 4-

fold higher mean levels of aCaspase-3 (107 U/mL) in comparison to all AMLs, while the levels of bcl-2 (744 U/mL) and ÒPARP (10.17 U/mL) were relatively low: a profile similar to observed in RAEB. Interestingly, the molecular analysis showed MLF1 mRNA expression in these cases. This finding might provide some evidence that at least some of AMLs result from the leukaemic transformation of a preceding undiagnosed MDS. *Conclusions*. By dividing MDS according to IPSS, definite patterns of key apoptosis related proteins can be identified. Intermediate and high risk MDS groups are associated with the parallel activation of apoptotic and anti-apoptotic mechanisms. The dysregulation of normal balance of cell death and survival might be one of the mechanisms of disease progression. Besides, although AML development is accompanied by a fall in pro-apoptotic versus anti-apoptotic parameters, the heterogeneity in the examined protein patterns indicates that additional factors might play a role in leukaemic pathogenesis and warrants further prospective studies. Acknowledgements: Study funded by the National Fund, Bulgarian Ministry of Education and Science.

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ENGRAFTMENT OF FLUORESCENTLY-LABELED BONE MARROW STROMAL CELLS INTO THE CEREBRAL CORTEX INJURY SITE IN THE RAT

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Backround. In recent years there has been an increasing interest in bone marrow stromal cells (BMSCs, also termed mesenchymal stem cells) due to their potential therapeutic function, also connected with the repair of central nervous system injury. Initial preclinical studies demonstrated the ability of BMSCs to migrate, engraft into the damaged tissue and differentiate into residential-like cells. *Aims*. The present study was undertaken to demonstrate the engraftment capacity and survival of BMSCs administrated intralesionaly after cerebral cortex injury. *Methods*. The experiment was carried out on female Wistar rats (all experimental procedures have been approved by the Local Bioethical Committee). Mechanical injury was induced with a drill in the left cerebral cortex. BMSCs (2×10^6/10 µL PBS)were administrated directly to the lesion site immediately after injury. Control group received the same volume of PBS. BMSCs were isolated from allogeneic bone marrow aspirate obtained from rat femurs and cultured for 14 days in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum and 120U/mL penicillin. 24 h prior to transplantation bis-benzimide (Hoechst 33342) was added to the medium in order to fluorescently label the BMSCs nuclei. Brain tissue was processed for preparation of sections, which were used for observation in fluorescence microscope. Cultured cells were observed under fluorescence and light microscope with Nomarsky contrast. *Results*. After 14 days of culture the cells approached confluency and at least three morphologically different types of cells could be seen: spindle-shaped cells, large flat cells and small round cells. Bis-benzimide labeled nuclei of almost all BMSCs. After intralesional administration large number of BMSCs was observed in the lumen of injury and also in the surrounding parenchyma 2 days post injury and were still visible after 30 days. Some BMSCs were found in contact with vessel walls. Engrafted cells were preferentially localized to the glial scar on day 30. BMSCs were not seen in the contrlateral hemisphere. *Conclusion*. Transplanted BMSCs engraft in the parenchyma of the injured brain and survive in the microenvironment of the injured tissue at least 30 days after intralesional administration. Long-term survival of these cells in the area of injury and their presence in the site of glial scar suggest that BMSCs are actively involved in the healing processes of the damaged brain.

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RISK FACTORS FOR BACTERAEMIA CAUSED BY EXTENDED-SPECTRUM β-LACTAMASE PRODUCING-ESCHERICHIA COLI IN HOSPITALIZED HAEMATOLOGICAL PATIENTS

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Introduction. Bacteraemia caused by extended-spectrum β-lactamase (ESBL) producing-E. coli has growing incidence and limited therapeutic options. The study of the risk factors for its development has not been patient-population stratified. The aim of this study is to describe the risk factors for the onset of bacteraemia by ESBL producing-E. coli (ESBL-EC) in the adult hospitalized haematological setting. *Material and methods*. Design: prospective study of cases and controls with two control groups between February 2005 to May 2006. Case definition: haematological inpatient with ESBL-EC bacteraemia. Control definition: group A: haematological inpatient with bacteraemia by E. coli not producting ESBL (1:3); group B: haematological inpatient without bacteraemia immediately hospitalized after one case (1:4). The following variables were analyzed: age, sex, type (urgent/programmed) of hospitalization, haematological disease, chemotherapy, central venous catheter, antibacterial prophylaxis, GCS-F administration, antibiotic treatment and neutropenia. Clonal relationship of the isolates was done using REP-PCR. Univariate and multivariate analysis of the risk factors were performed. *Results*. One hundred and twelve episodes of bacteraemia occurred: isolates were E. coli in 29 (26%) cases and ESBL-EC in 9 (31%) cases. There were not difference in the basal characteristics of cases (n=9) and controls of the groups A (n=20) and B (n=36), regarding to age, sex, and type hospitalization. ESBL-EC strains were polyclonal. The univariate analysis showed the following variables to be more frequent in the cases than in the group A of controls: diagnosis of acute leukemia (89% vs. 50%, $p<0.001$). *Conclusions*. 1. The rate of bacteraemia caused by extended-spectrum β-lactamase producing-E. coli was very high. 2. Previous profound neutropenia is an independent risk factor for bacteraemia caused by extended-spectrum β-lactamase producing-E. coli in hospitalized haematological patients.

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CONGENITAL FACTOR X DEFICIENCY: CASE REPORT

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Factor X (FX) deficiency is a rare disorder, with only 1 in 500 000 people affected. An autosomal recessive trait on chromosome 13 can cause varying degrees of severity. In this case report, we reported clinical findings and management of two cases children with congenital FX deficiency. Case I: A 3-year-old female presented at the age of 2 years with a FX level of < 1%.She had frequent epistaxis and oral bleeding. She had anaemia due to his frequent bleeding episodes and required red blood cell transfusion on one occasion. After two months she suffered to retroorbital hematoma, and after 4 months she was administered to hematoma in the back that were the result of trauma. Her bleeding episodes were always treated with activated prothrombin complex concentrates, FEIBA(Anti-Inhibitor Coagulant Complex, contains FXa, Baxter Healthcare) and fresh frosen plasma infusion. She had no bleeding or thromboembolic complications the duration of treatment. Case II:A 3-year-old male diagnosed with mild FX deficiency was referred for the operation of tonsillectomy and adenoidectomy to our hospital. This patient had a FX level of 16% at presentation.He was managed successfully with FEIBA during surgery. He had no bleeding or thromboembolic complications after surgery.

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CYTOPLASMIC LOCALIZATION OF NUCLEAR FACTOR-KAPPA B IN MULTIPLE MYELOMA CELLS

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Background. Nuclear factor-kappa B (NF-κB) is multifunctional transcription factor that regulates different signal transduction pathways such as cell survival and proliferation. A number of tumors display activated NF-κB, which contributes to promote cancer cell growth and resistance to chemotherapeutic drugs. NF-κB has been shown to be constitutively active in multiple myeloma (MM) cells, resulting in increased expression of Bcl-xL and IL-6. In addition NF-κB has an important role in regulation of IL-6 transcription in bone marrow stromal cells (BMSCs). *Aims*. we analyzed the localization of NF-κB in MM cells derived from 30 different patients with MM at presentation and in relapse, in BMSCs from two MM patients as well as in two myeloma cell lines (XG1, RPMI 8226). *Methods*. NF-κB localization was evaluated by either immunohistochemistry, immunofluorescence or immunoblot using a monoclonal mouse anti-human p65 (Rel A) antibody that recognizes the p65 subunit. *Results*. surprisingly, nuclear localization of NF-κB was (weakly) detected in only one MM sample from a refractory MM patient and in BMSC samples, while the other samples, including the MM cell lines, exclusive-

ly express the cytoplasmic (inactive) form of NF-κB. Moreover we analyzed the sensitivity of MM primary cells to different doses of the proteasome inhibitor Bortezomib (from 1 nanomolar to 10 micromolar), which is known to antagonizes NF-κB activity. We found a consistent dose and time-dependent antitumor activity against both chemoresistant and chemosensitive myeloma cells in all the samples analyzed, independently of NF-κB localization. *Conclusions*. our results indicate that Bortezomib is active in MM cells regardless the NF-κB localization and suggest the existence of other molecular targets of proteasome inhibitors in MM.

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PP2500 MRNA IS DOWNREGULATED IN LOW RISK MDS CELLS AND DURING MDS ERYTHROID DIFFERENTIATION

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Background. Ineffective erythropoiesis is a common feature of myelodysplastic syndromes (MDS), and anemia is the predominant symptom in these patients. Low-risk MDS, including refractory anemia (RA) and sideroblastic anemia (RARS), are characterized by increased apoptotic death of erythroid progenitors cells. Overexpression of proapoptotic Bax, Bid, and cytocrome c mRNAs in progenitor cells of MDS patients have already been described. However, the mechanism underlying the spontaneous apoptosis of MDS bone marrow progenitors remains unclear. ANKHD1, transcript variant 3, also named PP2500, (NM_024668), is a recently described gene that has been shown to be upregulated during erythroid differentiation of normal mononuclear haematopoietic cells, but its role in MDS has not been described. *Aims*. The aim of this study was to verify the expression level of PP2500 mRNA in bone marrow cells of MDS patients and normal donors. We also attempted to verify the modulation of PP2500 mRNA during erythroid differentiation of $CD34^+$ normal and MDS cells. *Methods*. Marrow aspirates were obtained from 7 normal donors and 16 patients with low-risk MDS out of treatment, FAB classification: 13 RA and 3 RARS (7 males, 9 females; 23-78 (median 64 years). The National Ethical Committee Board approved the study and informed-written consent was obtained from all patients and donors. Total cells were submitted to RNA extraction and the expression level of mRNA was detected by real time RT-PCR. The relative quantification value of gene expression was calculated using 2-DDCT. For erythroid differentiation, bone marrow samples from one MDS patient (RA) and one normal donor were collected and CD34+ cells were separated from mononuclear cells using MIDI-MACS immunoaffinity columns. $CD34^+$cells were plated on plastic culture dishes in methylcellulose medium with appropriate growth factors for 6 days. BFU-E, CFU-E and proerythroblasts were then cultured in α MEM for an additional 8 days. At days 6 and 14 cells were collected and submitted to real time RT-PCR and apoptosis analysis. Apoptosis was quantified with anexin V and propidium iodide; erythroblast differentiation was verified with transferrin receptor and glycophorin A and cells were analyzed by flow cytometry. *Results*. PP2500 mRNA expression was significantly lower in low-risk MDS compared with normal donors (0.0819 [0.0001-0.6134] vs 1.0000 [0.4398-1.892]; p=0.0004, Mann-Whitney Test). With regard to erythroid differentiation, $CD34^+$ normal hematopoietic cells were characterized by a low rate of apoptosis (8% at days 6 and 14) and an increasing expression of PP2500 mRNA (one fold at day 6 and twelve fold at day 14). However, $CD34^+$ MDS cells presented an increased apoptosis rate (8% at day 6 and 20% at day 14) and maintained a low expression of PP2500 mRNA. *Conclusions*. The low expression of PP2500 in low risk MDS cells, even during erythroid differentiation, suggests that this new gene is deregulated in MDS and may contribute to the high apoptosis rate observed in this disease. Finally, the identification of new genes involved in the pathophysiology of MDS is essential to better elucidate the mechanism underlying the spontaneous apoptosis of MDS bone marrow progenitors.

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EXPRESSION OF ANGIOGENIC FACTORS, HYPOXIA INDUCIBLE FACTOR (HIF-1A) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MULTIPLE MYELOMA (PRELIMINARY RESULTS)

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Various cytokines have been invoked as being responsible for driving the process of neovascularization seen in solid tumors and haematological malignancies. Vascular endothelial growth factor (VEGF) is the most potent inducer of angiogenesis and appears to have a particularly important role in the biology of multiple myeloma (MM). Recently, it has been widely accepted that VEGF expression is mediated by Hypoxia Inducible Factor (HIF-1a), during hypoxia. Also, HIF-1a expression correlates with VEGF expression and microvessel density in several solid tumors. The aim of this study was to investigate the relationship between expression of HIF-1a and VEGF in MM. Overexpression of HIF-1a may be proven a useful marker for predicting patient outcome. Total RNA was isolated from bone marrow and peripheral blood cells (4/6 samples respectively) from 10 patients (mean age 61 years, 4 men and 6 women) with MM at diagnosis (8 patients at stage IIIA and 2 patients at stage IIIB, according to Durie-Salmon Staging System). We used the Qiagen Kit (QIAamp® RNA Blood Mini Kit) for the RNA extraction. DNA-free total RNA was reverse transcribed (RT) with Superscript Reverse Transcriptase (Invitrogen®). In PCR a 595bp VEGF sequence, a 471bp HIF-1a sequence, and a 614bp sequence of the housekeeping gene b-actin were amplified with the appropriate primers. The PCR products were electrophorised in 2% agarose gel (Figure 1). Our study showed that HIF-1a and VEGF were expressed in all patients with MM at diagnosis. This can be explained considering the fact that VEGF contains a number of HIF-1a binding sites in each regulatory region and HIF-1a has been shown to activate the VEGF promoter *in vitro*. HIF-1a expression increases under hypoxic situation, and we should suppose that our results could be explained as all of our patients were anemic. Our results were confirmed with Real-Time PCR (Opticon 2 Real-Time PCR System-MJ Research), but a more precise quantitation of the exrression of VEGF and HIF-1a is under investigation, in our laboratory. Although, VEGF overexpression plays a crucial role in bone marrow angiogenesis in MM, there is little evidence about the role of HIF-1a in hematological malignancies. In several previous studies high expression of HIF-1a, in solid tumors, has predicted poor outcome, however, the opposite scenario has also been reported. The last decades, angiogenesis is implicated in prognosis of many hematological malignancies like MM, MDS, lymphoma. Many antiangiogenic drugs, like thalidomide, have added in the treatment of hematological malignancies with diverse response. HIF-1a may be a potential target for angiogenic therapy, and new drugs are under research.

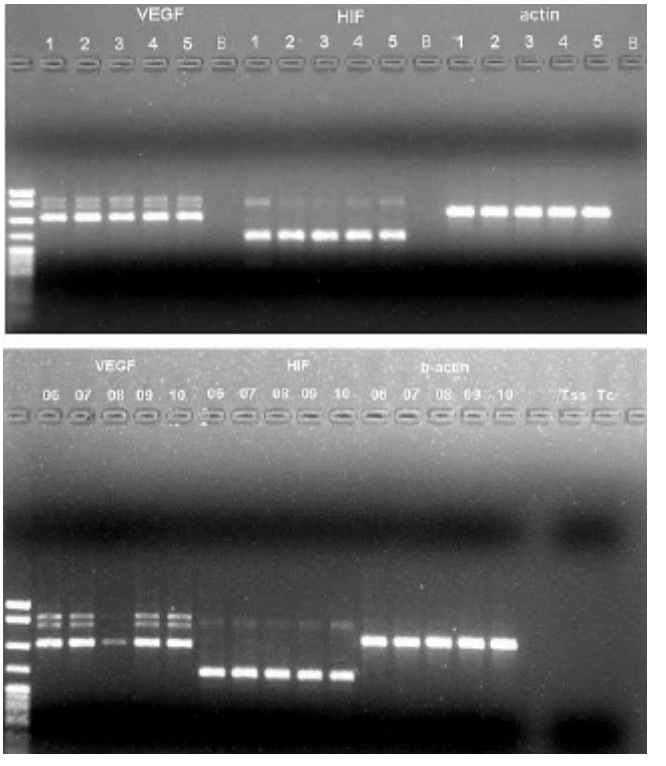

Figure 1. HIF-1a, VEGF mRNA expression in 10 MM patients.

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INTRAVENOUS (IV) BUSULPHAN IN CHILDREAN PRIOR TO AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION: 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 hematological malignancies or solid tumor 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 my 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 patients (pts), median age 37 (7-177) m, body weight 6-69 kg, affected by AML (5 in I CR), ALL (7; 2 in I CR, 3 in II CR, 2 in RR), NB (8; 2 in I CR, 6 in I PR), LCH (2), Ewing sarcoma (8; 2 in I CR, 4 in I PR, 2 in SR) underwent to autoSCT (18) or alloSCT (12; 1 haploidentical, 2 mismatched cord, 3 mismatched unrelated, 6 matched related), from December 204 through December 2006 and received i.v busulfan every 6 h for a total of 16 doses, as part of their conditioning regimen. Busulfan doses were based on accrual patient weight: <9 kg 0.95 mg/kg/dose; 9-16 kg, 1,2 mg/kg/dose; 16-23 kg, 1.1 mg/kg/dose; 24-34 kg, 0,95 mg/kg/; >34 kg, 0,8 mg/kg/dose. Development of hepatic veno occlusive disease (HVOD; modified Baltimore criteria) and engraftment (absolute neutrophil count $>0.5\times10^9$/L and platelet count $>50\times10^9$/L were evaluated. In 9 patients busulfan was followed by cyclophosphamide 21 h after the initiation of the last busulfan dose. No HVOD prophylaxis was given. To prevent busulfan-induced seizures, all patients received sodium valproate. 20 mg/kg/die 24 h before the first busulfan dose and continued until 6 h after last busulfan dose. *Results*. Only 1 (3%) patient developed HVOD, resolved after defibrotide and C-protein treatment. One patient evidenced seizures 24 h after last dose of busulfan and 16 h after the last dose of sodium valproate. One patient, that received an mismatched unrelated graft, died before engraftment for DIC. 29/30 patient demonstrated engraftment. Median time to neutrophil engraftment was 15 d (range, 10-19 d). Median time to platelet engraftment was 22 d (range, 12-25 d). Overall survival at day +100 was 94% (28/30). Regarding the alloSCT procedure, all the 11 valuable patient evidenced a Donor Complete Chimerism since day +30. After a median follow up of 12 m (range, 1-26 m), 23 patients (77%), are alive and well. *Conclusions*. Intravenous busulfan in children, administered on accrual patient weight, is safe and feasible. There was very limited toxicity and a rapid and sustained bone marrow function recovery. No patient underwent to alloSCT rejected nor evidenced primary or secondary graft failure. Moreover allows to avoid assumption difficulties in younger patients.

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TRASLOCATION (11;14) IN TWO CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA OF T-CELL ORIGIN

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Background. Cytogenetic analyses of acute lymphoblastic leukaemia (ALL) are sometimes used to define specific subgroups of patients with poor prognosis such as t(9;22) or t(4;11). For this reason, the patients with unfavourable cytogenetic pattern are treated with more aggressive protocols associated with bone marrow transplantation (BMT). At the moment, are not known the prognostic meaning of all abnormal karyotypic finding. In this study, we investigated the clinical course of two patients with T-ALL and t(11;14) at karyotype. *Patients and Results.* Between June 2000 and January 2007, 192 patients, with ALL, were enrolled in AIEOP ALL 2000 protocol in our Institution and 10 cases, 6 B-precursor ALL and 4 T-ALL presented a precocious relapse. Two of relapsed T-ALL showed a particular karyotype for t(11;14) (p13;q11). The characteristics of these 2 patients were very similar : both males with hyperleukocytosis at diagnosis, age respectively of 8 and 3 years, T-early immunophenotype, treated with the same protocol at high risk (HR) for poor prednisone response (PPR) at day 8. At diagnosis both patients showed large splenomegaly. Table 1 resume the characteristics of patients. All patients achieved a complete haematological remission (CR) at day 33 and 78, and negativity of minimal residual disease (MRD) at molecular biology.

Table 1. Characteristic of patients.

Characteristics Pts	*Pts 1*	*Pts 2*
Age (years)/Gender	8/M	3/M
WBC/µl at diagnosis	415.000	1.100.000
Immunophenotype	LLA early T	LLA early T
Treatment Protocol/Risk group	AIEOP LLA 2000/ HR	AIEOP LLA 2000/ HR
Extramedullary localization	Yes (splenomegaly)	Yes (splenomegaly)
Cytogenetic analysis	t(11;14) (p13;q11), 5p-, -13	t(11;14) (p13;q11)
Molecular Profile	Intrachromosomic microdelection del(1)	Negative
Time from diagnosis to relapse (months)	+13	+9
Site of relapse	BM and splenomegaly	BM, CNS and optic nerve
DFS from relapse (months)	+2† (Resistance)	+3 (CR)

Unfortunately, both children precociously presented a bone marrow (BM) relapse associated with extramedullar localization (spleen in the first patient and central nervous system (CNS) with optic nerve infiltration in the second). The time from diagnosis to relapse was 13 and 9 months, respectively. Both were enrolled in relapsed- ALL AIEOP protocol, followed by unrelated transplant. Patient 1 (Pts 1) showed resistance to treatment and died at + 2 months from relapse in progression disease (PD); Pts 2 was in CR at + 3 months and are to be undergoing a mismatch unrelated donor (MUD) transplant. *Conclusions*. The description of these similar cases suggest that a more aggressive therapeutic approach should be recommended in T-ALL with t(11;14) at karyotype. At the best of our knowledge, few cases of paediatric T-ALL with this particular translocation are described but no study reports the prognostic significance of this cytogenetic pattern. A larger series of cases is needed to confirm this finding but we believe that these patients, for the high risk of precocious relapse, must be treated with a more intensive protocol followed by familial or unrelated BMT in first CR, independently by MRD response.

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VON WILLEBRAND FACTOR AND PROPHYLAXIS OF THROMBOEMBOLISM IN PATIENTS WITH MULTIPLE MYELOMA

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Background. There is an increased risk of thrombotic complications in patients with multiple myeloma. Following risk factors play role in aetiopathogenesis: a) the presence of monoclonal immunoglobulin and related hyperviscosity syndrome with fibrine structures, b) production of pro-coagulation acting antibodies, c) effect of inflammatory cytokines to endothelium, d) frequent incidence of aquired activated protein-C resistance /Zangari 2002/, high level of both vWF:Ag /Minnema,2003// and FVIII /Zangari 2002/. The treatment with thalidomide especially in combination with dexamethasone and doxorubicine as well as therapeutic regimens containing high dose dexamethazone significantly increase the risk of thromboembolism. Prophylactic administration of LMWH in the risk groups of patients with multiple myeloma decreases the risk of thromboembolism by 50%. *Aims*. Evaluation of selected haemocoagulation parametres (vWF:Ag, F VIII:c) in patients in various stage of multiple myeloma and their possible relation with the prediction of thromboembolic risk. *Methods*. We examined 18 patients with multiple myeloma in clinical stage I.-III.(Durie, Salmon) for both vWF:Ag and F VIII and compared with group of 25 healthy controls. *Results*. The level of vWF:Ag was significantly increased in patients with multiple myeloma compared to control healthy group (p=0.00086). The level of F VIII:c was significantly higher (p=0.00020) in patients as well. *Summary*. Our

findings confirmed the significant increase of vWF:Ag and F VIII in patients with multiple myeloma, which contribute to the thromboembolic risk. The analysis of these results, taking also the other risk thromboembolic factors into consideration, may be helpful to decide the correct prophylaxis of thrombembolism. To confirm these results the larger randomized patient groups and following-up the changes of selected haemocoagulation parameters depending on activity of the disease are required.

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INDOLEAMINE 2,3-DIOXYGENASE (IDO) MAY NOT BE A MAJOR FACTOR FOR TUMOUR IMMUNE EVASION IN MULTIPLE MYELOMA

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Background. Indoleamine 2,3-dioxygenase (IDO) is a tryptophan catabolising enzyme expressed by several cancers that induces immune tolerance. High IDO expression has been linked with short survival in some cancers, but data on the possible role of IDO are not available as yet in multiple myeloma (MM). *Aims*. In this study we aimed to evaluate IDO expression in a series of previously untreated patients with multiple myeloma. *Methods*. We used conventional and quantitative (q) PCR to determine expression levels of IDO in CD138 sorted bone marrow (BM) cells from MM patients (n=17), MM cell lines (n=6) and MM BM stromal cells (SCs) (n=5). *Results*. Low level expression of IDO was found in the $CD138^+$ BM fraction of 17 myeloma patients using qPCR (median 0.52 fold compared to normal PBMNC; range, 0.08-15.03). Even in the patient with highest IDO expression, IDO mRNA levels were >100x lower than in DCs or HeLa cells stimulated with IFN-gamma. Similarly, 6 myeloma cell lines had low IDO expression by qPCR (median 0.04; range 0.001-0.68). Stimulation with IFN-gamma led to an upregulation of IDO in 2 of these cell lines, as shown by qPCR and Western blot, but again with expression levels being >100x lower than in activated DCs. Analysis of the tryptophan/kynurenin ratio in cell line culture supernatants furthermore revealed little sign of enzyme activity, even after stimulation. IDO expression could not be induced in the IDO- cell lines. Interestingly, when comparing $CD138^+$ and $CD138^-$ cell fractions from BM of 3 myeloma patients, a detectable, although weak, PCR band was demonstrated in the $CD138^-$ fraction only. By conventional PCR using purified cell subsets from the CD138- fraction, a weak band was amplified from monocytes and T-cells. In cultured BM SCs from myeloma patients, IDO expression was low at baseline, but could be upregulated by interferon-gamma. IDO also proved functional in this setting with reversal of the tryptophan/kynurenin ratio after 48 hours of interferon-gamma. BM-derived SCs from myeloma patients thus seem to have similar characteristics with regard to IDO expression as SCs from normal donors and do not appear to be a major source of IDO in myeloma when examined in isolation. *Conclusions*. IDO is weakly expressed in myeloma plasma and stromal cells and may not contribute to immune paralysis in this disease.

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REGULATORY EFFECTS OF PHYTOESTROGEN DAIDZEIN ON MURINE MYELOPOIESIS

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Background. previous work in this laboratory has shown that daidzein (4',7-dihydroxyisoflavone), a well-known phytoestrogen, can exert antitumour effects on neuronal cancers such as neuroblastoma. In addition, this naturally-occurring compound has also been reported to have various health benefits, such as chemopreventive and cardiovascular-protective activities. Despite its abundance in Asian foods, other biological effects of daidzein, especially its roles in cell development, are relatively undetermined. *Aims*. in this study, attempts had been made to investigate the potential regulatory role of daidzein on the process of myelopoiesis in the mouse. *Methods and results*. we began our studies on the matured macrophages. Daidzein was shown to have stimulatory effects on the thioglycollate-elicited murine peritoneal macrophages as it enhanced the phagocytic activity, nitric oxide production, and TNF-α expression in the treated cells. Moreover, daidzein exhibited no mRNA concomitant cytotoxic effect on the cells. We then continued our studies on the myelomonocytic cells. Daidzein was shown to inhibit the proliferation, induced G_0/G_1 phase cell cycle arrest and triggered apoptosis in the murine myelomonocytic WEHI-3B (JCS) cells. Interestingly, daidzein also induced the monocytic differentiation of the JCS cells, as judged by the increases in cytoplasm-to-nucleus ratio, superoxide anions production, and expression of the macrophage differentiation antigens (Mac-1 and F4/80). Furthermore, we also studied the possible effects of daidzein on the long term bone marrow derived murine myeloid progenitor cells, 32D. Daidzein treatment of 32D cells was shown to suppress the proliferation and increased the proportion of cells at the G2/M phase. In addition, daidzein also induced morphological changes (cell size and granularity) and increased the expression of macrophage differentiation antigen Mac-1 in the cells. *Summary/Conclusions*. taken together, our results hinted that daidzein possessed similar differentiation-inducing properties to colony stimulating factors (CSFs); but unlike CSFs, whose action was limited to cells at specific maturation status, the effects of daidzein was independent of that status. This interesting differentiation-inducing property of daidzein on myeloid cells should be further pursued to let us understand more about the mechanism(s) of myeloid cell development and possibly, the design of differentiation therapy for certain forms of myeloid leukemias.

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ONCE-DAILY INTRAVENOUS BUSULFAN PRIOR TO ALLOGENIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION: EFFICACY AND TOXICICY IN OUR CENTER

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Oral busulfan is a common component of pretransplant conditioning regimens but has an erratic and unpredictable bioavailability. Intravenous busulfan (IV Bu) has substituted oral busulfan for conditioning regimen before haematopoietic stem cell transplantation (HSCT). The aim of this study was to evaluate the early toxicity and efficacy of once-daily administration of IV Bu in our hospital. *Patiens*. We retrospectively evaluated 19 patients underwent allogenic HSCT between May 2005 to January 2007. All patients received intravenous busulfan in conditioning regimens. 10 patients with haematological malignancies suitable for conventional allogenic transplantation were treated with IV Bu (3.2 mg/kg/per day/four days) and cyclophosphamide. 9 patients received reduced intensity conditioning with IV Bu (3.2 mg/kg/per day/two days) and fludarabine. All patients received phenytoin prophylaxis. No pharmacokinetics study of IV Bu was made. No significant differences were observed between two groups respect age or other risk factors to develop pulmonary or hepatic toxicity. 11/19 patients had previous history of cigarette smoking and/or alcohol intake. Pre-transplant evaluation detected abnormalities in hepatic and pulmonary function test (reduced of CO diffusion), 7 and 4 patients respectively. All of these patients were asymptomatic. The stem cell source was bone marrow in 14 patients (8 allo-HSCT) and peripheral blood in 5 patients (2 allo-HSCT and 3 RIC). The median number of $CD34^+$ cell infused was 3.18×10^6 (1.23-8.65) and 3.61×10^6 (0.75-8.25) for allo-HSCT and RIC respectively. All patients received graft versus host disease prophylaxis with cyclosporine and methotrexate. *Methods*. We analysed clinical symptoms, laboratory test and radiological images to evaluated gastrointestinal, hepatic, neurological and pulmonary toxicity. (We used WHO score for classification toxicity grades). We also analysed haematological engraftment. We studied 100 days mortality rate in our group.

Table 1.

	Day 0 to < 500 ANC*	Aplastic (days)	>100 ANC* (days)	>500 ANC* (days)	>20000 platelets (days)	>50000 platelets (days)
BUCY	3 (1-6)	11 (8-19)	11 (9-17)	14.5 (11-20)	13.5 (12-20)	30 (16-66)
BUFLU	6 (4-12)	10 (6-16)	14 (13-16)	18 (15-23)	15.5 (13-21)	19 (14-25)

* ANC: absolute neutrophil count

Results. 15 patients developed mild transitory liver function tests abnormalities. Only one patient developed hyperbilirrubinemia and veno-occlusive disease. All patients developed gastrointestinal toxicity and were severe in five of them. Not patient developed neurology complications or interstitial pneumonitis. All patients achieved engraftment and haematopoietic recovery. No patients died due to toxicity related

with busulfan. Complete donor chimerism was achieved at 30 days after transplantation in 12 patients (10 allo-HSCT, 2 RIC). Mixed donor chimerism was achieved in the rest patients (7 RIC). Only 3 patients developed acute GVHD grade > 2. Overall survival evaluated on 100 day was 95%. Overall survival and disease-free survival with a median follow up of 19 months rates was 95% and 85% respectively. *Conclusions*. Once-daily administration of intravenous busulfan administrated with cyclophosphamide or fludarabine was effective and had short toxicity.

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CD52 ANTIGEN IS EXPRESSED AT DIFFERENT INTENSITIES ON TUMOR CELLS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA, SMALL LYMPHOCYTIC LYMPHOMA, MANTLE CELL LYMPHOMA AND ON CD34+ CELLS

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Background. Monoclonal antibody anti-CD52 (Campath-1H) is used for treatment of B- and T-lymphoproliferative diseases. The success of the treatment is influenced by, among other factors, the target antigen expression level on tumor cells. Small lymphocytic lymphoma (SLL) and B-cell chronic lymphocytic leukemia (B-CLL) are often considered as two clinical manifestations of one type of disease. The presence of CD52 antigen on $CD34^+$ cells is not yet clear and quantitative flow cytometry could contribute to its clarification. *Aims*. The goal of the work was to analyze the expression intensity of the CD52 antigen in patients with B-CLL, mantle-cell lymphoma (MCL) and SLL and to compare them with CD52 expression on B-lymphocytes of a healthy population and $CD34^+$ cells in peripheral blood stem cells (PBSC) grafts. *Methods*. Recently diagnosed and previously untreated patients were evaluated. Furthermore, expression of CD52 antigen on $CD34^+$ cells from graft of PBSC was analyzed. The CD52 antigen level was measured on tumor cell populations in patients and on the B-lymphocytes of a control group. The intensity of expression was expressed in molecules of equivalent soluble fluorochrome units (MESF) and antibody binding capacity (ABC). *Results*. In the group of patients with chronic lymphocytic leukemia, the CD52 level on B-CLL lymphocytes (245000 MESF; 107000 ABC) was significantly lower than on B-lymphocytes of the control group (446000 MESF; 194000 ABC; $p<0.001$) and tumor cells of SLL (526000 MESF; 229000 ABC; $p<0.001$). The CD52 antigen was expressed on a majority of $CD34^+$ cells from a graft, but its intensity of expression was low (101000 MESF; 44000 ABC). *Conclusions*. Our data demonstrate differences in the intensity of the CD52 antigen expression between B-lymphocytes and tumor lymphocytes of B-CLL patients, and, more importantly, between B-CLL and SLL tumor cells. This difference could become helpful in differential diagnostics of these two similar malignancies. This work was supported by grant IGA MZ CR No. NR9023-3.

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EFFICACY AND SAFETY OF NEW PRODUCT OF RECOMBINANT HUMAN RHU-GRANULOCYTE COLONY-STIMULATING FACTOR (FILGASTRIM) TO TREAT CHEMOTHERAPY-INDUCED SEVERE NEUTROPENIA (GRADE IV)

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Background. Exogenous growth colony-stimulating factors (GCSF) is used widespread to treat patients with chemotherapy-induced severe / life threatening neutropenia. Most of GCSF products available in market are quite expensive causing limitation of their use, especially in developing countries. Recently, a new and more economic product of rHu-GCSF has been produced in China (Shandong Kexing Bioproducts Co., Ltd.) by recombinant genetic engineering using Escherechia coli. A multi center, pre- and post-study without control has been performed to evaluate the efficacy and safety of this alternative product of rHu-GCSF to treat severe chemotherapy induced neutropenia in Indonesian population. *Methods*. Thirty-six patients with solid tumors, non-myeloid blood malignancy, receiving standard chemotherapy regimen were enrolled in this study. After chemotherapy, the patients received 5µg/kg BW rHu-GCSF as a single dose subcutaneously when leucocyte count <2000/µL or neutrophil count <500/µL for 14 days or stopped when the leucocyte count reach a stabile level of >3000/µL for 3 consecutive days. The efficacy was accessed by duration of leukopenia or neutropenia (grade IV), the onset to reach leucocyte count >3000/µL or neutrophil count >1500/µL, duration of rHU-GCSF administration until the leucocyte count >3000/µL or neutrophil count > 1500/µL, and the evidence of infection induced severe neutropenia. Safety was assessed by clinical signs and symptoms of adverse events. *Results*. Severe leukopenia and or neutropenia could be observed at the first, second, third, fourth, fifth, and sixth cycles of chemotherapy, and in 44% of cases this adverse events occurred at the first cycle. The administration of rHu-GCSF was followed by leukocytosis (median: 18,350/µL; range: 3,100-68,700/µL) and neutrophilia (median: 14,225/µL, range: 1,600-61,200/µL). Severe leucopenia or neutropenia mostly occurred for 2 days (39%) with the duration mean was 2.32±1.30 days. Mostly, rHu-GCSF was administered for 2 days (26%) and 3 days (28%) to increase leucocyte count >3000/µL or neutrophil count >1500/µL. Infections (febrile neutropenia, stomatitis, acute respiratory infection) developed in leucopenia / neutropenia state before the administration of rH-GCSF were observed in 39.58% of 54 cycles. However, after the administration of rH-GCSF no mortality was reported due to those infections . The adverse events suggested due to rH-GCSF included bone pain (5 patients) and fever (5 patients). Eight patients experienced serious illness. Five patients were hospitalized due to the thrombocytopenic bleeding and stress ulcer and 3 patients died of the disease progressiveness. However, all those serious illness were not related to the rHu-GCSF administration. *Conclusion*. The administration of the more affordable rHu-GCSF has shortened the duration of leucopenia / neutropenia as well as has demonstrated the efficacious and safe treatment of severe chemotherapy induced leucopenia/neutropenia in Indonesian population.

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KARYOTYPIC AND FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ANALYSES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AT DIAGNOSIS: THE GREEK PROFILE

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Background. Acute lymphoblastic leukemia (ALL) represents about 85% of childhood leukemias. The current cure rate is nearly 80%, reflecting the remarkable progress in identifying and treating resistant subtypes of the disease. A large number of established structural and numerical chromosome rearrangements have now been described, for which the underlying genetic alterations and prognostic significance are well known. These include the t(12;21) with the TEL/AML1 (ETV6/RUNX1) fusion, hyperdiploidy ≥50, p16 gene (9p21) deletion and rearrangements of 11q23 involving MLL. *Aims*. We investigated the cytogenetic profile of pediatric ALL in Greece. The karyotypic findings were integrated with the interphase FISH results and further explored by metaphase FISH. *Patients and Methods*. Sixty eight Greek pediatric patients with ALL at diagnosis (64 B-ALL, 4 T-ALL) were included in the study. The age ranged from 1 to 17 years and the male to female ratio was 3:2. Bone marrow specimens were initially studied by conventional cytogenetics and further tested by FISH for the presence of the TEL/AML1 fusion gene, rearrangements of the MLL gene, p16 (9p21) gene deletion and hyperdiploidy. The molecular cytogenetic study was conducted (interphase FISH, iFISH; metaphase FISH, mFISH) using the commercially available probes LSI TEL/AML1 ES Dual color translocation, LSI MLL Dual color Break Apart rearrangement, LSI p16 (9p21)/CEP 9 Dual color (Vysis, Downers Grove, IL, USA) and a-satellite probes specific for the centromere of chromosomes 4, 6, 10, 17, 18, X (Vysis, Downers Grove, IL, USA) and chromosomes 13, 16 and 21 (Cytocell, Cambridge UK). *Results*. Conventional cytogenetic analysis was successful in 61/68 cases (89.7%) and detected clonal abnormalities in 22/61 cases (36.1%). FISH revealed that 13/68 (19.1%) patients were positive for the TEL/AML1 fusion gene. Additional genetic changes were present in 9/13 (69.2%) of the $TELAML1^+$ cases and consisted of deletion of the unrearranged TEL gene (5/13, 38.5%), an extra der(21)t(12;21) (4/13, 30.8%), an extra AML1 gene (3/13, 23.1%) and heterozygous deletion of the MLL gene (2/13, 15.4%). More than one additional genetic change was observed in 2/13 (15.4%) of these cases. Moreover, using the TEL/AML1 probe we detected one case 1/68 (1.47%) carrying amplification of the AML1 gene. The p16 gene deletion occurred at an incidence of 20.7%, while no structural MLL rearrangement was detected in our sample. The use of centromeric probes revealed clones with hyperdiploidy in 20/68 patients (29.4%). Overall, combining karyotypic analysis with FISH, the abnormality detection rate was increased to 70.6%. *Summary/Conclusions*. Our cytogenetic study on Greek pediatric ALL suggests that the incidence for the most common chromosomal abnormalities are very similar to those reported in the literature. The detection of secondary genetic changes in $TEL/AML1^+$ cases at diagnosis may imply

a less favorable prognosis. Interestingly, our findings concerning the amplification of the AML1 gene in one case and the heterozygous deletion of the MLL gene in two TEL/AML1+ cases, agree well with recent reports considering these aberrations as new non-random recurrent abnormalities in childhood ALL.

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THE THROMBOCYTOPENIA DOES NOT EXCLUDE THE INCIDENCE OF DEEP VEIN THROMBOSIS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

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The role of platelets is well established in the development of arterial thrombotic disorders but questioned in venous thrombosis. We present 5 case reports of deep vein thrombosis (DVT) which occurred in patients (pts) with thrombocytopenia (grade 3/4) in the course of haematological neoplasms. The patients were admitted to our department because of : acute myeloid leucaemia (n=3) (M2, M3) , mantle cell lymphoma (n=1) and lymphocytic lymphoma (n=1). Thrombocytopenia resulted from marrow infiltration (n=2) or post -chemotherapy marrow aplasia (n=3). The following thrombotic risk factors were found : immobilization (n=5), long term central venous catheter (n=2), chemoterapy (n=3). All pts presented with typical clinical signs of thrombosis and the diagnosis of DVT was confirmed by duplex Doppler ultrasonography in all cases. Localisation of thrombosis included deep veins of lower limbs (n=5) as well as at the site of central venous catheter insertion (n=2). Patients were not exposed to heparin (icluding intravascular catheter *flushes*) before the first thrombotic episode. Standard treatment with i.v. unfractionated heparin was applied maintaining aPTT within the therapeutical range, followed by secondary prophylaxis with a medium dose of enoxaparine s.c. or oral anticoagulant (INR 2.0-3.0). No severe bleeding complications were observed but in two still thrombocytopenic pts, DVT relapsed during anticoagulation treatment at other site then their previous episode. Fortunately, the course of DVT was not fatal but it prolonged hospitalization and led to delayed treatment of primary haematologic disease. *Conclusions*. Thrombocytopenia dose not prevent deep vein thrombosis in the course of haematological malignancies and standard secondary prophylaxis is not effective in some cases. Further studies are needed to evaluate the trigger factors for clot formation in thrombocytopenic patients.

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WHO OVERCOME PVSG DIAGNOSTIC CRITERIA FOR PATIENTS OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

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The Registro Italiano Trombocitemia (RIT), that is a GIMEMA project, has been activated in order to registry Italian ET patients, to improve the diagnosis appropriateness (WHO criteria), to promote the acquisition of biological data, to evaluate the compliance to the therapeutical guidelines of the Italian Society of Haematology (SIE), to monitor in particular the ET patients receiving Interferons α and Anagrelide, to evaluate cases of pregnancy, paediatric age and familiarity, to define the prognostic value of the biological factors, to create a network for activation of new clinical and biological studies. The RIT, co-ordinated by the Haematology Unit of Reggio Emilia, is a web-based registry that, beside a public area, comprehends a database of Italian ET patients. The data, with respect of the privacy rules, are object of validation and analysis by various RIT expert subcommittees. Eighty-six haematological Institutions adhered to the RIT, 1242 patients have been registered since June 2005 and 1060 of them are object of the present clinical-haematological analysis. The use of the WHO classification is interestingly increasing (27% before 2004 and 61% in the year 2006). The patients, 651 females (61%) and 409 males (39%), F:M ratio 1.6, showed median age 60 years (males 64, females 58), age below 40 yr (16%), 41-60 yr (35%), 61-70 yr (19%), over 70 yr (30%). At diagnosis the platelet count (109/L) was 400-600 (13%), 601-1000 (66%), 1001-1500 (17%), over 1500 (4%) with a mean 864±315 and a median value 787 (males 758, females 804). The haematocrit value (Hct, %) was significantly higher in males (median 44.4, over 48 in 17% of cases) than in females (median 41.8, over 48 in 6% of cases). The WBC count (1012/L) was 9.1± 2.7 with no difference between males and females. Thrombosis and haemorrhage were observed before/at diagnosis in 19% e 5% of cases, respectively. At diagnosis the patients presented disease related symptoms and general thrombotic risk factors in 42% and 79% respectively; the splenomegaly according to physical examination and ultrasound evaluation was reported in 21% and 44% of cases, respectively. The bcr/abl evaluation was always performed and the kariotype study documented a random abnormality in 3.4% of the valuable cases. To improve the diagnostic approach, the RIT has promoted the bone marrow biopsy centralized revision (WHO criteria) and the acquisition of the new biological parameters (JAK2, CD34+ peripheral cells). The 115 reported pregnancies are object of a separete abstract. Antiplatelet drugs were administered in 75% of patients and cytoreduction was performed in 70% of cases, with use of Hydroxyurea (51%), Anagrelide (12%), Interferons α (12%), Pipobroman (3%), Busulfan (2%). A separate analysis for patients treated with Anagrelide and Iterferons α is in progress. The follow-up data are presently not reported.

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THE TYPE AND SCREEN PROCEDURE: EFFECTIVENESS AND APPLICATION IN AN EMERGENCY DEPARTMENT

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Background. The Type and Screen (TS) procedure is a part of the pretransfusional testing strategy, and is based on the determination of ABO-Rh type as well as screening for unexpected antibodies. TS strategy was initially conceived as an alternative to crossmatching in scheduled surgery. However, literature is scarce concerning its effectiveness in Emergency Departments, where request forms for *crossmatch and hold* blood for patients in situations such as digestive bleeding, multiple trauma, etc… are common and frequently wasteful of donor blood. *Aims*. To evaluate the results obtained following the implementation of a TS strategy in our hospital's Emergency Department. We previously organized continuous educational workshops on the subject, aimed at both medical and nursing staff. *Methods*. We revised transfusion requests and effective transfusion indexes throughout the previous two years (stage 1) as well as the two years following implementation of the TS strategy in our hospital's Emergency Department (stage 2). *Results*. In stage 1, 870 TS requests were registered in our hospital. In stage 2, a total of 1,314 were ordered, 487 of which (37.1%) came from the Emergency Department. Of these, only 111 (22.7%) cases eventually required blood crossmatching. If we accept an average of two packed red cell units per transfusion request, a total of 752 crossmatching tests would be avoided. The transfusion percentage for this department in stage 1 was 69.3% (1,486 units were transfused from 2,144 requested), with a crossmatched / transfused ratio, C:T ratio, of 1:44. In stage 2, 1,695 units were crossmatched, 1,344 of which were transfused, with and transfusion index of 79.3% and a C:T ratio of 1:26, significantly lower than that in stage 1. If we had crossmatched all 752 units that were saved, the global hospital transfusion percentage would have been 67.2% (against the actual 75.1%). With regards to only the Emergency Department, the index would have been 54.9% (against the actual 79.3%). The global hospital transfusion percentage in stage 1 was 70,1% (C:T ratio 1:43, with 7,729 units requested and 5,414 transfused). In stage 2, the index was 75.1% (C:T ratio 1:33), with 4,815 units transfused from 6,411 requested. Assuming a 17€ cost for every unit that is tested, the global saving totals 12,784€, when considering only these two years in this single department. *Conclusions*. 1. The Type and Screen procedure has proved to be effective in a non-surgical area, such as in an Emergency Department, where the holding of blood is a common practice, even in situations when transfusion is not finally required. 2. Its implementation has resulted in a remarkable cost saving, the optimization of human resources in the Blood Bank, and most importantly, an improvement in the use of donor blood. 3. The above-mentioned fact, which is difficult to quantify, gives blood banks higher operational effectiveness in real emergency situations, with no associated reduction in transfusional safety. 4. The procedure's management has been possible thanks to the close cooperation between members of the hospital's Transfusion Committee, whose main aims are in ensuring proper transfusional practice.

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SPLENIC MARGINAL ZONE LYMPHOMA WITH VILLOUS LYMPHOCYTES AND SYSTEMIC AL AMYLOIDOSIS: A PREVIOUSLY UNREPORTED ASSOCIATION

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Here we report a patient diagnosed with a splenic marginal lymphoma with villous lymphocytes and systemic AL amyloidosis. To the best of our knowledge this association has never been described. Typically AL amyloidosis is a plasma cell dyscrasia. Systemic AL amyloidosis can occur alone or in association with multiple myeloma or, much less often, with non-Hodgkin´s lymphoma. In particular, lymphoplasmacytic lymphoma (LPL) has been associated with both systemic and localized AL amyloidosis. It has also been reported with chronic lymphocitic leukemia. Marginal zone B-cell lymphomas have been associated to localized AL amyloid in a few reports, but its association with systemic AL amyloidosis has never been described. *Case report*. A 60-year-old woman, with a previous history of recurrent malaria was admitted to our hospital due to nephrotic syndrome. She had a 6 months history of abdominal pain and fluid retention. On physical examination she had small bilateral axillary lymphadenopathies and hepatosplenomegaly. Laboratory data were as follows: hemoglobin 10.3 g/dL ,Platelets 258.000/mm^3. Leukocytes 28.500 with 70% lymphocytes (40% were villous lymphocytes). IgG 327, IgA 673, IgM 109. She had severe proteinuria (13 g/day) and severe hypoalbuminemia (albumin 0.5 gr/dL). Monoclonal IgA-kappa and IgG-lambda were detected by serum immunofixation. Urine immunofixation showed two monoclonal bands: IgA-kappa and IgA-lambda.. Bone marrow aspirate showed an heterogeneous population of small lymphocytes frequently with villous morphology. Atypical plasma cells were also seen (20%). Bone marrow biopsy yielded infiltration by a low grade lymphoproliferative syndrome with villous lymphocytes and mild plamocytosis (15-20%) with two monoclonal populations, IgA kappa and IgA lambda. AL amyloidosis was shown with specific stains. Monoclonal B lymphocytes were positive for CD19,CD20, CD22, FMC-7, CD11c+, and negative for CD5, CD79b, CD23, CD10, CD38, CD103, CD25 and CD138. A computed tomography yielded small axillary and abdominal lymphadenopaties and hepatosplenomegaly. Troponin, pro-BNP and an echocardiography were normal. Free light chains were not available at our institution at that time. Initially she was treated with two cicles of Rituximab-CVP. As no response was seen, we started melphalan and dexametasona cycles adding rituximab. We had little response after three cicles. IgA, kappa/lambda ratio, and serum and urine monoclonal bands remained unchanged. The nephrotic syndrome did not respond with proteinuria > 10 g/ day and albumin <0.7 gr /dL. The patient condition deteriorated with severe anasarca and anuric acute renal failure, and she finally died. Autopsy showed severe amyloid infiltration in spleen, kidneys, liver, heart, and lymph nodes. The features and clinical curse of AL associated with NHL are not well characterized and the optimal therapy remain undefined. Treatments aimed at both lymphoid and plasma cell components appear warranted .Some good responses have been described with rituximab-based therapies (R-CVP and R-CHOP) and with autologous transplantation. Combinations of rituximab, chemotherapy and dexamethasone with or without autologous transplantation have been proposed and merit further investigation. We did not have response neither with a lymphoma directed treatment nor with a amyloidosis directed treatment.

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CONTRIBUTION OF FLOW CYTOMETRY IMMUNOPHENOTYPING (FCI) TO THE DIAGNOSIS OF LYMPHOMA IN FINE NEEDLE ASPIRATE (FNA) AND TISSUES BIOPSY (TB) SPECIMENS

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Background. Immunophenotyping is a fundamental step in the diagnosis of non-Hodgkin's lymphoma (NHL). There have been several recent studies supporting the usefulness of FCI in the diagnosis of lymphoma on samples obtained by surgical specimens or FNA. The most appreciated advantage of this technique is the possibility of applying a broad panel of antibodies and to provide a very quick diagnosis. *Aims*. To analyze the diagnostic value of FCI of lymph node and extranodal tissue specimens submitted directly to our laboratory with a diagnosis of suspected lymphoma and to correlate it with the final cytology-histology diagnosis given by the pathologist. *Methods*. We investigated 59 specimens from 55 patients (pts), 26 FNAs (25 pts) and 33 TBs (32 pts), submitted over a 2-year period. The first pass was to prepare FNA smears or touch imprints of tissue for Wright's stain that were seen immediately. The microscopic features and clinical data, when available, were used to select the panel of antibodies to apply in each sample. FC three-color panel included CD4/CD8/CD3, TdT/CD79ac/CD3c, CD45/CD5/CD19, CD22/CD23/CD19, CD10/CD20/CD19, CD38/CD34/CD19 and kappa/lambda/CD19. Both our cytology and FCI findings were interpreted together to diagnose and sub-classify NHL according to WHO classification as far as possible. *Results*. FNAs: In 10 cases the material was inadequate because of scanty blood mixed aspirate or cellular necrosis. In the rest of them, FNA cytology confirmed 5 cases of B-LNH, 10 cases of reactive lymphoid hyperplasia (RLH) and 1 case of carcinoma. FC was concordant in 14 cases (87.5%). Only one case was *negative* by FC due to a small population of monoclonal B cells in sample. Another case was a false-positive, diagnosed of follicular lymphoid hyperplasia with lambda chain restriction. In 2 of 3 cases of low-grade B-NHL, FC could correctly sub-classify them. Specificity and sensitivity of FCI was 90% and 83.3%, respectively. TBs: FCI couldn't be helpful in 3 cases due to inadequate material. FCI was consistent with the diagnosis in 73.3% of all samples. FCI gave correct diagnosis in 100% of 8 RLH cases and in 100% of NHL cases (7 low-grade B-NHL and 6 high-grade NHL). None of the 8 cases of Hodgkin's lymphoma (HL) were diagnosed by FCI due to scarcity of tumor cells but, in 5 samples, the touch imprints morphology suggested the diagnosis. 75% of B-NHL cases could be sub-classified. Specificity and sensitivity was 100% and 63.6%, respectively. *Conclusions*. 1.-FC is a suitable tool allowing a very rapid and sensitive diagnosis of lymphoproliferative diseases and RLH 2.-FCI data in combination with microscopic exam of samples increase the diagnosis accuracy, mainly in HL and in sub-classifying NHL.

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PLASMA CONCENTRATION OF ASYMMETRIC DIMETHYLARGININE IN HAEMATOLOGICAL MALIGNANCIES

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Background. Asymmetric dimethylarginine (ADMA) is a product of protein turnover and competitive inhibitor of nitric oxide (NO) synthase (NOS). It is a new independent risk factor for endothelial dysfunction and cardiovascular diseases. Diseases associated with elevated ADMA blood levels include: coronary artery disease, chronic renal failure, peripheral arterial occlusive disease, chronic heart failure, diabetes mellitus, lipid disorders and preeclampia. The role of angiogenesis and endothelial cells activation in haematological malignancies is well established. There have been no study that investigated the ADMA concentration in haematological malignancies. *Aims*. The aim of our study was to examinate plasma level of ADMA in patients with various haematological malignancies.

Table 1. Mean plasma levels of ADMA, argininr and ratio arginine/ADMA in group with hematological malignancies and in control group. (SD-standard deviation).

	Mean plasma levels in group with hematological malignancies [SD]	Mean plasma levels in control group [SD]	p
ADMA	1,59 [±0,79]	0,74 [±0,29]	<0,001
arginine	17,56 [±8,45]	12,96 [±6,27]	0,005
arginine/ADMA	13,79 [±11,08]	21,63 [±17,05]	0,013

Material and methods. 43 patients with various haematological malignancies were evaluated (16 females and 27 males). The median age of patients was 52 years. All patients were in active phase of disease. There were 11 patients (25%) with chronic lymphocytic leukemia (CLL), 10 patients (23%) with non Hodgkin's lymphoma (NHL), 7 patients (16%) with acute myeloid leukemia (AML), 5 patients (12%) with Hodgkin's

disease (HD), 4 patients (9%) with acute lymphoblastic leukemia (ALL), 4 patients (9%) with multiple myeloma (MM), 1 patient (2%) with lymphoproliferative syndrome (LS) and 1 patient (2%) with acute biphenotypic leukemia (BL). The healthy control group include 43 age-matched persons (19 females and 24 males). Plasma concentration of L-arginine and ADMA were measured using high-performance liquid chromatography (HPLC) and precolumn derivatization with o-phtaldialdehyde (OPA). For statistical analysys Student's test was used. $p<0,05$ was considered statistically significant. *Results*. The results are showed in Table1. *Conclusions*. We conclude that ADMA level is increased in haematological malignancies in active phase of disease.

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THE SIGNIFICANCE OF ABERRANT CYTOGENETIC CLONES, EMERGING DURING TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA WITH IMATINIB MESYLATE

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Introduction-Aims. Emergence of aberrant cytogenetic clones during treatment of patients with bcr/abl+ chronic myelogenous leukemia (CML) with imatinib mesylate has been reported. We investigated the significance of this phenomenon in a cohort of 101 patients with CML. *Patients and Methods*. Patients were 39 females and 62 males, with a median age of 57 years (range 19-81 years). Imatinib was given as initial treatment in 41 patients, in early chronic phase in additional 26, in late chronic phase in 24, in accelerated phase in 5, in blastic phase in 4 and as post-transplant salvage treatment in 1 patient. All patients were bcr/abl positive. Cytogenetic follow-up was performed roughly every 6 months initially and once a year after the achievement of molecular remission. Molecular monitoring was performed every 3-6 months. *Results*. Cytogenetic analysis at diagnosis was available in 98/101 patients and revealed the typical t(9;22) translocation in 92 cases in total (94%), but as the only abnormality in 86 cases (87.8%). A complex translocation with the involvement of chromosomes 9 and 22 was found in 3 cases, additional chromosomal abnormalities over the dominant clone in 3 cases, additional subclones of the initial t(9;22) translocation in 3 cases, a normal karyotype in 2 cases, and 20q- alone in 1 case. Cytogenetic follow-up was performed in 95 patients, 6-48 months after treatment start. At 6-12 months following initiation of treatment with imatinib, disappearance of the leukemic clone was observed in 45 cases (50%) and persistence of the t(9;22) translocation alone in 28 (31.1%). In 13 patients, new cytogenetic abnormalities, not present initially, were emerged, and these were superimposed over the dominant t(9;22) clone in 7 cases, but they were present over a normal karyotype in 5 cases. A new complex translocation was emerged in 1 case, whereas in 3 cases no metaphases were available for analysis. Later on, 2 additional patients developed new aberrant clones, in the absence of t(9;22) abnormality. At their latest evaluation 72 patients were in complete cytogenetic and molecular remission, and among them 4/7 with the additional subclones emerged over the main clone. Similarly, the new aberrant clones had disappeared in 5/7 patients (+8 in two cases, t(2;5)(p21-23;q12) in one, +1q in one and -Y in one) and persisted in 2 (one case with -Y, and another with -Y,+15). However these two patients had normal blood counts and they did not manifest any sign of a clonal hematopoietic disorder. Three out of the totally 4 patients exhibiting a complex translocation with the involvement of 9 and 22 chromosomes were resistant to imatinib and progressed to accelerated or blastic phase. *Summary-Conclusions*. The emergence of additional cytogenetic clones, both over Ph^+ and on Ph^- hematopoiesis during the treatment of CML patients with imatinib is not uncommon, however in the majority of cases these clones are transient. The existence or emergence of complex translocations should be carefully monitored, as they may be associated with imatinib resistance and disease progression.

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PROGNOSTIC FACTORS IN CHRONIC MYELOMONOCYTIC LEUKAEMIA: A RETROSPECTIVE ANALYSIS OF 113 PATIENTS

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Background. Chronic myelomonocytic leukaemia (CMML), initially recognized by the FAB classification as a myelodysplastic syndrome (MDS), is currently included in the new category of myelodysplastic / myeloproliferative disorders according to the WHO classification. CMML is characterized by an increased number of monocytes in peripheral blood and in bone marrow, associated with various dysplasia. Clinically, 50% of patients present with an organ involvement. CMML is a heterogeneous disease with no cytogenetic signature in which the most common chromosomal defects are monosomy 7 and trisomy 8. The prognosis can be evaluated using the International prognostic scoring system (IPSS) but other prognostic factors have been reported by F Onida *et al* in 2002. *Aims and methods*. to apply these prognostic factors to our series of patients (pts), we retrospectively analysed 113 CMML pts recruited between October 1991 and July 2005. *Results*. Median age was 73 years (46-94), sex ratio was 44F/ 69M. 15 pts presented with hepatomegaly, and 23 pts with splenomegaly. 23 pts progressed to AML. The median overall survival was 19 months. LDH level was available for 39 pts and 27 were abnormal. Karyotypes were performed in 72 pts and 29 were abnormal, with trisomy 8 in 7 pts, -7/7q in 5 pts, 11q23 rearrangements in 5 pts, complex karyotypes in 3 pts, -X in 3 pts, -Y in 3 pts, -5/5q in 2 pts, 17p defects in 2 pts and trisomy 14 in 1 pt. IPSS could be evaluated in 71 pts (25 low risk, 28 intermediate1, 10 intermediate 2, 8 high risk), even for those with proliferative CMML. Univariate analysis showed that high intermediate2/ high risk IPSS, hemoglobin level <10g/dL, marrow blasts >10%, peripheral blasts >5%, monocytosis >4.109/L and presence of circulating immature myeloid cells were poor prognostic factors but not abnormal LDH level, white blood cell count >10.109/L, platelets level <100.109 /L. In conclusion, the characteristics of our patients were similar to those published previously. The prognosis was strongly linked to IPSS. The other factors not involved in IPSS which could easily be used in routine practice are the percentage of peripheral blasts, monocytosis and the proportion of immature myeloid cells.

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MILD RENAL FAILURE IN MULTIPLE MYELOMA PATIENTS : ARE YOU SURE MYELOMA IS THE CULPRIT?

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Background. Renal failure carries a poor prognosis to patients newly diagnosed for multiple myeloma (MM). Free light chains play a key role in causing specific renal lesions. However, few patients are investigated for a differential diagnosis of their renal involvement. *Aims*. To identify potential other causes of renal impairment at presentation and to compare renal function according to whether Bence Jones Proteinuria (BJP) is present or not. *Methods*. Patients referred for MM in our Haematology Unit between 1997 and 2006 were retrospectively evaluated for MM characteristics and renal function at presentation. Patients concurrently presenting with acute renal failure or hypercalcaemia were excluded of the analysis. *Results*. 59 patients were recruited. Sex ratio: 31males/28 females. Mean age: 68.7±10,3 years. Durie-Salmon clinical stage: III n=29 (49%); II n=13 (22%); I n=17 (29%). Isotype: IgG n=38 (64.5%). IgA n=16 (27%). Light chain disease n=4 (6.8%). IgD n=1 (1.7%). Osteolytic lesions: 39 patients (66%). Haemoglobin<10 g/dL: 10 patients (17%). Corrected Calcemia: 2.32±0.15 mmol/L. Albumin: 39.1±5.1 g/L. β2 microglobulin: 3.3±1.9 mg/L. BJP: 27 patients (45.8%). Creatinine: 91.6±38 µmol/L. Creatinine clearance: 75.8±26 mL/mn. Creatinine clearance < 60 mL/mn: 13 patients (22%). Diabetes: 10 patients (17%). High blood pressure: 22patients (37.3%). Dyslipaemia: 8patients (13.5%). Obesity: 2patients (3.4%). Prior cardiovascular events: 15patients (25.4%). Prostatic obstruction: 3patients (5.1%). Group A (BJP present): Creatinine clearance: 72.6±33.2 mL/mn. Group B (BJP absent): Creatinine clearance: 71±25.2 mL/mn. Age and cardiovascular risk factors are equally distributed in the two groups. *Summary/Conclusions*. It is usually admitted that renal failure at presentation is related

to a poor prognosis in MM patients, reflecting a high tumour burden. Renal damage is uncommonly described in the absence of BJP. However, high prevalence for older age and cardiovascular disease in our population suggest potential other causes of renal involvement. So, renal impairment in the MM population needs a particular attention. Even in the absence of BJP, a mild renal dysfunction must be worthwhile examined for adequate haematological, cardiovascular and renal care. Cardiovascular disease and older age are common in our newly diagnosed MM patients. Either BJP is present or absent, renal function is comparable. Mild renal dysfunction in MM patients could appear as a multifactorial entity, needing a multidisciplinary careful analysis for adequate assessment and treatment.

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VALGANCICLOVIR IS SAFE AND EFFECTIVE AS PRE-EMPTIVE TREATMENT FOR CMV REACTIVATION IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Despite significant advances in prevention and therapy, cytomegalovirus (CMV) infection still represents an important cause of morbidity and mortality in patients undergone allogeneic haematopoietic stem cell transplant (HSCT). The standard pre-emptive treatment is based on intravenous administration of Ganciclovir (GCV). Valganciclovir (VGC), the pro-drug formulation of GCV is characterised by an excellent bio viability, making this drug suitable for oral administration. *Aims*. To evaluate the safe and the efficacy of Valganciclovir as pre-emptive treatment for CMV reactivation in allogenic hematopoietic stem cell transplantation. *Methods*. Since March 2003 patients enrolled in our Institute to undergo allogeneic HSCT have been started CMV-pre-emptive treatment with VGC, administrated in outpatient setting. Thirty patients were followed by weekly monitoring both of CMV/PCR and pp65/assays. Patients resulted positive (3 cells pp65+ or 1000/100000 PCR^+) started oral treatment with VGC 900 mg bid, for the first fourteen days, followed by 900 mg q.d. up to at least seven days after assays negativization. *Results*. Overall 15 episodes of CMV positivity were detected in seven patients (all with sibling donors). In these patients, the median duration of therapy was 21 days (range 10-21 days), with a response rate (RR) of 100%, as confirmed by the assays negativization within fourteen days. No significant toxicity was encountered. In two patients the oral VGC therapy was changed to the intravenous administration of Foscavir, because of concomitant neutropenia and acute GvHD. *Conclusions*. Pre-emptive treatment of CMV reactivation with VGC is safe and effective. Furthermore, the oral administration of this drug in outpatient setting, reduces significantly the costs compared with a therapy that needs hospitalization as intravenous Ganciclovir.

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COMPARISON OF TWO METHODS OF HEMATOPOIETIC STEM CELL ISOLATION AND EVALUATION OF THE EX VIVO CULTURED AND DIFFERENTIATED CELLS WITH CDNA MICROARRAYS

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Background. $CD34^+$ cells are clinically used in transplantation of bone marrow or peripheral blood stem cells (PBSC) after conditioning regimens in patients with diagnosis of leukemia or lymphoma. The $CD34^+$ cell count increases in peripheral blood after stimulation with granulocyte colony-stimulating factor (G-CSF). Hematopoietic stem cells are found within the compartment of $CD34^+$ cells and they are able to regenerate hematopoiesis in all of its lineages, as well as renew themselves. *Aims*. We wanted to assemble a suitable method for evaluating gene expression in enriched populations of hematopoietic stem cells and compare their biological properties during *ex vivo* culture in time. *Methods*. We used two ways of immunomagnetic separation: positive selection of $CD34^+$ cells and negative selection of Lin^- cells. $CD34^+$ and Lin' cells were enriched from PBSC grafts of patients with non Hodgkin's lymphoma. After the immunomagnetic separation, the cells were cultivated in the presence of cytokines stem cell factor, interleukin-3, interleukin-6, Flt-3-L and G-CSF in serum-free media in order to induce granulocytic differentiation. At days 0, 4, 6, 8, 10, 12 and 14 cells were harvested and analyzed by cDNA microarrays. Other analyses included colony forming assays, immunophenotyping of the surface differentiation antigens (CD13, CD33, CD34, CD36, CD65, CD116, CD117, CD123, CD124 a CD133), electron microscopy, vitality, total cell and $CD34^+$ cell counts. *Results*. The purities of $CD34^+$ cells after $CD34^+$ and Lin^- separation procedures were comparable. $CD34^+$ expression was the highest in the beginning of the cultivation and decreased rapidly in time. 23.94±10.29 (average±s.d.) fold expansion of the CFU-GM and 2.56±3.31 fold expansion of CFU-Meg in $CD34^+$ cultures were reached. In Lin^- cultures, 30.39±16.64 fold expansion of the CFU-GM and 1.28±1.93 fold expansion of the CFU-Meg were observed. Comparison of gene expression maps of two different types of hematopoietic cell enrichment pointed to a suitability of cDNA microarray analysis of *ex vivo* cultured and differentiating hematopoietic cells. Equivalence of the two enrichment methods from PBSC samples was demonstrated. *Summary/Conclusions*. Microarray analysis of the short-term *ex vivo* cultured hematopoietic cells enabled us to investigate up- and down- regulation of the gene expression during granulocytic differentiation. This methodological approach is helpful in characterizing *ex vivo* cultured cells, but it is also suitable for more general purposes. Equivalence of $CD34^+$ and Lin^- selection methods from PBSC samples proved by cDNA microarray may have an implication for graft manipulation in an experimental setting of hematopoietic stem cell transplantation.

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AN AUDIT ON THE USE OF 20% HUMAN ALBUMIN IN A TERTIARY CARE HOSPITAL

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Background. The use of human albumin has been looked into for almost a decade. Most of the evidence we have comes from systemic review of randomised-controlled trials as well as meta-analysis. The main conclusions from such evidence were that current data suggest the lack of evidence of mortality benefits from human albumin and increased cost when compared to cheaper alternatives such as crystalloids. Moreover, fluid resuscitation with albumin or saline produced similar outcomes irrespective of patients' baseline serum albumin concentration. Certainly the use of albumin in the United Kingdom decreased by at least 40% following these publications. Nevertheless there is growing evidence to support the use of 20% human albumin in terms of mortality reduction in specific patient populations such as following large volume paracentesis in diuretic refractory ascites and in preventing renal failure in spontaneous bacterial peritonitis associated with cirrhosis. *Aim*. Based on this evidence, we ask through this audit how far does our hospital practice comply with the current data and what possible economic repercussions may result from inappropriate use. *Methods*. We carried out a retrospective review of all the requests for 20% human albumin, which North East of Scotland Blood Transfusion Service (NESNBTS) received from 1st May 2005 to 31st April 2006 from the Aberdeen Royal Infirmary; a tertiary care hospital. The use of 20% human albumin was deemed appropriate in: 1. Ascites for drainage 2. Hepatorenal syndrome. 3. Spontaneous bacterial peritonitis. *Results*. NESNBTS received a total of 461 orders from the 1st May 2005-31st April 2006 from different subspecialties at the Aberdeen Royal infirmary, which was mainly from the gastroenterology ward-around 79% (n=364). 130 adult patients aged 19-92 years received a total of 1-77 vials. A total of 1056 total vials (each of which was 100ml) were issued . 4% (n=43) of the vials had to be discarded, as they were not required after being dispensed to the ward (as per MRHA regulations). Indications ranged variably but paracentesis in cirrhotic patients was the most common - 66% (n=304) of the total orders. 81% (n=855) of the total orders were considered appropriate. 14.96% (n=158) did not comply with evidence to support its use and this comes mainly from medical wards. The total expenditure for the amount of human albumin issued over the audit year was approximately £38300. It is estimated that approximately £4000 worth of human albumin has been wasted through discarded and inappropriately used vials. *Summary*. Our hospital practice is reasonably appropriate. This might well be due to the controlled 20% human albumin prescription by the medical officer at the NESNBTS. Although these results are encouraging there still remains space for improvement through further educational measures. The use of 20% human albumin remains controversial even to this date. We still require more well-conducted, large scale, randomised controlled trials of sufficient statistical power to determine its indications. However till then, we are bound to justify and rationalise its use.

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HEMOPHAGOCYTIC LYMPHOHYSTIOCYTOSIS PRESENTED AS A COMPONENT OF MULTIPLE ORGAN DYSFUNCTION SYNDROME IN PEDIATRIC INTENSIVE CARE UNIT

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Background. In Pediatric Intensive Care Unit (PICU) multiple organ dysfunction syndrome(MODS) is a frequent diagnosis. Most of the patients with MODS are secondary to sepsis. Hemophagocytic lymphohystiocytosis is a rare disorder which can cause a sepsis like syndrom with multiple organ dysfunction. *Aims*. Here we describe 7 children presented with HLH and MODS to PICU of a tertiary care center to notice that HLH can be presented as a component of MODS. Unless the diagnosis of HLH is considered and appropriate treatment started it can be fatal. *Methods*. All patients records who admitted to PICU of Ege University Hospital between December 20005 and February 2007 were reviewed. The records of the patients presented with MODS and HLH at the admittion to PICU were evaluated retrospectively. *Results*. Seven children (female/male= 4/3) were admitted to PICU of Ege University Hospital because of prolonged fever of unknown origin, pancytopenia and MODS between December 2005 and February 2007. The median age at the admittion was 13 months (range 3 months -15 years). Five of the patients had 6 organ dysfunction, one had 5 and the other had 3. The lowest PELOD score was 12 and the highest was 62 with a median of 51. All of the patients met the diagnostic criteria of Hemophagocytic lymphohystiocytosis (HLH) and in all patients Bone marrow aspiration revealed hemophagocytosis without malignancy. All the patients required mechanical ventilation, 6 out of 7 were supported by inotropic±vasopressor agents. Neurologic system involvement was seen in 6 patients and all had a Glascow Coma Score below 7, and two had seizure at the admittion to PICU. Severe clinical hemorrhage was seen in all patients at the presentation. Of the 7 children, 4 (%57.1) died. Two of these children expired just after they were admitted to PICU. They had the highest PELOD scores in this small series (62 and 61). Other 2 patients expired during the first days of HLH- 2004 treatment protocol. Among the patients survived, one with brucellosis was given only anti Brucella treatment and IVIG. His clinical and laboratory findings resolved rapidly. This patient had the lowest PELOD score at the time of diagnosis. Other two patients were put on HLH- 2004 protocol and they are still in remission. *Conclusions*. HLH is a rare disorder with a high mortality rate. HLH can share the same pathophysiologic elements with MODS and severe sepsis, septic shock. Clinician should be aware of the diagnostic criteria of HLH. In the presence of prolonged fever and pancytopenia in PICU patients who presented with MODS the diagnosis of HLH should be recognized.

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CLINICAL EFFECTS OF 5-AZACITIDINE FIVE DAYS/MONTHLY SCHEDULE IN THREE SYMPTOMATIC LOW-RISK (IPSS: 0-1) MYELODISPLASTIC PATIENTS

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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 75mg/mq/day subcutaneously 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 tolerability in low risk MDS patients. Between May and December 2006 we treated five patients affected by refractory anemia (RA) with Low Risk IPSS (score 0-1). Age at diagnosis ranged between 66 and 73 years. All patients failed EPO therapy and were in chronic red blood cell (RBC) supportive care with a median transfusions requirement of 4 units/monthly. The 5-Aza five days/monthly schedule was administered for a total of 8 courses. The response treatment criteria was according to International Working Group (IWG) as reported by Cheson *et al*. Two months after the end of therapy (8 courses) the evaluation of response was completed in 3 out of 5 patients. An hematologic improvement (HI) was observed in two patients, both reaching a major erythroid response (major HI-E), with no longer needed transfusions and RBC transfusion independence of 20 and 16 weeks respectively. The third patient obtained a transitory major HI-E after the 2th course of treatment, maintaining a transfusion independent time for 16 weeks and increasing haemoglobin greater than 2 g/dL; he failed after the 7th course of therapy. Quality of life (QOL) measured by the FACT-An score improved in all patients. Extrahematologic toxicity was mild and consisted in nausea and vomiting (WHO grade I) in two patients and flu-like syndrome with fever (WHO grade I) in one patient. Hematologic toxicity consisted in neutropenia (WHO grade III) and thrombocytopenia (WHO grade II) in one patient; it was transitory and no delay of treatment was necessary. Our preliminary results show that the 5-Aza five days/monthly schedule is very well tolerated and it appears to have an efficacy similar to the seven days/monthly schedule, at least in low-risk MDS setting. Considering that the optimal schedule and duration for demethylating agents has not yet been established, further MDS patients recruitment is warranted to confirm the efficacy of this alternative 5-Aza low dose regimen.

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APOPTOTIC CHARACTERISTICS OF MESENCHYMAL STROMAL CELLS FROM BONE MARROW OF CHILDREN

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Background. Mesenchymal stromal cells (MSC) represent a novel promising field in transplantation. Their relatively easy and quite effective *in vitro* expansion makes them even more attractive. The aim of the study was to assess the effect of pro apoptotic conditions and that of long - term expansion through serial passages on the characteristics of MSC from children. *Material-Methods*. Sixteen bone marrow (BM) mononuclear cell cultures from children with benign hematological disorders (n=10) and solid tumours without BM involvement (n= 6) have been initiated and were assessed for 10 passages. The expression of CD105, CD146 and CD95 as well as apoptosis by 7AAD staining, were evaluated by flow cytometry. In every passage CFU-F assay was performed and the cell doubling time was calculated. Cell cycle characteristics were assessed by propidium iodide staining at P2 and P6. *Results*. CFU-F counts ranged from 40.71+4.3 in P1, to 15.5 + 6.7 in P10. The cell doubling time was found to be 2.01±0.14 days at P1, increasing to 3.5±1.19 days at P8. A low percentage of apoptotic and dead cells was detected by 7AAD staining at P2 (3.0±0.6%) and this did not change until P10. In order to test if CD95 is functional, induction of apoptosis was triggered by the addition of different concentrations of an agonistic antibody (20ng and 1 microg/mL, anti-Fas CH-11) under standard culture conditions. A minor effect on MSC survival was observed at the highest anti-Fas concentration used. Additionally, serum deprivation of MSC for up to 72 hours revealed no substantial apoptotic effect. According to the cell cycle analysis, cells at P2 are mostly at GoG1 phase (72.9 + 8.4%, S phase: 19.6 + 7.4%) while at P6 there is a slight increase in the cells that have entered cell cycle (GoG1 64.5+7.5% and S 24.4 + 5.8%). *Conclusions*. It seems that MSC from children with benign hematological diseases and solid tumors without BM involvement, retain their functional characteristics throughout serial passages and are very stable under conditions that usually cause apoptosis although progression in passages results in a lower number of cells maintaining quiescence. This could be very useful in the setting of transplantation as long as serum deprivation for up to 72 hours does not seem to affect *in vitro* expanded MSC, while, in the clinical setting, avoiding the use of serum protects from the possible risk of immune responses attributable to serum contamination. These features make MSC, especially those of early passages, optimal candidates for use in transplantation and cellular therapy.

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EPIDOMIOLOGIE STUDY OF ACUTE LEUKEMIA IN TUNISIA (MULTICENTRIC NATIONAL STUDY)

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This is a retrospective multicentric national study, in which we present epidemiologic data concerning Acute Leukemia cases followed up in all haematology departments in Tunisia during a ten year period (1995-2004). 1801 acute leukaemia cases are recorded : 1037 acute myeloid leukaemia (AML) (57,6%), 752 (41,8%) acute lymphoblastic leukaemia le (ALL), 5(0,3%) biphenotypic and 7 (0,4%) undifferentiated. The national incidence is 1,9 cases per 100 000 habitants per year ; i.e 1,1 for AML and 0,8 for ALL. The incidences in the differents *gouvernorats* (districts) vary from 1 per 100 000 inhabitants per year in Kebily (south) to 2,66 in Beja (north), the difference being statistically significant ($p<$

0,001). The mean annual number of cases is 180 (104 AML, 75 ALL), and it varies from 166 to 197 per year. The monthly number of cases is 150, and it varies from 121 cases in September to 179 cases in May, the difference is not significant. The number of cases at different ages shows a great incidence at the ages of 0-5 years (11%), 6-10 years (11,8%) , 11-15 years (11,4%), and 16-20 years (10,9%). As compared with the incidence at older ages, the difference is statistically significant. When the cytological types are considered, we noted that for the ALL, the type L1 was the most frequent, (63,9%), the difference between type 1 and type 2 incidences is statistically significant (p<0,001). For the AML, the most frequent types were M2=31,6% , M1:25,7%, and M5 : 16,7% . There was not a significant relation between the AML type and the age. The incidence of the various types of ALL and AML resembles that noted in the literature. The incidence of AML reported here is lower than that noted in the literature (>3 for 100 000 habitants). This is probably because a number of cases of AML in old patients are not diagnosed and not recorded. In contrast, the incidence of ALL in our series is very close to that reported in the literature.

1299

JAK2 MUTATIONAL STATUS IN THE DIAGNOSTIC WORK-UP OF PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS

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Background. Splanchnic vein thrombosis (SVT) including Budd-Chiari syndrome (BCS) and extrahepatic portal vein obstruction, is associated with thrombophilic abnormalities and/or underlying chronich myeloproliferative disorders (CMPD). Whereas the diagnosis of thrombophilia is relatively simple, that of CMPD is sometimes difficult due to concomitant hypersplenism and/or hemodilution, that mask the clinical and laboratory features of classic CMPD. Using the classical criteria for the diagnosis of polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (IMF), an overt CMPD can be recognized in about 30% of patients with SVT, while in 25% of patients no underlying cause is found and these forms are therefore referred to as idiopathic SVT. However, based on the results of erythroid and megakaryocytic colony studies, it has been suggested that latent CMPD can be identified in about 80% of idiophathic SVT. Recently, a novel point mutation in the autoinhibitory JH2 domain of the JAK2 gene (V617F) has been found in about 90%, 75% and 50% of PV, ET and IMF patients, respectively. The high specificity of such genetic lesion for CMPD can be used for the diagnostic work-up of occult CMPD in SVT patients.

Table 1.

PT	SEX	AGE	DIAGNOSIS	JAK-2 V617F	EEC
1	M	32	BCS	POS	POS
2	F	43	BCS	POS	POS
3	F	47	BCS	POS	POS
4	F	38	BCS	POS	N.A.
5	F	30	BCS	POS	N.A
6	M	25	BCS	NEG	NEG
7	F	46	BCS	NEG	NEG
8	F	43	Portal vein thrombosis	POS	POS
9	F	41	Portal vein thrombosis	POS	POS
10	M	41	Portal vein thrombosis	POS	N.A
11	F	25	Portal vein thrombosis	NEG	NEG
12	F	57	Inferior mesenteric vein thrombosis	NEG	NEG
13	F	66	Inferior mesenteric vein thrombosis	NEG	NEG
14	M	56	Inferior mesenteric vein thrombosis	NEG	NEG
15	M	37	Portal cavernoma	NEG	NEG
16	M	37	Portal cavernoma	NEG	NEG
17	F	14	Portal cavernoma	NEG	NEG
18	F	41	Splenic vein thrombosis	NEG	NEG

Aims. The aim of this study was to evaluate the incidence of occult CMPD in a cohort of 18 patients with idiopathic SVT. *Methods*. DNA was extracted from peripheral blood and the JAK2 mutational status was investigated by allele specific PCR. All patients were negative for thrombophilia screening including genotyping for prothrombin (G20210A), and factor V Leiden (G1691A) mutations, anti-phospholipid antibodies, hyperomocysteinemia, and antigenic assay for protein C and S and ATI-II. SVT was diagnosed by doppler ultrasonography, CT-scan or venography. *Results*. The main clinical data, JAK2 mutational status and results of endogenous erythroid colony (EEC) study in the analysed series are shown in the Table 1. All patients had normal blood counts. The JAK2 V617F was detected in 8/18 patients, including 5/8 with BCS and 3/4 with portal vein thrombosis. In JAK2 mutated cases, EEC were positive in 3/5 patients with BCS and 2/3 patients with portal vein thrombosis. EEC were negative in all patients wild type for JAK2. Bone marrow histology was performed in 6 patients, (5 JAK2V617F and 1 JAK2 wild type) and was consistent with a diagnosis of PV, undefined CMPD and IMF in 2, 2 and 1 cases, respectively, while it was non diagnostic in one case. *Conclusions*. These results confirm the value of JAK2 mutational screening to unravel occult CMPD and suggest a high prevalence of this genetic aberration in patients with SVT.

1300

THE CALM INTERACTOR CATS, WHICH INFLUENCES THE SUBCELLULAR LOCALIZATION OF THE LEUKEMOGENIC FUSION PROTEIN CALM/AF10 IS A MARKER FOR PROLIFERATION

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CATS is the CALM interacting protein expressed in thymus and spleen. The CATS interaction region of CALM is contained in the leukemogenic fusion protein CALM/AF10. CATS increase the nuclear localization of CALM/AF10 and sequesters the fusion protein in the nucleolus. The murine Cats is highly expressed during embryogenesis where it is enriched in the CNS and expression decreases gradually while embryos develop to later stages and is confined to thymus and ovary of the adult organism. Cats is widely expressed in the hematopoietic compartment and an striking up-regulation of Cats transcript was observed in $B220^+$ positive cells derived from a CALM/AF10 murine bone marrow transplant model in comparison to the same population of a non-leukemic mouse. We confirmed expression of endogenous CATS in human thymus and show strong expression in leukemia, lymphoma and tumor cell lines but not in non-proliferating T-cells. CATS expression is regulated in a cell cycle dependent manner and is induced by mitogens. The clear upregulation of CATS protein upon mitogenic activation and its high expression in proliferating but not in quiescent cells suggest a role of CATS in the control of cell proliferation. CATS-CALM/AF10 interaction might thus play an important role in the CALM/AF10-mediated leukemogenesis.

1301

R-FMD VS R-CHOP TREATMENT AS FIRST LINE THERAPY FOR FOLLICULAR LYMPHOMAS: A SINGLE CENTER EXPERIENCE

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Background. High response rates in follicular lymphoma (FL) with the *FMD* protocol have been previously reported. The monoclonal anti-CD20 antibody rituximab has been shown to induce a high response rate in FL patients and to improve outcome when associated with classic regimes (CVP or CHOP). *Aims*. We have evaluated the impact of R-FMD as compared to R-CHOP as a first line therapy in patients with follicular lymphomas, in terms of: complete response (CR), overall survival (OS), feasibility, toxicity and the efficacy of PCR molecular analysis in predicting clinical and molecular remission. *Methods*. Between September 2002 and June 2006, 40 pts with FL were enrolled in the study. Twenty patients (M/F: 10/10, median age 54 years) received R-FMD treatment in stage II-IV, FLIPI score: intermediate grade 10 pts, high grade 10 pts. R-FMD regimen was administered every 28 days for six cycles: Fludarabine 25 mg/m^2 e.v. days 1-3, Mitoxantrone 10 mg/m^2 day 1, Dexamethasone 20 mg days 1-3 and Rituximab 375 mg/m^2 day 1.Granulocyte colony-stimulating factor and Pneumocystis Carinii prophylaxis was given. PCR molecular analysis was performed in 12 patients at diagnosis, showing in 16 (80%) of them bcl-2 rearrangement. Twenty patients (M/F: 8/12, median age 58 years) received R-CHOP treatment in stage II-IV, FLIPI score: intermediate grade 8 pts, high grade 12 pts. The CHOP regimen consisted of doxorubicin 50 mg/m^2 on day 1, cyclophos-

phamide 750 mg/m^2 on day 1, vincristine 1.4 mg/m^2 on day 1, and oral prednisone 100 mg/d on days 1 to 5, preceded on day 1 by Rituximab 375 mg/m^2. This regimen were repeated every 21days for six cycles. Granulocyte colony-stimulating factor (G-CSF) and Pneumocystis Carinii prophylaxis was given. PCR molecular analysis was performed in 14 patients at diagnosis showing in 14 (70%) of them bcl-2 rearrangement. *Results*. Arm R-FMD: Out of all patients, 19 and 1 achieved CR and PR, respectively. The patient in PR achieved CR after Zevalin. Actually, after a median follow up of 28 months, all patients resulted in CR. At the end of treatment, bcl-2 appeared to be negative in 15/20 pts (75%). The toxicity was mild with grade 3-4 neutropenia in 6 pts (30%). CMV infection was observed in 1 patient. *Arm R-CHOP*: Ninenteen patients achieved CR and 1 resulted non responder. Out of all 19 pts in RC: 1 died in CR for infection and 1 relapsed after 23 months. After a median follow up of 28 months, 18 patients are still alive and 17 of which are in continue CR. Grade 3-4 neutropenia was observed in 4 (28%) pts. At the end of treatment bcl-2 appears to be negative in 6/9 pts (66%). *Conclusions*. Our data demonstrate that both frontline R-FMD and R-CHOP treatments produce high rate of response in terms of CR, OS and molecular remission (although a more positive trend was observed in R-FMD Arm) and had very low toxicity. A prolonged follow-up will be needed to determine the long-term efficacy of these combinations.

1302

R-COMP VS R-CHOP IN THE TREATMENT IN NEWLY DIAGNOSED ELDERLY PATIENTS WITW AGGRESSIVE NON-HODGKINS LYMPHOMA

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Background. Conventional anthracyclines are active in non-Hodgkin's lymphomas(LNH), but cardiotoxicity related to the cumulative dose may limit their use. Preclinical studies determined that encapsulating conventional anthracyclines in liposomes can reduce the incidence and severity of cumulative dose-related cardiomyopathy while preserving antitumor activity. *Aims*. In this study we have evaluated the impact of R-COMP as compared to R-CHOP as a first line therapy in newly diagnosed ederly patients with aggressive LNH, in terms of complete response (CR), overall survival (OS) and toxicity (especially cardiac safety) substituting for conventional doxorubicin with non-pegylated liposomal doxorubicin (Myocet) in the CHOP regimen. *Methods*. Between January 2005 and June 2006, 40 pts with aggressive LNH were enrolled in the study. Twenty patients : 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. Baselin median left ventricular ejection fraction (LVEF) was 58(range 50-68). R-COMP regimen was administered every 21 days for six cycles: Rituximab 375 mg/m^2 day 1, Cyclophosphamide 750 mg/m^2 day1, Myocet 50 mg/m^2 day 1, Vincristine mg/ m2 day 1, and oral Prednisone 100 mg/d on day 1-5. *Twenty patients*: M/F 8/12, median age 66 years (range 57-70) received R-CHOP treatment in stage III-IV, IPI score: intermediate grade 14 pts, high grade 6 pts. Baseline median LVEF was 62% (range 55-70). The CHOP regimen consisted of Doxorubicin 50 mg/m^2 on day 1, Cyclophosphamide 750 mg/m^2 on day 1, Vincristine 1.4 mg/m^2 on day 1, and oral Prednisone 100 mg/d on days 1 to 5, preceded on day 1 by Rituximab 375 mg/m^2. This regimen were repeated every 21 days for six cycles.Granulocyte colony-stimulating factor and Pneumocystis Carinii prophylaxis was given in both regimen. *Results*. Arm R-COMP: An overall response 15 (75%)CR, 4 (20%)PR and 1(10%) NR was achieved in all patients. After a median follow up of 13 months, all 20 (100%) pts resulted alive. At the end of treatment, LVEF median was 57,50% . Any cardiac event in relationship to the therapy.The toxicity was mild with grade 3-4 neutropenia in 4 pts (20%). Arm R-CHOP: 14 pts (70%) achieved CR , 4(20%) PR, and 2(10%) resulted non responder. Out of all 14 pts in RC: 1 died in CR for infection, 1 relapsed after 10 months. After a median follow up of 13 months, 19 (95%) pts are alive, 12 (60%) of which are in continue CR. Grade 3-4 neutropenia was observed in 4 (28%) pts. At the end of treatment LVEF median was 58%. *Conclusions*. The substitution of Doxorubicin with Myocet into the R-CHOP regimen (R-COMP) at an equivalent dose is anapplicable and active regimenn in elderly patients with newly diagnosed aggressive LNH and the significant difference in median post-chemotherapy LVEF (R-COMP 58>57,5; R-CHOP 62 > 58), show a lower cardiotoxic effect. A long-term follow up and more patients will be necessary to confirm our preliminary observations.

1303

OVER AND DOWN EXPRESSION OF GENES TRIGGER THE ACTIVATION OF DIFFERENTIATION AND PROLIFERATION OF SIGNALLING PATHWAYS IN PLASMA CELLS OF MULTIPLE MYELOMA

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Background. The molecular mechanisms involved in multiple myeloma (MM) are still not completely clarified. Recent leaps in comprehension of the intracellular pathways and the complex interaction with the bone marrow microenvironment have contributed for elucidating its physiopathology. We previously studied the global gene expression in malignant plasma cells (MPC) by serial analysis of gene expression (SAGE) and found abnormalities in expression of several genes potentially involved in disease. *Aims*. In the current study, we selected and quantified the expression of nine genes (IL6-ST, CD19, CD40, PRDM2, CFOS, FOSB, DUSP1, CJUN and CCND1) with potential action in MPC maturation, proliferation, and survival. *Methods*. Purified samples of MPC and normal plasma cells (NPC) were obtained from 14 MM patients and one healthy control using the CD138 antibody MACS microbeads and column magnetic sorting. The gene expression was investigated in MPC and NPC by quantitative polymerase chain reaction real-time (RT-PCR) using the B-actin and GADPH genes to normalise reactions. The cut off ratio for the comparison of profiles was set as 2-fold over expressed or 2-fold under expressed for each gene, with a statistical likelihood of $p<0.01$. *Results*. We observed that six out of nine genes were over expressed (IL-6-ST, CFOS, FOSB, DUSP1, CJUN, CCND1) and the remaining three genes were down expressed (CD19, CD40, PRDM2) in MPC. The over expressed genes were related mainly to PC proliferation. The under expressed CD19 and CD40 genes in MPC were previously related to differentiation of B cells into NPC, and the PRDM2 gene was described as an inhibitor of the tumour suppressor gene RB. We postulated that binding of interleukin-6 to its receptor (IL-6-st), up expressed in our study, trigger the activation of proliferation signalling pathways, the janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3), the Ras/mitogen-activated protein kinase (RAS/MAPK), and the phosphatidylinositol-3 kinase/protein kinase B (PI-3K/AKT) by activation of CFOS, FOSB, DUSP1, CJUN and CCND1 genes, which were over expressed in our study. In contrast, the lower activity of the CD19, CD40 and PRDM2 genes, seen in our cases, may compromise MPC maturation and proliferation by inhibition of CDC42, XBP-1 and RB pathways in MM, respectively. Farnesyltransferase inhibitors and rapamycin represent novel classes of signal transduction inhibitors targeting principally the RAS/MAPK and PI-3K/AKT pathways, and resveratrol inhibits proliferation, induces apoptosis and overcomes chemoresistance through under regulation of JUN-FOS family genes and JAK/STAT3 pathway. An anti-IL-6 monoclonal antibody has also been shown to have effects in MPC in both animal models and human preclinical trials. These findings suggest that specific therapies for MM could be develop based on abnormal expression of genes in MPC. *Conclusions*. We identified abnormalities in expression of several genes involved in differentiation and proliferation signalling pathways in MPC in this study. We also described, for the first time, the over expression of DUSP1 and down expression of PRDM2 genes in the disease. These findings may contribute to comprehension of the MM physiopathology as well as to the identification of new therapies for the disease.

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1304

EARLY FDG-PET SCAN IN ADVANCED STAGE HODGKIN LYMPHOMA TREATED WITH BEACOPP-14

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Background. Therapy of Hodgkin lymphoma (HL) is designed to prolong progression-free survival and minimize toxicity. The best regimen to achieve this has not yet been defined. the German Hodgkin Study Group (GHSG) developed the BEACOPP regimen for further improving the outcome of patients with advanced Hodgkin's lymphoma (HL). Since then, BEACOPP has been introduced in 3 different prospective randomized clinical trials of the GHSG to find an equilibrium between maximal efficacy

and least toxicity with the BEACOPP principle for the treatment of advanced stage HL. Recently, an interim FDG-PET study was suggested that FDG-PET after two cycles of CT is the most powerful tool available for predicting treatment outcome in HL *Purpose*: To evaluate the efficacy of a time-dense regimen BEACOPP-14 in naïve patients with advanced HL They were assigned to therapy according to defined risk. Patients were defined depending on the International Prognostic Score (IPS). Those with IPS of 3 or higher received 2 cycles of BEACOPP-14 making a FDG-PET after them in order to decide the total cycles. Design: observational, prospective trial in a consecutive and previously untreated patients diagnosed of HL non Lymphocyte Predominant with advanced stage in one centre. Exclusion criteria: HIV positivity, other malignancies and CNS involvement. *Patients and Methods*. Since September 2005 to December 2006, 10 patients were included in a bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP-14) regimen administered in outpatient setting. At baseline assessment: demographic data, clinical and physical exam, blood counts, serum and urine biochemistry, body scan, bone marrow biopsy. Patients were classified according IPS and clinical stage. All patients receiving prophylaxis with pegilated granulocyte factor on day +4. Response was evaluated by FDG-PET after 2 cycles and classified as: complete remission (CR), partial remission (PR), and non response (NR). Patients with CR received 2 additionally cycles and patients on PR 6 cycles. Adverse effects were registered. Statistical analysis: Survival analysis was performed using Kaplan-Meier and Cox regression. Overall survival (OS), relapsed free survival (RFS). *Results*. 9 valuable classic HL patients Mean age 27.6 y (17-51), male 6 and female 4. IPS 4 (3), 5(4). Bulky disease 2 patients. 1). FDG-PET after 2 cycles: positive 4 patients, negative 5. Total cycles: 4 in 4 patients, 8 in 3. Response (5 valuable patients): 3 CR, 1 PR, 1 NR. Toxicity: anaemia 6 (all received erythroid agents), neutropenia 1, respiratory infection 2, erythema 1, peripheral neuropathy 1, acute hearth infarction 1. Cycles delayed: 10 in 5 patients. Early relapse: 1 (on 6 month). No death have been observed. Mean OS was 6.6 months (2-12) and mean RFS 5 (1-9). *Conclusions*. Overall response has been 80% (CR60%). Adverse events are frequent (78%) and we had to delay cycles in 50% of patient by myelotoxicity. It necessary more patients and a longer follow-up to know the effectiveness of BEACOPP-14 and the value of FDG-PET after 2 cycles of BEACOPP-14 to predict response.

1305

RESPONSE TO BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA COHORT

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Background. Bortezomib has been shown to be effective in multiple myeloma (MM), but there is limited experience in response to re-treatment. *Aims*. To evaluate the efficacy and safety of Bortezomib in an every day clinical use in refractory/relapsed MM between December 2003 to November 2006 in a single institution. *Patients and Methods*. 43 patients with relapsed/refractory MM receiving Bortezomib alone (1,3 mg/m^2 on days 1,4,8,11 in a 21-day course) as second or more line of therapy. The response was evaluated according EGBMT criteria (Bladé J, Samson D, Reece E *et al.*). Patients without response after 4 courses and patients that relapsed after reached CR or PR with Bortezomib alone, a combination of bortezomib + dexamethasone (BD) or bortezomib+melphalan+prednisone (BMP) were administered. Adverse effects were registered. *Results*. 43 patients (males 41.8%), mean age 67.3 years (34-89), over 65 years (51.1%). Bortezomib was administered in second line: 14 (32.5%), in third or more: 29 (67.4%). Overall response: 31 (77.5%); CR+PR: 29 (72.5%); MR: 2 (5.0%); CR: 16 (40.01%); CR-EIF negative: 11 (27.5%); failure: 9 (22,5%), mean courses to reached response: 3.6. No relation to response and presence or not chromosomal aberrations. At 36 months on follow-up, of 40 valuable patients, 18 (45%) are in stable response without therapy. Seven patients (16.3%) do not reached response after 4 courses and received a Bortezomib combination therapy, 10 patients (23.2%) were relapsed after a mean of 18 months follow-up in stable response. In 12 patients (70.5%) a combination of BD was applied and 5 patients(29.5%) received BMP by relapse or progression. Responses: group BD 1 CR EIF+, 7 PR, 4 F; group BMP 1 CR-EIF-, 2 PR, 1 F, 1 NV. 14 patients died by progression or infectious complications (35%). *Adverse events*. thrombocytopenia 40% (grade III: 20), fatigue 25%, peripheral neuropathy 37.7%, constipation 35%, diarrhoea 22.5%, ZHV 15%, non documented infection 35%, fever 12.5%, hypotension 5%, leucopenia grade 3 15%. Only 3 patients (7.5%) need disrupted therapy by toxicity. No more adverse events were observed in patients treated with bortezomib in combination. *Comments*. In our experience a high response to Bortezomib in an every day clinical use in relapsed/refractory MM was observed. In addition re-treatment with Bortezomib in combination induces response (64.7%). The safety is good with tolerable adverse effects. It is necessary prolonged follow-up time and to perform more studies in order to establish the best schedule for relapsed/refractory MM.

1306

EXPRESSION OF THE MICROVESSEL DENSITY, GP-130, VCAM-1, AND KI67 WITHIN THE BONE MARROW COMPARTMENT IN MULTIPLE MYELOMA

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In multiple myeloma (MM), the interaction between myeloma cells and bone marrow microenvironment has an important role in the pathogenesis of MM. The functional interplay between the myeloma cells and the surrounding microenvironment within the bone marrow (BM) includes increased activity of endothelial cells resulting in neovascularisation, and enhanced sensitivity to the IL-6 as a main growth factor in multiple myeloma (MM). This cytokine, as a member of gp130 family, binds on the surface of myeloma cells to the IL-6 receptor gamma chain that associates with the gp130 transducer chain providing the proliferation signal to the tumor cells. VCAM-1 is adhesion molecul from the immunoglobulin gene superfamily. High proliferative rate has been associated with worse survival. The aim of study was to investigate the correlation between expression of BM angiogenesis estimated as microvessel density (MVD), and expression of the transmembrane signal transducer, gp130, VCAM-1 , and proliferative rate of the myeloma cells in the bone marrow of MM patients (pts). The study included 60 newly diagnosed MM pts (33 male and 27 female pts, mean age 60 years, range 35-75). According to the clinical stage (CS, Salmon&Durie), distribution of MM pts was as follows: I 8pts, II 22pts, III 30pts. There were 35pts with IgG monoclonal (m) protein, 12pts with IgA, and 12pts with secretion of kappa/lambda chain. Renal failure had 17 pts. All pts were treated with standard chemotherapy regimens. BM vessels were visualized by immunohistochemical staining for CD34 on slides of formalin-fixed, paraffin-embedded BM biopsies. MVD was calculated by the number of vessels per 400x high-power microscopy field in the area of the most dense vascularization. All samples were further analyzed for the immunohistochemical expression of the gp130, VCAM-1 which showed cytoplasmic and membrane localization. The intensity of these stainings was graded as weak (0-30% myeloma cells), moderate (31-60% myeloma cells), and strong (>60% myeloma cells). We also analyzed the percentage of Ki67$^+$ MM cells. According to the CS of myeloma, positive correlation was found between MVD and expression of GP130 in myeloma cells. The expression of MVD was significantly higher in MM pts in III CS than in pts in I CS of myeloma (15 vs. 7,5/ x400 field, $p< 0.001$). Similarly, significantly higher expression of gp130 was found in pts in III CS of myeloma comparing to the MM pts in I CS (32 vs.15%, $p<0.05$). The percentage of Ki-67$^+$ cells in MM pts in III CS was higher comparing to CSI, II (10 vs. 5%, $p<0.001$). Pts with high VCAM-1 expression had significantly shorter survival (36 vs. 18m, log rank, $p<0.001$). These findings of increased angiogenesis and proliferation index found in IIICS of myeloma pointed out significantly shorter survival of those pts (26 vs. 43,5 m, log rank, $p<0.05$). Strong activity of angiogenesis in myeloma, combined with high IL-6 sensitivity by immunohistochemical expression of gp130, increased proliferative rate and high VCAM-1 expression, represents possible predictive factors of poor prognosis.

1307

THE CLINICAL SIGNIFICANCE OF T-CELL RECEPTOR GENE REARRANGEMENT IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Chronic lymphocytic leukaemia (CLL) is heterogenous disorder predominantly involving the B lymphocyte. However, clonal/oligoclonal T cell expansion has also been detected at a low frequency in B-CLL using a range of methodologies including Southern blotting and flow cytometric analysis. The underlying cause of this phe-

nomenon is unclear. It has been suggested that the observed T cell clonality may simply be related to the normal aging process or that it may result from the immune response to malignant cells or viral infection. During normal T cell maturation in the thymus, the T cell receptor (TCR) genes undergo a complex process of V(D)J segment rearrangement to produce a wide repertoire of antigen specificity. As it is an early event in T cell development, TCRG gene rearrangement is commonly used as a marker of clonality. The TCRB gene is also routinely investigated due to its greater combinatorial diversity. Lack of sufficiently sensitive detection methodology is often a limiting factor in the discrimination between clonal and polyclonal T-cell populations. *Aims*. The aim of this study was to use high resolution heteroduplex gel and fluorescence GeneScan analysis to screen for TCRG and TCRB gene rearrangements respectively in a cohort of B-CLL patients and to ascertain if the clonal rearrangement occurred in either B cells or T cells. *Methods*. B and T cells were purified from peripheral blood using a $CD19^+$ or $CD3^+$ magnetic bead system (autoMACS). DNA was extracted from purified B or T cells from 34 B-CLL patients and the TCRG and TCRB genes were amplified by BIOMED-2 multiplex PCR assays (InVivoScribe Technologies). Heteroduplex and GeneScan analysis were then performed to detect monoclonal T-cell expansion. *Results*. Clonal TCR gene rearrangements were detected in11/34 (32%) of cases in purified T cell fractions. No clonal TCR gene rearrangements were found in purified B cell fractions. Clonal TCRB rearrangements were found in 10/34 (29%) and clonal TCRG in 8/34 (23%). In most instances a weak clonal pattern was observed. Clonal TCR rearrangements were detected in 7 males and 4 females. Five patients had mutated IGVH genes, while the remaining 6 possessed unmutated IGVH genes. Seven patients presented with more advanced clinical stage (Binet B or C), and 8 patients received treatment. *Summary/conclusions*. These results are in agreement with previous work demonstrating that T-cell clonal/oligoclonal expansions occur frequently in B-CLL patients. However this is the first report to demonstrate that the clonal expansions occur in T cells and not in B cells. In most instances a weak clonal pattern was observed which was probably due to minor clonal T-cell populations. It is possible that T-cell clonality may be associated with a poorer prognosis in this disease category. Of interest, we found that 8/11(73%) patients in our B-CLL sub-group with T-cell expansion also had advanced stage disease and/or unfavourable molecular (IgVH gene usage/mutational status) markers. We are currently investigating the possibility of a specific antigenic stimulus resulting in this clonal T-cell expansion.

1308

INCIDENCE OF SECOND NEOPLASMS IN CHRONIC LYMPHOCYTIC LEUKAEMIA: INFLUENCE OF PROGNOSTIC BIOMARKERS

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Background. B-cell chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in the west, with a median survival of approximately 10 years. It is a heterogenous disorder with a highly variable clinical course and it is well recognized that patients with CLL are at a higher risk of developing second malignancies when compared to age and sex-matched controls. IGVH mutational status provides prognostic information in CLL, with unmutated IGVH status conveying a poor prognosis in comparison to mutated IGVH genes. *Aims*. To determine what influence the mutational status of the IGVH gene, gene usage and acquired cytogenetic aberrations play in the development of a second malignancy in patients with CLL. *Methods*. Three hundred and twenty patients were recruited from the Haematology Outpatient Clinic and surrounding regional hospitals. Clinical staging, immunophenotyping, lymphocyte doubling time (LDT) and time to treatment (TTT) were available on all patients. IGVH mutational status and gene usage were determined using multiplex BIOMED-2 primers (InVivoScribe Technologies) and sequence analysis. Chromosomal abnormalities were determined using interphase fluorescence *in situ* hybridisation (FISH). *Results*. Results revealed that solid tumours occurred in 57 patients (18%) of which 24 were second malignancies and 33 were malignancies occurring before the diagnosis of CLL. The second malignancies occurred in the following sites: skin (14) lung (4), breast (2), bowel (2), prostate (1), and 1 case of stomach cancer. Median time from diagnosis of CLL to second malignancy was 48 months (10-204 months). Eighteen patients were male and six were female. Interestingly 14 patients had mutated IGVH genes, while the remaining 10 possessed unmutated IGVH genes. No specific gene usage was associated with second malignancies. Material for FISH analysis was available on all cases. The mutated case showed no detectable abnormality (n=6) and biallelic/del13q14 (n=8). The unmutated sub-group consisted of: biallelic/del 13q14 (n=6) and trisomy 12 (n=4). Nine patients presented with more advanced clinical stage (Binet B or C), and 12 patients received treatment. *Summary/conclusions*. It is well documented that patients with CLL have an increased risk of developing second malignancies, over and above that expected for a matched age group. This may be due to several factors, either singly or synergistically, including defective immune surveillance due to an impaired adaptive immune system associated with CLL, or it may be treatment related, associated with, for example, the use of alkylating agents such as chlorambucil. Our data shows that the second malignancies occur in both treated and untreated patients. Secondary neoplasms are also found in mutated and unmutated cases. Our data suggests that the development of a secondary neoplasm is dependent on the length of disease duration rather than therapy duration.

1309

THE REVERSE CORRELATION OF $CD38^+$/CD62L- VERSUS $CD62L^+$/CD38- IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. CD38 is a transmembrane glycoprotein expressed on the surface of leukemic cells in a significant percentage of patients with B-cell chronic lymphocytic leukemia (B-CLL). A recent study suggested that CD38 expression has negative prognostic value in CLL.The leukocyte selectin (CD62-L) is expressed on hematopoietic stem-progenitors and mediates adhesive interactions with other receptors. *Aims*. The aim of this work was to study the correlation between the expression of $CD38^+$ and $CD62L^+$ on the pathogenic B-CLL cells. *Methods*. Peripheral blood samples from 40 patients with B-CLL were analyzed by flow cytometry for CD38 and CD62L expression on $CD5^+CD19^+$ leukemic cells. We chose to consider only typical B-CLL patients, having a score of 4-5, according to the classification of typical/atypical B-CLL proposed by Matutes *et al.*1994. The analysis was performed by the FACScan Flow cytometer (Becton-Dickinson, Mountain View, CA) and CellQuest software (Becton-Dickinson). In our patient cohort, CD38 expression was evaluated as a classical diagnostic marker. Considering the CD38 antigenic expression, the patients were classified into two groups: those with > or = 20% were considered positive ($CD38^+$) and those with < 20% were considered negative (CD38-). Our study focused on the expression of CD62L on these two groups. *Results*. CD38 was expressed in 20% or more of leukemic cells in 17 patients (42,5%), while 23 patients (57,5%) were negative for CD38. The over-expression of CD62L was detected on 17 patients (74%) who were negative for CD38. Six patients (26%) of this group had low expression of CD62L.On the other hand patients who were positive for CD38 had absolute lack of expression of CD62L (100%). Patients with $CD38^+$ samples have significantly aggressive disease regardless of their clinical stage. The group of the patients with expression of CD62L has good clinical progress by far. *Conclusions*. The over-expression of CD62L is usually associated with the absence of CD38 and represented the immunophenotypic signature of good prognosis in B-CLL. CD62L is an adhesion molecule, which is involved in the cross-talk between B-lymphocytes with neighboring endothelial and/or T-cells within the lymph node microenvironment. CD62L may be used as a diagnostic/prognostic marker for B-CLL, but more studies are necessary

1310

AQUAGENIC PRURITUS IN POLYCYTHEMIA VERA (PV): HOW IT INFULENCES QUALITY OF LIFE AND HOW IT CAN BE TREATED: FIRST RESULTS OF A WRITTEN SURVEY OF 123 PATIENTS IN GERMANY

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Background. Aquagenic pruritus (AP) is a debilitating condition occuring in patients with PV. It is characterised by strong itching or stinging following contact with water without visible changes of the skin. Treatment of this condition is difficult, but very important as it affects quality of life in affected patients and is responsible for most of the suffering in polycythemia vera. Very little is known about the frequency of this symptom, its precise description, its influence on the quality of life and its optimal treatment. *Aims*. For these reasons we intended to document

the clinical features of AP and its impact on quality of life in a large cohort of German patients with PV. *Methods*. Hematologists in Germany were invited to distribute anonymous questionnaires to their PV patients. The questionnaires contained questions to assess PV status, AP characteristics and treatment, as well as a standardised EORTC QLQ-C30 questionnaire to assess quality of life. Also, an electronic web-based version was created and provided to a patient support group. *Results*. 123 patients (67 females, 56 males; mean age 61 years (range: 28.13 - 87.42)) with PV responded and returned the questionnaires. Of these 81 had a history of AP. In 52 patients (64.2 percent of AP patients and 42.3 percent of all patients), AP occured prior to diagnosis of PV, but only in 5 patients this led to the diagnosis of PV. 5 patients developed AP after diagnosis of PV. AP is perceived as an itching sensation in 64 patients (79 percent), tickling in 15 (18.5 percent), stinging in 18 (22.2 percent) and burning in 14 (17.3 percent). 36 patients (44.4 percent) report warm water to cause stronger pruritus than cold water, whereas only 7 patients (8.6 percent) report the contrary and 31 patients (38.3 percent) do not feel a difference. In 83 percent of patients pruritus starts less than 10 minutes after water-contact. Only in 36 patients (44.4 percent), pruritus has improved or ceased with therapy of PV (only 7.4 percent reported cessation of pruritus). Overall quality of life is decreased in patients with AP (56 points out of 100) as compared to PV patients without AP (65 points out of 100). *Conclusions*. AP is a frequent symptom in PV which has a strong impact on quality of life and can only be resolved in 7.4 percent of patients by PV therapy. Also, AP seems to be a predictor of PV, occuring in 42.3 percent of all PV patients before diagnosis of PV. The small number patients in which this actually led to diagnosis of PV gives reason to concern and room for improvement.

1311

THE ABO, RHESUS AND KELL SYSTEM POLYMORPHISM AND THE DISTRIBUTION OF WEAK D PHENOTYPES OF RHESUS BLOOD GROUP SYSTEM IN THE GREEK POPULATION

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The ABO and Rhesus blood group systems are involved in the newborn's haemolytic disease, the transfusion reactions, and the autoimmune haemolytic anemia. D antigen of the Rh system is now considered to be a mosaic of epitopes. Among Europeans 1% carries RHD alleles as weak D and partial D. These different phenotypes have distinct immunohematologic characteristics. During the last years several DNA typing methods have been developed to complement routine serological typing for determination of polymorphisms in the ABO, RH, Kell, JK and FY blood group genes. The purpose of this study was the RHD genotyping in a Greek population sample and also the study of phenotype and gene frequencies of ABO, RhD and Kell systems among 4034 blood donors. Since 2003-2005, 4034 samples from a Greek population were phenotyped by standard serologic techniques for the ABO, RhD, Kell blood group systems and genotyped with the use of PCR-SSP techniques by an established polymerase chain reaction protocol (weak D-SSP, INNO-train). Data were collected for their age and ancestry. From the 4034 individuals, nine with Rh phenotypes Cce, ce, Cce, Cce, Cce Cce Cce Cce Cce, were characterized as weak D (ID-Card Anti-D (human), DiaMed) and were further investigated using ID-Partial RhD-Typing Set and genotyped with the use of PCR-SSP techniques (weak D-SSP, INNO-train). Genomic DNA was extracted from whole blood collected with EDTA and six RhD specific primers sets were used. The PCR products were visualized via horizontal gel detection. Five of them were genotyped as weak D type 1, two as weak D type 3 and two couldn't be typed with those primers and they needed further investigation. Gene frequencies were found as follows: ABO*O = 0,623, ABO*A = 0,274, ABO*B = 0,104, RH*D = 0,6668. Kell allele frequencies were: K: 0,031 and k: 0,969. The frequency of the Cw antigen in our samples was 2,4%. Phenotype frequencies for the blood groups were in agreement with Hardy-Weinberg equilibrium expectations. Important variations have not been observed between different regions of the country. Our survey is reported in the hope that it may find some use as reference for studies of blood group systems and indicates as many others studies that molecular classification of weak D offers a more reliable basis than serotyping and is relevant to optimal D transfusion strategies.

1312

FREE-LIGHT CHAIN CONTRIBUTION TO THE EVALUATION OF RESPONSE AFTER STEM-CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Background. The increase in complete remission (CR) rate in Multiple Myeloma (MM) after stem cell transplantation (SCT) according with the EBMT criteria raise the interest for the application of a quantitative test for clonality. *Aims*. To determine the serum concentration of free light chains and its ratio kappa/lambda (k/L) to determine clonality and compare it to immunofixation (IF). *Patients and Methods*. We studied retrospectively 12 patients in remision after auto-SCT and 1 patient in CR after a sibling allo-SCT. Most of them presented with bone lesions and a median of 45% plasma cells in bone marrow. M-component distribution: IgGk (6), IgGL (1), IgAk (1), IgDL (1), BJk (2), BJL (2). Standard evaluation 3 months after the procedure were as follows : 8 patients (pts) were in CR, 4 pts in near CR with >90% reduction in initial M-component (including one with engraftment's failure),and one patient with only 2 months follow-up in partial remission (PR). Serum free-light chain were determined between 2 and 86 months after SCT, the median being 26 months. *Results*. Both light chains were high (policlonal) in a single case in CR, who had active neumonitis. Both were low in the patient with engraftment's failure, who relapsed shortly thereafter. One of the chains was elevated in 6 cases, all in agreement with the initial M-component, 4 proved to be clonal according to k/L ratio and 2 showed normal clonality after 23 and 86 months of follow-up. The k/L ratio as test of clonality was normal in 9 cases and showed clonality k in two cases and clonality L in another two, altough one of the latter was very close to the normal limit. There was a concordance with immunofixation in 12 /13 cases. A single patient was discordant having a marginal k/L ratio of 0.23 with negative IF, and remains in CR 17 months postransplantation. There was a concordant case, evaluated too early (2 months) who had marginal clonality k (1.66) with positive IF, and showed progression with a rising M-component in the subsequent visit. *Conclusions*. Determination of serum free-light chains allows a quantitative measure of clonality, which are in agreement with the results of standar IF (a qualitative assay) in a high percentage of cases. Therefore this test might afford increased accuracy in the follow-up of these patient's CR status.

1313

HEMATO-ONCOLOGICAL DISORDERS IN ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) PATIENTS FROM VENEZUELA

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Background. AIDS patients, besides suffering from opportunistic infections, are prone to develop hematological and oncological disorders, mainly anemia, cytopenia, lymphoma and Kaposi sarcoma. *Aims*. Our objective was to investigate the frequency of these disorders in a series of Venezuelan patients studied during the period 2003-2007. *Patients and Methods*. Patients were diagnosed at the Institute of Hematology and Oncology (Ministry of Health-Central University of Venezuela, Caracas) by clinical presentation and lab tests (number of $CD4^+$ and $CD8^+$ T lymphocytes, positive serology and confirmatory Western blot). All patients (108) received antiretroviral treatment and the corresponding treatment for opportunistic infections. Sixty-seven patients were male and forty-one female; the median age was 38.8 years (range: 23-61 years), without significative difference between sexes. The most common risk factor was heterosexual contacts followed by male homosexual contacts. Routine hematology tests were performed and when indicated, bone marrow aspirate and biopsy as well as lymph node biopsy. *Results*. Sixty-seven patients (62%) developed anemia during the course of the study: 16 with Hb level below 10 g/dL, 23 between 10 and 12 g/dL, and 28 with Hb between 12 and 14 g/dL. The number of CD4 T cells at diagnosis was 257±64 cells/µL and viral load of 67,254±8,950 RNA copies/mL. Most cases were associated with treatment and were managed by optimizing antiviral therapy. A few cases responded to erythropoietin; none required transfusion. Of the anemic patients, four also developed neutropenia which was classified as mild (Absolute Neutrophil Count: 1000-1500/µL, 4 cases); moderate neutropenia (ANC 500-999/µL) was observed in two other patients without associated anemia; no severe cases of neutropenia were observed. Six patients (5.6%) devel-

oped mild thrombocytopenia (platelet count between 50.000 and 100.000/µL) without clinical manifestations; of these, three cases were associated with anaemia. In this series of AIDS patients we observed 11 cases of malignancy (10,2%): 6 Kaposi sarcomas (5 males, 1 female), 3 non-Hodgkin lymphomas (histologically of the diffuse large cell type), 1 Hodgkin lymphoma and 1 multiple myeloma. They were treated with chemotherapy according to the established protocol and staging. *Summary*. In our series of AIDS patients the most common hematological complication was anemia (62%), in a few cases associated with neutropenia or thrombocytopenia. Isolated neutropenia or thrombocytopenia was uncommon. Kaposi sarcoma was the most frequent malignancy observed (6 cases), followed by non-Hodgkin lymphoma (3 cases).

1314

COMBINATION OF FLUORESCENCE IMMUNOPHENOTYPING AND *IN SITU* HYBRIDIZATION TO DETECT CHROMOSOMAL ABERRATIONS IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is the prototypic monoclonal B-cell neoplasm that is derived from the autonomous proliferation of plasma cells and associated with paraprotein production and osteolytic bone lesions. Chromosomal abnormalities are among the most important prognostic parameters for patients with MM. However, conventional karyotyping has been hampered by the slow growth of MM cells in cell cultures, and chromosomal abnormalities are often missed by this technique, showing normal karyotypes in around 50 to 70% of cases. The importance of chromosomal abnormalities in order to determine prognosis and evolution of the disease justifies the need to detect these abnormalities in all patients. Rutine FISH (fluorescent *in situ* hybridization) improves somewhat this percentage showing aberrations in up to 85% of patients. Fluorescence Immunophenotyping and interphase Cytogenetics as a Tool for the Investigation Of Neoplasms (FICTION) is a new technique that permits the study and counting of only plasma cell nuclei, therefore improving the sensitivity of the FISH technique. *Aims*. The aim of this study is the comparison of standard FISH technique to FICTION technique for the detection of chromosomal abnormalities in multiple myeloma and monoclonal gammopathy of undetermined significance (MGUS). *Methods*. Bone marrow (BM) samples from 6 patients with MM, 3 patients with MGUS, and one normal bone marrow, used as a control, were obtained. For the detection of chromosomal aberrations, FISH on interphase nuclei with commercially available probes for 13q14, 11q22.3, p53 gene (17p13) and IgH rearrangements was performed. For all probes applied, 200 nuclei were counted. Combination of fluorescence immunophenotyping, using an anti-K?L Ig light chain antibody, and *in situ* hybridization was applied to all patients, analyzing also 200 nuclei, except for two cases with specially low percentage of plasma cell infiltration, in which, all plasma cells present in the smear were counted.

Table 1.

	P1 F/FC	P2 F/FC	P3 F/FC	P4 F/FC	P5 F/FC	P6 F/FC	P7 F/FC	P8 F/FC	P9 F/FC	P10 F/FC
17p13	0/ 10	2/ 0	2,5/ 75	25/ 80	1/ 0	0/0	0/0	0/2	0/0	0/0
11q22	0/0	0/0	0/ 75	0/0	22/ 99	0/0	0/ 80	0/0	0/0	0,5/ 0
13q	0/ 60	12/ 92	0/0	0/0	0,5/0	0/0	0/0	0/ 80	0/0	0/0
IgH	1/ 60	0/0	0/0	25/ 76	0/0	0/0	0/0	6/ 72	0/0	0/0

Results. Median plasma cell infiltration was 25% (1-89%), of note, 6 patients presented with less than 25% of infiltration. FISH detected 5 chromosomal aberrations in 4 cases (44%) 2 of them with less than 25% of infiltration. FICTION was able to detect 12 chromosomal aberrations in 7 patients (77, 7%). In three patients, the FICTION revealed a genomic aberration that hadn´t been detected with FISH (one of them with only 4% of infiltration). FISH sensitivity was very low compared to FICTION sensitivity, being the aberration rates 6-22% in FISH and 10-99% in FICTION analysis. FICTION revealed that only plasma cells presented chromosomal aberrations. Table 1 shows percentages of abnormal cells. Results higher than 4% have been considered as positives. P:patient, F:FISH, FC:FICTION. *Conclusions*. In conclusion, the combination of immunophenotyping and FISH on a single cell level demonstrated that only plasma cells bore chromosomal aberrations and that combined techniques allow us to increase the sensitivity of the specific genomic aberration.

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THE EFFECT OF BLOOD VISCOSITY ON ERYTHROPOIETIN SECRETION

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Background. Erythropoietin (Epo) is produced primarily in the adult kidney under the control of an oxygen-sensing mechanism. Besides hypoxia, there are several factors that modulate Epo production, such as hypoglycemia, increased intracellular calcium, insulin release, estrogen, androgenic steroids, and various cytokines. *Aims*. To demonstrate our hypothesis that the blood viscosity modulate as well Epo production. *Methods*. Considering blood viscosity's importance for kidney function we have evaluated the serum erythropoietin's level in subjects with Waldenstrom's disease and multiple myeloma before starting the plasmapheresis program and at 21 days after the serum viscosity was normalized. The analyzed lot had viscosity between 4cp and 12cp and need plasmapheresis as a regulator of their severe hyper-viscosity syndrome. The results we obtained denoted constant decrease to the low level of normal values and under normal limits of serum Epo concentration for the evaluation made before plasmapheresis. We observed a high correlation between the high level serum viscosity and the severity of Epo's decrease level. The reevaluation of the serum erythropoietin concentration 21 days after serum viscosity stabilizing, indicated a constant increase of the serum Epo with 68% to 230% (referring to initial values), the average increase being 114%. Our conclusions of this stage are: the serum viscosity is involved in the Epo's release regulation; the hyper-viscosity represents an inhibitory factor of the Epo's release.

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THE RELATION BETWEEN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA AND THYROID AUTOANTIBODIES

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Background and Aim. The association between thrombocytopenia and hyperthyroidism or thyroid autoimmune diseases (TAD) has been reported but its mechanism is unclear. Thrombocytopenia may be observed in patients with TAD, especially those with hyperthyroidism. We designed a prospective study to investigate the prevalence of antithyroid and antigliadin autoantibodies in patients with idiopathic thrombocytopenic purpura (ITP), and to determine the frequency of overlapping autoimmune disorders. *Patients and Methods*. Patients admitted with chronic ITP to the Dicle University Hematology policlinic between June 2003 and December 2006 were tested at study entry and followed for the presence of antithyroid antibodies, antiendomisium and antigliadin antibodies. Thyroid, endomisium and gliadin autoantibodies performed in 68 patient with chronic ITP compared with 92 healthy individuals that have a normal platelet counts. *Results*. Thyroid antithyroid autoantibody (TATA) positivity detected in 26 (38.2%) out of 68 patients with chronic ITP, and 3 out of 92 (3.2%) healthy controls. Thyroid antimicrosomal autoantibody (TAMA) positivity found in 14 out of 68 (20.58%) patients with chronic ITP, and 2 out of 92 (2.1%) healthy controls. Both antithyroid autoantibodies are significantly different from control subjects ($p<0.0001$). Antigliadin autoantibody positivity was also significantly different from control patients. 14 out of 68 (20.58) patients and 3 of 92 (3.2%) healthy controls have positive antigliadin autoantibody test ($p=0.0001$). On the other hand there is no significant difference in antiendomisium autoantibody positivity between patients and controls. Four out of 68 (5.8%) patients with chronic ITP and 2 out of 92 (2.1%) healthy controls have positive antiendomisium test ($p=0.211$). *Conclusions*. We found statistically meaningful difference in antithyroid and antigliadin autoantibodies between patients with chronic ITP and healthy controls. However, none of patients with positive thyroid autoantibodies and antigliadin antibody exhibited overt TAD or cheliac disease during 14 months average follow-up period. Therefore we suggest these autoantibodies are not a strong marker of primary diseases that they mostly related. Large-scala studies are needed to confirm this observation and to investigate these presented strong relation.

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IDIOPATHIC THROMBOCYTOPENIC PURPURA: PRELIMINARY RESULTS IN 30 PATIENTS OLDER THAN 65 YEARS

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Background. Idiopathic thrombocytopenic purpura (ITP) is not well-documented in elderly patients. *Aims*. Here, we describe data and management experience of ITP in elderly patients. *Methods*. The study was conducted retrospectively among 30 elderly patients (i.e. ≥65 years) followed since 1985 in the University Hospital of Strasbourg (France). The analyzed data includes: clinical characteristics, therapies used, their response rates and side effects at the 6th month. *Results*. The mean age of the patients was 71 years (range, 65 to 82); 12 patients were older than 75 years. The initial presentation included: thrombocytopenia revealed by a routine blood count in 6 patients (20%), bleeding limited to the skin in 7 cases (23%) and bleeding in one or more other sites (mucosa or visceral) in 17 patients (57%). The mean platelet count was 47×10^9/L (range, 1 to 120). During follow-up, 3 patients (10%) died. Initially, a response to oral corticosteroid therapy was obtained in all the treated patients (n=14), but only one third of the patients were responders after 6 months of follow-up. Adverse effects of corticosteroid therapy were reported in 100% of the patients. Intravenous immunoglobulin represented the initial treatment in 3 patients; none of them presented any response. Initially, all splenectomized patients (n=6) showed response to the splenectomy. But after 6 months of follow-up, none of these patients was still in complete response. Postoperative complications occurred in 4 patients, including one fatal issue (septic shock). Danazol was given to 5 patients with a response in 60% of the cases. Moderate to severe elevation of serum aspartate or alanine aminotransferase was reported in all these patients. *Summary/Conclusions*. The present results show that ITP seem to be more severe in elderly patients. They confirm that the age influences the response and adverse effects of various conventional therapies and that Danazol may be a potentially effective therapeutic alternative to splenectomy for elderly ITP patients.

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COAGULATION, FIBRINOLYTIC SYSTEM ACTIVATION AND ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH MITRAL STENOSIS IN SINUS RHYTHM

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Introduction. Systemic embolism is a important complication in patients with mitral stenosis. Anticoagulation treatment can prevent this serious complication in patients with mitral stenosis in atrial fibrilation, but in sinus rhythm the place of this treatment is in argument. In this study, our aim is to determine the hemostatic parameters of mitral stenotic patients in sinus rhythm and also to compare the systemic hemostatic parameters of patients both in atrial fibrilation with left atrium spontaneous echo contrast(LASEC) and without LASEC and normal population. *Material and methods*. 46 patients with mitral stenosis contributed to this study. 28 patients were in sinus rhythm and 18 patients were in atrial fibrillation. None of the patients had left atrial thrombus in transesophageal echocardiography. We studied systemic venous fibrinogen, D Dimer, antithrombin, tisuue plasminogen activator(tpa), plasminogen activator inhibitor-1, von Willebrand factor, platelet factor 4(PF4) in these patients. Fibrinogen, D Dimer, antithrombin, plasminogen activator inhibitor-1, von Willebrand factor were mesured by automated coagulometer(BCS System, Dade-Behring, Germany). Tpa (Biopool, Sveden) and PF4 (Stago, France) were measured by ELISA method. The patients were divided into subgroups, first according to their rhythm as sinusal and atrial fibrillation than those with LASEC and atrial fibrillation, those without LASEC and sinus rhythm, those without LASEC and atrial fibrillation. All of these groups were compared. *Results*. Our results suggest that fibrinogen, DDimer, antithrombin, von Willebrand factor, platelet factor 4 levels were greater in sinus rhythm and atrial fibrillation groups than the control group. This was significant statistically ($p<0.05$). Also in presence of LASEC both in sinus rhythm and atrial fibrillation fibrinogen, DDimer, antithrombin, von Willebrand factor, platelet factor 4 levels were significantly higher compare to control group ($p<0.05$). Without LASEC fibrinogen, antithrombin, von Willebrand levels were high only in atrial fibrillation than sinus rhythm than controls ($p<0.001$). Only PF4 was also high in atrial fibrillation than sinus rhythm ($p<0.05$). We studied also tissue plasminogen activator and tissue plasminogen activator inhibitor-1 levels. Only tissue plasminogen activator levels were higher in atrial fibrillation group than sinus rhythm and control group($p<0.05$). *Conclusions*. In patients with mitral stenosis but in sinus rhythm, especialy the patients with LASEC, coagulation activation, platellet activation and endothelial dysfunction are similar in patients with atrial fibrillation.

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IS BLASTOCYSTIS HOMINIS A NEW ETIOLOGICAL FACTOR OR A COINCIDENCE IN IRON DEFICIENCY ANEMIA?

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Background. Iron deficiency anemia (IDA) is the most frequent anemia in medicine. Blastocystis hominis (BH) is a widespread protozoon in the developing countries. BH may contribute to anemia only in children aged between 8-10 years. *Aim*. To investigate the frequency of BH in patients with IDA. *Methods*. One-hundred ninety-one patients (18 male and 173 female) with a mean age of 41±15 years with IDA and 73 healthy persons (11 male and 62 female) with a mean age of 45±17 years were enrolled to this study. The diagnosis of IDA was based on serum iron, iron binding capacity, ferritin and iron saturation. Three consecutive parasitic investigations with direct microscopy using native lugol method were done in two groups. Statistical analysis was performed with Chi-square and student's t tests. *Results*. Hematological parameters of the patients were a hemoglobin level 9.7±1.5 g/dL, serum iron 20±11 µg/dL, total iron binding capacity 320±46 µg/dL, iron saturation 7±4% and ferritin 5.5±4 ng/dL. BH was found in 53 patients (31%) and 4 healthy persons (6%), respectively. This rate was significantly different ($p<0.001$). *Conclusion*. In this study, the frequency of BH in the patients IDA was higher than controls. Although it was no shown that BH invade the colon mucosa in case-controlled studies, edema and inflammation were detected. It was detected in one study which BH may use the cationic ferritin for endocytic pathway. It may be considered that BH may contribute alone or together with factors to IDA.

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LONG-TERM NORMALISATION OF PLATELET COUNT AFTER ONE INCOMPLETE DOSE OF RITUXIMAB IN A GIRL WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Rituximab, a chimeric monoclonal antibody directed against mature B-lymphocytes, has been used in the treatment of refractory chronic immune thrombocytopenic purpura (cITP). The standard dosing schedule of rituximab in the treatment of ITP is a weekly dose of 375 mg/m^2 four times. Taube challenged this scheme and treated 22 children with only one single dose of rituximab with a response rate comparable to that achieved by the standard dosing schedule.[1] We report on 10-year-old-girl suffering from cITP treated with only one incomplete dose of rituximab of 54 mg/m^2. A 10-year-old girl presented with bruising, petechiae and nose-bleeds since several months. The blood cell count showed thrombocytopenia (platelets 6×10^9/L). Leucocytes, activated thromboplastin time and prothombin time were normal. Specific anti-platelet antibodies were positive. Several courses of prednisone only resulted in short periods of normal platelets and after one year relapses occurred during the step-down fase of prednisone. A intravenous immunoglobulin G course was not effective and was tolerated badly. Because of repeated episodes of severe menorrhagia we decided to start a course of rituximab at a platelet count of 8×10^9/L. After one half-hour of i.v. rituximab the girl developed urticariae. After antihistamine i.v. and a 30 minutes pause the complaints had disappeared and the rituximab was re-started. Again after about 30 minutes paleness, nausea and hypotension developed. The rituximab was stopped, only 90 mg (54 mg/m^2) of the planned 625 mg of rituximab was given. No additional therapy was given after the treatment with rituximab. After three weeks the platelet count was 16×10^9/L, another 3 weeks later the platelet count was normalized to 253×10^9/L, and one year after the incomplete Rituximab platelet counts are still normal. Rituximab is a monoclonal antibody that targets CD20 antigen on B-lymfocytes. After Saleh *et al*. showed that only higher doses of rituximab (375 mg/m^2) were effective no other dose-finding studies were published. Taube recently challenged the scheme of four weekly doses in children and used only a single dose of 375 mg/m^2 and showed equal results compared with reports in the literature (1). Our report with a complete remission of cITP after only a sin-

gle dose of 54mg/m^2 rituximab further stresses the need for further studies to define the optimal dose and schedule for rituximab therapy in cITP.

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HEMOGLOBINOPATHIES AND CONGENITAL HEMOLYTIC ANEMIAS. A COMPARATIVE STUDY OF AUTOCHTONOUS VS IMMIGRANT POPULATIONS IN SOUTHERN SPAIN. A TEN YEAR FOLLOW-UP

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Background. An estimated 30% of the world's population suffers from anemia. Structural hemoglobinopathies and thalassemias are the monogenic disorders with the highest prevalence. Migrational social changes in our country are resulting in a remarkable increase in the occurrence of these abnormalities, considering both mild (heterozygous) or severe (homozygous or compound heterozygous) forms. The immigrant population in Almería (Andalusia, southern Spain) makes up approximately 17.4%, 110,637 out of 635,850 inhabitants. *Aims*. To describe the cases of congenital hemolytic anemias occurring in the immigrant population in our area, mostly coming from The Maghreb and Sub-Saharan Africa, compared to those detected in the autochtonous Mediterranean population. *Material and methods*. Anemia work-up protocols over a ten years period (from January 1997 to January 2007) have been reviewed. Since October 2002 we have used the Hi-AUTO A1c 8160 (Menarini-R) high-pressure liquid cromatography (HPLC) system, which allows the detection of abnormal variants of hemoglobin, with their final characterisation made by acidified medium electrophoresis. *Results*. Out of the 3,149 anemia protocols reviewed, 484 corresponded to immigrant patients (15.4%). We detected 81 cases of hemoglobinopathy (16.7%), 11 cases of GP6DH deficiency (2.4%) and 10 cases of hereditary spherocytosis (2%). Among hemoglobinopathies, we found: 20 β thalassemia minor; 18 hemoglobin (Hb) AS; 13 Hb AC; 6 homozygous HbS (HbSS); 6 heterozygous α thalassemia; 5 β thalassemia intermedia; 5 double heterozygous HbSC; 5 HbCC; 1 delta β thalassemia; 1 HbSS + α thalassemia and 1 HbSS + β thalassemia. Since we started using the HPLC abnormal hemoglobin band detection system: 60 HbAS; 15 HbAC; 4 β thalassemia minor; 2 persistence of fetal hemoglobin (HPFH) and 1 HbCC have been added. Considering the autochtonous population, 2,665 anemia protocols were carried out, which detected 131 hemoglobinopathies (4.9%), 59 hereditary spherocytosis (2.2%) and 7 GP6DH deficiency (0.3%). Among the hemoglobinopathies were: 68 β thalassemia minor; 23 β thalassemia intermedia; 23 α thalassemia; 14 delta β thalassemia; 1 HbAC; 1 HPFH and 1 HbAS or HbAD that we were unable to characterise. Using the HPLC system, we added: 6 Hb AD; 3 Hb AS; 3 Hb AC; 2 delta β thalassemia; 2 Hb AS or AD;1 β thalassemia intermedia; 1 β thalassemia minor; 1 PHHF and 1 Hb C or E. *Conclusions*. 1) Of the anemia investigation protocols carried out, 15.4% were on the immigrant population, which is similar to the percentage of the estimated immigrant population in Almería overall. 2) In immigrants, we have found hemoglobinopathies, even without anemia, that are unusual in local patients, and shouldn't be underdiagnosed. 3) An abnormal hemoglobin band detection system, such as HPLC, is of great usefulness in the diagnosis of anemia. 4) Data shown here is general in nature. It would be of great interest to classify these patients according to their specific origin. 5) Hereditary spherocytosis, the most prevalent hemolytic anemia among caucasians, should not be ignored when investigating anemia in immigrants.

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THE USE OF COLLAGEN COATED BLOOD SAMPLING BOTTLES TO DETECT FUNCTIONAL AND NON FUNCTIONAL PLATELETS IN NORMAL SUBJECTS AND PATIENTS SEEN IN ITU, HAEMATOLOGY CLINICS AND UNDERGOING RENAL DIALYSIS

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Platelet function is an important factor to prevent bleeding irrespective of circulating number. Classically, platelet function has been assessed by Simplate bleeding time measurement, platelet aggregometry and, more recently, PFA100 technology. Despite this, patients often have surgery cancelled due to the fact that they are taking Aspirin or similar agents eg. Clopidogrel. In the ITU setting, clinicians often face the situation where patients are bleeding but the humoral coagulation and platelet count are not apparent issues. This is particularly the case where the patient is on extra-corporeal circulation. The concept of *exhausted* platelets is real but cumbersome to prove. Therefore, the availabilty of a techique which can be performed at the bedside which is reliable and reproducible in enumerating the patient's *functional*platelet count would be potentially invaluable. In the last three years Helena Laboratories, Beaumont, Texas, US have produced blood sampling bottles which are coated with 10 ug collagen (equine tendon) and 3.2mg sodium citrate (Plateletworks). Our group is the first in Europe to use these bottles in association with a *point of care testing* system (POCT) using the Horiba ABX Pentra five part differential analyser (Chicksands, Bedfordshire, England). POCT full blood counts were performed on samples of blood taken from normal subjects, renal dialysis patients, cardiac catheterisation patients, ITU patients and haematology patients with myelodysplasia and myeloproliferative disorders. A small cohort of patients were on regular low dose aspirin and some of the normal subjects volunteered to take aspirin prior to testing. One normal subject was on antihistamines. Blood samples were taken synchronously into K2EDTA and collagen bottles and both were handled identically in terms of mixing and analysis on the ABX Pentra. All staff undertaking the study had been trained by Plateletworks in sample handling. The K2EDTA platelet count represents the total platelet count whereas the measurable platelet count in the collagen coated bottle represents the non functional compartment of the individual's platelet count (the functional platelets having aggregated onto the inside wall of the collagen tube). Data on 54 normal subjects showed that the interquartile range of functional platelets was between 89.9-95.9%. Median value was 93.8%. This implies that even in healthy subjects a small proportion of platelets are non functional. Cardiac catheterisation patients (n=10) all of whom were on Aspirin and Clopidogrel had 76.5% functional platelets which is statistically significant when compared to control subjects (p=0.0009). Surprisingly, one subject who had taken 600mg of Aspirin 24 hours earlier had 70% functional platelets using this assay system. Finally renal patients who were already thrombocytopenic had significantly lower functional counts than expected such that platelet transfusion might be considered. The data presented here have been generated using collagen coated tubes only. This, at best, is a screening tool where the most appropriate assessment of Aspirin effect may be achieved using Arachidonic Acid. Plateletworks is looking at producing such tubes shortly. Nonetheless, the philosophy of measuring the functional platelet count is still valid and may provide haematologists with a valuable tool in deciding treatment in the future.

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RELATIONSHIP BETWEEN PAI-1 POLYMORPHISM AND DEVELOPMENT OF VASOPLEGIC SYNDROME ASSOCIATED WITH CARDIOPULMONARY BYPASS AFTER CARDIAC SURGERY

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Introduction. Vasoplegic syndrome (VS) is a recognized complication following cardiac surgery using cardiopulmonary bypass (CPB) and is associated with increased morbidity and mortality. Intensity of VS can vary from mild to severe and it occurs with an incidence ranging between 5% and 15%. Although its etiology is not completely understood, risk factors as temperature, duration of CPB and preoperative treatment with angiotensin-converting enzyme (ACE)-inhibitors have been reported.[1,2]

Our aim was to investigate a possible role of several genetic polymorphisms in patients with VS after elective CPB. *Methods*. We performed a nested case-control study of 50 patients undergoing CPB, 27 men and 23 women, median age 66.5 years (SD 9.6). VS was defined as the conjunction of a systemic vascular resistance index lower than 1600 dyn seg cm 5 m^2 and a cardiac index greater than 2.5 L/min/m^2, both occurring within the first 4 hours after surgery. We recorded data related to hemodynamic parameters at three different postoperative time points (at Intensive Care Unit admission, and after 4 and 24 hours from surgery) and the polymorphism of the following genes: plasminogen activator inhibitor-1 (PAI-1) and β-tumor necrosis factor (β-TNF). In addition, 22 neutral markers were genotyped to follow genomic control strategies that would detect spurious associations due to population substructure. We used Pearson χ^2 test and binary logistic regression, by means of a SPSS v12.1 informatics software. *Results*. We observed 17 patients (34%) with vasoplegia criteria, 11(65%) men and 6 (35%) women, median age 67 years (range 61-72). Only PAI-1 polyporphism was found to be associated with vasoplegia, and its distribution in the whole study population was: 4G/G genotype in 10 patients (20%), 4G/5G in 26 (52%), and 5G/G in 14 (28%). According to PAI-1 polymorphism, vasoplegia criteria were found in 1(6%) 4G/G carrier, in 6 (35%) 4G/5G carriers and in 10 (59%) 5G/G carriers. (p=0.012). The post-hoc power for PAI-1 polymorphism and vasoplegia was 0.85. Once controlled the temperature, clamping time, body mass index and ACE-inhibitors, 5G/G genotype was independently associated with vasoplegia (p=0.017); OR: 24.6 (CI95%: 1.8-342). *Conclusions*. PAI-1 polymorphism (specifically homozygous 5G/G) was independently associated with the onset of vasoplegic syndrome. For the best of our knowledge, do not exist other publications establishing a possible relationship between fibrinolysis and vasoplegic syndrome. Although we consider very interesting these findings, further studies are needed to confirm them.

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IMMUNE THROMBOCYTOPENIA FOLLOWING INFLUENZA VACCINATION IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Thrombocytopenia is a common complication of chronic lymphocytic leukaemia, owing to bone marrow infiltration or immune disturbance. Influenza vaccine is administered to older population as well as younger people with occupational risk. It is an infrequent cause of thrombocytopenia. *Case report*. We report the case of an 83 year old female diagnosed of chronic lymphocytic leukaemia in stage A/0, requiring no therapy. Ten days after influenza vaccination she was admitted because of massive bruising and oral mucosal bleeding. Vaccination had been with a polyvalent vaccine (Leti laboratories, Tres Cantos, Spain) containing inactivated fraccionated viruses from H1N1 and H3N2 strains. Physical examination showed only pethequiae, bruising and hemorrhagic bullae in oral mucosa. No enlargement of lymph nodes nor spleen or liver was found. Platelet count was 1×10^9/L, Haemoglobin 122 g/L and leucocytes $21{,}4\times10^6$/L, with a predominance of lymphocytes due to chronic lymphocytic leukaemia. In bone marrow examination megakariocyte hyperplasia was remarkable, as well as a lymphoid infiltrate of 74% of leukemic cells. Therapy with intravenous methylprednisolone produced a quick response with disappearance of bleeding and platelet count reaching 140×10^9/L in just five days. Once steroid therapy has been tapered, platelet count remains to date within normal limits. *Discussion*. In Spain, influenza vaccination is administered to the whole population over 65 years. Thus it is a frequent confounding factor in investigation of thrombocytopenia. In patients with immune disturbances, such as chronic lymphocytic leukaemia or transplantation, several agents can trigger autoimmune phenomena. Influenza vaccine is one of these agents which can induce a short lived thrombocytopenia with an excellent response to steroids. *Conclusion*. When facing an acute thrombocytopenia, past history of drugs and vaccines must be taken into account, even in the setting of other diseases as chronic lymphocytic leukaemia where thrombocytopenia is not uncommon. Thus recent influenza vaccination must be recorded as a cause of immune thrombocytopenia.

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COMBINED BAND 3 DEFICIENCY AND α I/74 SPECTRIN VARIANT RESULT IN A CLINICAL PATTERN COMMON TO HEREDITARY SPHEROCYTOSIS AND ELLIPTOCYTOSIS

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Background. Hereditary spherocytosis (HS) and elliptocytosis (HE) are highly heterogeneous haemolytic anaemias caused by defects of vertical interactions between RBC cytoskeleton and integral domain (Spectrin, Ankyrin, Band 3 and Protein 4.2 deficiency), and by abnormalities of horizontal interactions among membrane components (Protein 4.1 deficiency, or Spectrin variants impairing the dimer self-association site). Although a variety of abnormalities have been described in all the RBC membrane proteins, rare are the cases with combined spherocytic and helliptocytic defects. *Aim*. We describe a case of congenital haemolytic anaemia due to the copresence of HS and HE. *Case*. The propositus was a 68 yrs male of Southern Italian origin with a life-long history of haemolytic anaemia (Hb 8-9 g/dL), jaundice since birth, splenomegaly and gallstones. At the age of 37 he was diagnosed as HS. In the last two years, anaemia became more severe requiring occasional transfusions. *Methods*. The diagnosis of membrane defect based on clinical history, physical examination and results of laboratory tests: complete blood count, blood smear examination, reticulocytes count, bilirubin concentration, RBC osmotic fragility tests, flow-cytometric eosin-5-maleimide (EMA) test, and by the exclusion of other causes of haemolysis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of RBC membrane proteins, and functional studies of spectrin were performed to establish the biochemical defect. The codifying region, promoter and flanking intronic regions of Band 3 gene (SLC4A1), and exon 2 of α-spectrin (SPTA1) were amplified and sequenced on an ABIPRISM 310 capillary sequencer to identify the molecular defect. *Results*. At the time of the study Hb was 6.9 g/dL, MCV 84.7 fL, reticulocytes 337×10^9/L, unconjugated bilirubin 3.6 mg/dL, LDH 1059 U/L, haptoglobin <5 mg/dL. Plasma free haemoglobin was increased and haemosiderin was present in urine. The peripheral blood smear examination showed microspherocytes (15%), schistocytes (10%), elliptocytes (7%) and a few mushroom-like RBCs. Erythrocyte osmotic fragility was increased, screening test for unstable haemoglobins was negative. EMA binding test displayed a double peak due to residual transfused red cells; the patient's RBCs showed a marked reduction of fluorescence intensity (MCF=5.2 n.v.:9.1-12.3). SDS-PAGE of RBC membrane revealed a Band 3 defect (Spectrin/Band 3 ratio=1.25, n.v.:0.95-1.21). Spectrin peptide map after limited trypsin digestion displayed the presence of α I/74 variant; spectrin dimers in the 0°C extract were increased (42.3%, n.v.<15.9) and Ka decreased (1.0e5 M-1, n.v.:1.6-6.0). Family studies allowed to trace back the spectrin defect in the paternal family, the maternal family was not available. Molecular characterization showed the presence of mutation CGT-CAT (Arg28Asp) in the SPTA1 gene, associated to α I/74 phenotype. In the SLC4A1 gene we did'nt detect any abnormality except for the nucleotidic substitution D38A described as polymorphic site *Darmstadt* (Miraglia del Giudice, 97) and an intronic substitution IVS5(+27)t. The effects of these two abnormalities, *per se* not associated to Band 3 deficiency, remain to be established.

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C807T GP IA SINGLE NUCLEOTIDE POLYMORPHISM DETERMINATED EXTENSIVNES OF MYOCARDIAL NECROSIS DURING ACUTE MYOCARDIAL INFARCTION

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Introduction. Increased platelet inhibition is achieved when clopidogrel is added to aspirin (ASA). A broad variability in platelet inhibition profiles during the early phases of treatment has been demonstrated and may be attributed to ASA and/or clopidogrel resistance. Platelet adhesion to fibrillar collagen via the membrane glycoprotein (GP) Ia-IIa is a crucial event in the pathogenesis of arterial occlusive disorders. The C807T single nucleotide polymorphism of the integrin α 2 (ITGA2) gene has been shown to correlate with the platelet GPIa/IIa density. *Study population*. 48

patients (pts), 36 male, with acute myocardial infarction treated by stent placement (PCI). After PCI all patients received dual anti-platelet therapy (ASA-75mg/d and clopidogrel-initial dose 600 mg and next 75 mg/d). *Methods*. Following platelet functions were assessed: aggregation induced by ADP (3.5 and 5.0 umol/L) or collagen (2 ug/mL) as well as the closure time in PFA-100. The C807T polymorphism of platelet GPIa was investigated using the PCR method introduced by Santoso *et al*. Myocardial destruction was assessed by measurement of troponin I serum level. *Results*. In the whole study group the frequency of the T allele was 52,5%, and in the anti-platelet therapy resistant subgroup (40% of pts) this polymorphism has been found in 56,3% of pts. There were no differences of age, infarction location and number of coronary artery lesions between the groups with CC and CT polymorphism. In pts with the T allele we observed a higher level of troponin I in the anti-platelet therapy resistant subgroup in comparison with pts with the C allele. This difference was not significant in non-resistant pts (Figure 1). *Conclusions*. This study suggests a strong association between the presence of platelet GPIa T807 allele and myocardial damage during acute coronary syndrome, especially in anti-platelet therapy resistant patients.

Figure 1.

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UTILITY OF RETICULATED PLATELETS VALUE IN DIFFERENT HEMATOLOGIC DISEASES

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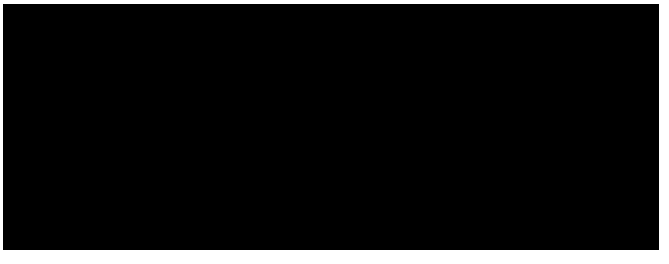
Reticulated platelets are the youngest platelets in the circulation and contain residual mRNA in their cytoplasm. mRNA can be measured using flow cytometry. A new automated method to asses reticulated platelets, the immature platelet fraction (IPF), has been recently incorporated to the Sysmex XE-2100 hematology autoanalyzer. IPF reflects thrombopoiesis in bone marrow. Some authors have communicated that IPF was significantly high in patients with idiopathic thrombocytopenic purpura (ITP) and recovery phase of post-chemotherapy, while other investigators think that IPF may be useful to differentiate essential thrombocytosis from reactive thrombocytosis (RT). The aim of the study was to obtain the normal values of IPF in our laboratory in healthy donors and in apheresis product, compared to patients diagnosed with ITP, myeloproliferative disease (MPD) and RT.

Table 1.



Material and Methods. Peripheral blood (PB) samples in EDTA tubes from 92 healthy donors, 11 ITP, 13 RT, 6 MPD and 55 platelet apheresis product were acquired in a Sysmex XE-2100 autoanalyzer employing XE-Pro Series software. *Results*. Table 1. Sensibility and specifity of IPF were excellent for ITP diagnosis when we apply a cutt-off value of 8.6% All ITP patients show high IPF at diagnosis and it decreased when platelet counts rise. *Discussions*. Results demonstrate the value of IPF determination in ITP differential diagnosis with high sensibility and specificity, but not for RT and MPD. More samples must be studied to confirm these results.

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EFFECTS OF COMBINED THERAPY WITH DEFERIPRONE AND DEFEROXAMINE ON GONADAL FUNCTION IN MALE PATIENTS WITH β-THALASSEMIA MAJOR

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Background. Hypogonadism, mainly of hypogonadotrophic origin represents one of the major iron-induced complications in patients with β-thalassemia major. β-thalassemia major leads to variable pituitary iron overload and thus hypophyseal damage. *Aims*. The purpose of this study was to investigate the effects of 5 years of intensive chelation with combined deferoxamine and deferiprone regimen on pituitary- testicular axis in 9 eugonadal and 7 hypogonadal thalassaemic men, aged from 22 to 44 years (29.8±2.03, mean±SEM) who were previously maintained on subcutaneous deferoxamine monotherapy. *Methods*. The protocol of biochemical investigation included basal serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone and gonadotrophins response after a gonadotrophin-releasing hormone (GnRH) test. All patients were initially assessed before the beginning of combined therapy (Between January and October 2001) and reassessed on July 2006. Substitution therapy was discontinued for 40 days before the biochemical assessments. *Results*. According to our results, testosterone levels were significantly increased in eugonadal patients (7.67±0.63 vs. 5.71±0.55 ng/mL, $p<0.001$). LH levels were also increased in all times during the GnRH test, particularly at 30mins (15.67± 2.96 vs. 12.07± 1.91, $p<0.05$). The quantitative LH secretion, as it is assessed by the calculated area under curve, was increased but without reaching statistical significance (1124±195 vs. 962±139, $p=0.17$). No differences occurred in the excretion of FSH. In hypogonadal patients testosterone remained unchanged (3.23± 1.33 vs 3.27± 0.98). However, FSH levels significantly improved in times: 0' min (2.64±0.75 vs. 2.14±0.59, $p<0.05$) and 30' min: (2.45±0.59 vs. 2.09±0.49, $p<0.05$). Two patients had normal testosterone levels and normal pituitary response during the second assessment suggesting that, in some patients, iron-induced dysfunction of the pituitary gonadotrophic cells might be reversible. *Conclusions*. Although the number of participants was small, the present study shows that intensification of iron chelation might be of benefit in regards to the pituitary-testicular axis.

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IRON OVERLOAD IN HCV+ PATIENTS TREATED BY BLOOD LETTING, HEMATOLOGICAL PARAMETERS AND HEPATIC INFLAMMATION

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Background. Hemosiderosis is a metabolic pathology characterized by tissue deposition of iron determining fibrosis and functional failure of involved organs (liver, heart and endocrine glands). Iron overload can be genetic or secondary; in fact about 30% of HCV+ patients is affected with an hemosiderosis, that, increasing fibrogenesis, aggravates the liver damage. If contraindications do not exist (anemia, hypoproteinemia, cardiovascular diseases), hemosiderosis is treated by phlebotomy or erythrocytapheresis. *Aims*. Aim of this study is to evaluate the clinical and laboratory results in patients affected with hemosiderosis secondary to C hepatitis during a therapeutic cycle of blood letting. *Methods*. 19 patients, enrolled and treated in University, were submitted to a cycle of weekly phlebotomy (taking away, in every sitting, 400-450 mL of whole blood), in order to obtain a complete iron depletion. Clinical and laboratory screening were performed before starting the therapy (time 0), 7 days after the end of treatment cycle (time 1) and after a short follow-up (time 2). *Results*. At time 0, patients showed these results (mean±SE): RBC=4.9±0.1×10^6/μL; Hb=15.6±0.3 g/dL; PLT=197±13×10^3/μL; PT= 99.5±2.9%; Ferritin=398.9±56.1 ng/mL and Transferrin Saturation = 42.5±2.5%, ALT=110 U/L. The therapeutic cycle was composed by 4.3±0.5 phlebotomies (range 1-9). At time 1: RBC=4.3±0.13; Hb= 13.4±0.3; PLT=228±15; PT=98.5±1.7; Ferritin=51.8±4.7 and Transferrin

Saturation=16.7±2.8, ALT=76. The follow up was conducted for 47.7±13.9 days; at its end: RBC=4.7±0.11; Hb=14.4±0.4; PLT=231±17; PT=97.8±2.4; Ferritin=39.5±5.6 and Transferrin Saturation=25.9±3.7, ALT=73. gammaGT was respectively 62, 52 and 55 U/L (p=0.011 between time 0 and 1; p=0.043 between time 0 and 2). Total bilirubin significantly decreased between time 0 and 1 (0,81 and 0,56 mg/dL, p=0.002) and gone up at time 2 (0.56, p=0.055) but not significantly. Plasmatic albumin, prothrombinic activity and ALP showed not significant variations. From a clinical point of view, patients reported to tolerate the treatment, to feel a generic sensation of well-being and to feel itself less tired an*lighter*; main problems were: the difficulty in finding the vein, the pain linked to vein puncture, a sensation of malaise and an headache during and immediately after the phlebotomy. *Conclusions*. On the basis of our experience, with a weekly frequency of phlebotomy it is possible to complete all therapeutic cycles without causing an anemia in patients an avoiding complications and adverse reactions. Final follow-up showed that iron depletion is maintained for a long time thanks to stimulation of bone marrow hemopoiesis, due to iatrogenic anemia. Moreover we have registered a very positive action on hepatic inflammation parameters, probably linked to removal of the iron excess that acts as further pathogenic factor upgrading the cytopathic effects of HCV. Although clinical response and laboratory results must be always monitored, we can affirm that blood letting, in removing a liver cytopathic factor as iron overload, can be favorable for patients affected with hemosiderosis secondary to hepatitis; moreover, using opportune and appropriate sagacities, iron depletion does not represent a limiting factor for specific antiviral treatments determining an anemization of patient.

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REMISSION OF REFRACTORY CHRONIC IMMUNE CYTOPENIAS WITH RITUXIMAB

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Background. Chronic inmune cytopenias refractory to conventional treatment represent a therapeutic challenge. Recent studies have shown that rituximab might be useful in the treatment of these patients due to its B-cell depleting effect. *Aims*. The objective of this study was to evaluate the effect of rituximab in immune cytopenias. *Patients and Methods*. Twenty-eight (28) patients with chronic immune cytopenia refractory to other treatments were treated with rituximab: 15 patients with chronic immune thrombocytopenic purpura (ITP), 1 patient with thrombotic thrombocytopenic purpura, 9 with recurrent autoimmune hemolytic anemia (AIHA) and 3 with Evans syndrome. Patients (5 children and ten adults) with ITP for up to 21 years (2 of the patients also with diagnostic of LED), 6 to 78 years-old, with platelet counts <40,000 /µL, with platelet antibodies, normal bone marrow cellularity and megakaryocyte count, received Rituximab at a dose of 375 mg/m^2, once weekly for 4 weeks. Platelet response was characterized as complete remission (CR) if a count >150,000/µL was achieved and partial (PR) if 50,000 to 150,000/µL. Nine adult patients (21-78 years-old) with refractory autoimmune hemolytic anemia, eight of them with direct Coombs test (IgG) positive and one patient with direct Coombs test (IgM) positive and three patients (14 to 43 years-old) with Evans syndrome were also treated with Rituximab. In these cases CR was characterized as complete if normal Hb and Hto for their sex and age was achieved and PR if their Hb increased at least two grams. Study of CD20$^+$ B cells was done with monoclonal antibodies by flow cytometry. RESULTS: Thirteen patients (11 adults and 2 children) with refractory ITP responded to rituximab *de novo* (86.7%). Four of them had been splenectomysed. Twelve (12) patients had been in CR for 6 months to 2.7 years (8 pts in CR for more than a year) and 1 patient in partial remission for 1 year. Nine (9) out of 12 patients (75%) who entered in CR relapsed after *de novo* treatment with rituximab. Seven of these patients were retreated with rituximab and all of them have responded for up to 1.7 years. The two children with ITP did not respond. Eight patients with AIHA have been in CR for 1 to 2 years. One patient 21 year old with AIHA had a CR only for one month. Only one patient with AIHA has relapsed; he was retreated responding again to rituximab. Three patients with Evans syndrome have been in CR for 2 months to 2 years. Therapy was well tolerated. The CD20$^+$ count decreased to less than 1% after rituximab. *Conclusions*. In our series, most patients with refractory chronic immune cytopenias responded to *de novo* rituximab treatment or to retreatment, even if they have been splenectomized. Rituximab may be considered a good treatment option, allowing the withdrawal of steroids when the patients enter in CR and thus reducing steroid side effects for a reasonable period of time. The AIHA patients had less number of relapses with rituximab than the ITP patients. The fall in CD20 count talks in favor of depletion of CD20 positive B cell as a mechanism of action of rituximab.

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METASTATIC EPITHELOID HAEMANGIOENDOTHELIOMA IN A 22 YEAR OLD PATIENT

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Background. Eptheloid hemangioendothelioma (EH) is a rare neoplasia of vascular origin involving soft tissue and visceral organs. Lesions occur in almost all ages except early childhood without preference in gender. Usually EH develops as solitary tumour of the soft tissue either of superficial or visceral origin involving skull, axial skeleton or lower extremities and low progression. Because of the rare occurrence of the disease and its granulomatous histology, approximately 2/3 of EH patients may be misdiagnosed initially. *Patient's characteristics*. We report a 22 year old male patient who presented with multiple granulomatous lesions of lung and liver. Initially the patient complained pain of the right arm and progredient dyspnoe for almost 2 years. Radiologic examination of the chest showed bilateral lymphadenopathia. During the last 20 months the patient had underwent several biopsies of lung and liver in regional hospitals, and sarcoidosis had been diagnosed. The patient had been treated with prednison for 12 months without any improvement of cough or dyspnoe or regression of the lymphadenopathy. Finally, liver biopsy revealed diagnosis of EH. In March 2007, we started chemotherapy according to the sarcoma protocol with ifosfamide, adriamycine, actinomycine and vincristine, *Conclusion*. Even if EH is a rare tumour, it should be considered in patients with granulomatous lesions of soft tissue. It is not proven whether new monoclonal antibodies might improve prognosis in metastatic disease.

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IS THERE A PREDICTIVE ROLE FOR GA-67 SCINTIGRAPHY IN PATIENTS WITH LYMPHOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION?

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Aims. The aim of this study is to evaluate Gallium-67 scintigraphy (GS) and CT for predicting clinical outcome in lymphoma patients who undergo autologous stem-cell transplantation (ASCT). *Methods*. Forty patients undergoing ASCT, had GS before and at 100 days post-transplantation (D-100). Patients were followed for 6-61 months and 15 had repeat GS at 200 days post-transplantation (D-200). All patients underwent CT imaging. *Results*. Out of 40 patients, 15 were diagnosed with HD and 25 with NHL. Fifteen had pathologic D-100 GS, where 7 were correlated with CT. Out of 8 patients with normal CT, 6 had control studies. Two patients returned to normal, 1 showed persistence and 3 were accepted in relapse due to progressive lesions on GS with new appeared lesions on CT. Four patients with pathologic findings on D-100 CT but normal GS, were in remission on follow-up. Of the patients 85.7% with negative and 28% with positive D-100 GS were disease-free (median follow up: 30 months). The PPV, NPV and accuracy for GS were 64%, 88% and 77.5%, respectively, and 37.5%, 75% and 75% for CT, suggesting importance of D-100 GS in the prediction of outcome in lymphoma patients who undergo ASCT. *Conclusions*. The results of our findings suggest that GS is superior compared to CT for the prediction of progression, so it can be used in centers where PET imaging is not available.

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SERUM UREA, A SURROGATE MARKER FOR IMPENDING VASOOCCLUSIVE CRISIS IN SICKLE CELL DISEASE

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Background. Vasoocclusive crisis (VOC) is the outcome of several interactions between sickle red blood cells, the endothelium and leucocytes

resulting alterations of the endothelium with release of vasoactive substances and cytokines. Recognizing clinical subsets in sickle cell disease (SCD) with biomarkers, is a useful strategy in deciphering secondary factors involved in modulating the SCD phenotype. There is no single clinical or laboratory test that can prognosticate SCD patients, making it is difficult to stratify patients in relation to disease severity and outcome of several complications. *Aims*. The aim of this study was to assess the significance of alterations in the serum urea concentrations in steady state and VOC's in patients with SCD and use it as a surrogate marker to monitor VOC. *Methods*. SCD patients (n=58; 48 SS homozygotes;10 S,+ Thal double heterozygotes) admitted from the A/E in VOC were enrolled prospectively after informed consent. All patients were also studied later in steady state. The period between the painful crises, during which the patient feels well, is called steady state. i.e no acute illness or VOC or infection in the previous 3 months. Complete blood counts were obtained with an electronic cell counter (Abbott CELL-DYN 4000, Abbott Diagnistics, Abbott Park, IN). A fresh hemolysate was prepared from each sample and subjected to cation-exchange high-performance liquid chromatography (Bio-Rad VARIANT, Bio-Rad Laboratories, Hercules, CA) to reconfirm the hemoglobin phenotype. C-reactive protein (CRP) was estimated by rate nephelometry. Several biochemical parameters of renal and liver function were measured by Beckman Synchrom CX7 analyzer. Serum urea was estimated in all of these patients in the steady state, at the time of presentation of acute painful crisis (before receiving any fluid hydration), at 48 hrs of the VOC episode, and before discharge. *Results*. The patients comprised of 25 male [29 episodes] and 33 female [44 episodes]. Four males and five females had two episodes, while 3 females had 3 admission episodes. Patients age ranged from 14 year to 43 years (mean ± SD, 22.15±6.24). It was observed that mean serum urea (±SD) in the steady state, at 2.8 (±0.07) dropped significantly at the time of presentation of VOC to 1.8 (±1.1; $p< 0.0005$; Student's t-test) (Table 1). *Summary/Conclusions*. Our study strongly indicates the value of monitoring serum urea concentrations during the VOC episodes. We therefore hypothesize that the urea cycle is shifted towards a reduced urea production, which also leads to a decreased production of nitrous oxide. This compound is a well known vasodilator and its reduced availability is recognized to play an important role in the pathogenesis of SCD crisis. It is hypothesized that serum urea levels will correlate with the L-arginine nitric oxide pathway and can be a useful biomarker to monitor the progress of VOC episodes. Furthermore, it can be also useful in prognostication of SCD complications.

Table 1. Mean S. urea in Steady state, at Admission, 48 hrs Inpatient and at Discharge.

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LOW DOSE THALIDOMIDE AS MAINTENANCE IN MULTIPLE MYELOMA

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Background. Autologous hematopoietic stem cell transplantation (ASCT) following chemotherapy improves survival and decrease relapse rate in patients with multiple myeloma. However, patients not eligible for ASCT relapse earlier demanding search for new treatment modalities. *Aim*. was to evaluate the effect of low dose thalidomide as a maintenance treatment in patient with first complete remission not candidate for autologous bone marrow transplantation, and its effect on disease free survival. *Patients*. the study was carried out on 102 patients randomized in two groups, group I (44 patients) and group II (48 patients). Group I received 50 mg thalidomide daily, and group II did not receive any maintenance treatment. *Results*. the follow up period was 40 months, median age for group I was 62 years and 64 years for group II. Males to females ratio was 3:1 for both groups. Regarding types of myeloma; IgG kappa myloma represent 70% in group I and 75% in group II, IgG lambda 20% and 19% in group I and II respectively. Out of 44 patients in group I, only 12 patients relapsed during follow up period, 9 of them died, while 40 patients relapsed from group II, 26 of them died with a significant difference between both groups ($p<0.01$). *Conclusion*. from the present study we concluded that maintenance with low dose thalidomide may show beneficial results over those without maintenance treatment in preventing relapse. However, further studies must be done to compare low dose thalidomide and ABMT following CR

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CURRENT HEMATOLOGICAL FINDINGS IN COBALAMIN DEFICIENCY. A MONO STUDY OF 201 CONSECUTIVE PATIENTS WITH DOCUMENTED COBALAMIN DEFICIENCY

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Background/Aims. With the introduction of automated assays for measuring serum cobalamin levels over the last decades, the hematological manifestations related to cobalamin deficiency have been changed from the description reported in *old* studies or textbooks. *Patients and Methods*. We studied the hematological manifestations or abnormalities in 201 patients (median age: 67±6 years) with well-documented cobalamin deficiency (mean serum vitamin B12 levels 125±47 pg/mL) extracted from an observational cohort study (1995-2003). Assessment included clinical features, blood count and morphologic review. *Results*. Hematological abnormalities were reported in at least two-third of the patients: anemia (37%), leukopenia (13.9%), thrombopenia (9.9%), macrocytosis (54%) and hypegmented neutrophils (32%). The mean hemoglobin level was 10.3±0.4 g/dL and the mean erythrocyte cell volume 98.9±25.6 fL. Around 10% of the patients have life-threatening hematological manifestations with documented symptomatic pancytopenia (5%), *pseudo* thrombotic microangiopathy (Moschkowitz) (2.5%), severe anemia (defined as Hb levels <6 g/dL) (2.5%) and hemolytic anemia (1.5%). Correction of the hematological abnormalities was achieved in at least two-third of the patients, equally well in patients treated with either intramuscular or oral crystalline cyanocobalamin. *Conclusions*. This study, based on real data from a single institution with a large number of consecutive patients with well documented cobalamin deficiency, confirms several *old* findings that have been previously reported before 1990's in several studies and in textbooks.

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MMP-2 CONCENTRATION LEVEL IN AML AND ITS PROGNOSTIC RELEVANCE

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Matrix metalloproteinases (MMPs) were postulated to have important implication in progression and invasiveness of many malignant disorders. On the other hand the biological role of MMP-2 in acute myeloid leukemia is not clear. Serum MMP-2 concentration levels was determined before and after induction chemotherapy using enzyme linked immunosorbent assay and were compared with from normal control group. The pretreatment sMMP-2 levels was significantly lower as compared to post induction level (p=0.000) and control levels (p=0.007). Patient who had high pretreatment levels of sMMP-2 had particularly poor outcome. High sMMP-2 had an independent adverse influence on survival, it was entered as a factor into multivariate analysis together with other prognostic factors (relative risk 9.0, confidence interval 0.94-86.0; p=0.01) *Conclusions*. High pretreatment levels of sMMP-2 is associated with poor survival in patients with AML.

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THE PRELIMINARY REPORT FOR THE EARLY PREDICTION OF THERAPEUTIC RESPONSE IN MULTIPLE MYELOMA PATIENTS BY INTERIM 18F-FDG-PET/CT

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Background and Aims. 18F-FDG-PET/CT (PET/CT) has been known to be useful for staging work up as well as therapeutic response in cancer patients. We prospectively studied the usefulness of interim PET/CT for early prediction of therapeutic response in multiple myeloma (MM) patients. *Methods*. Until now, nine patients were included (M: F=2:7, median age= 56 y). 6 patients were at stage I, 1 at stage II, and 2 at stage

III by international staging system (ISS). PET/CT was performed at initial diagnosis and 7th day after 1st chemotherapy (D7), respectively. The number of hypermetabolic lesion (HML), maximum SUV and functional volume of HML with cut off value of SUV of 2.5 were measured and the changes between 2 time points of PET were evaluated. The responses of PET/CT were classified as complete response (CR, >95% reduction for the individual parameters), partial response (PR, >50% reduction), minimal change (MC, <50% reduction), and progressed disease (PD >25% of aggravation). The responses on PET/CT were compared with clinical responses based performance status and laboratory findings. For the clinical responses, 7 patients were assessed at the end of complete chemotherapy cycles (5.6±2.4 cycles) and 2 patients at the time after 2nd cycle of chemotherapy. *Results*. The therapeutic responses of MM patients were CR of 3, PR of 3, MC of 1, and PD of 2 patients by clinical response assessments according to EBMT criteria and CR of 2, PR of 5, MC of 1, and PD of 1 patient by D7 PET/CT. The changes for the maximum SUV and functional volume of HML had a good correlation with clinical response (rho=0.695, and rho=0.782, $p<0.05$). Especially, the volume change of HML had a strong positive correlation with clinical response in 6 patients of stage I (rho=0.833, $p<0.05$). A weak change for the numbers of HML was shown on D7 PET/CT. *Summary/Conclusions*. This preliminary report suggested that the changes of intensity of FDG uptake and functional volume on D7 PET/CT after 1st cycle chemotherapy were able early to predict therapeutic responses in MM patients.

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PLASMA ENDOSTATIN LEVELS IN CHRONIC LYMPHOCYTIC LEUKEMIA: RELATIONSHIP TO PROGNOSTIC FACTORS

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Background. Angiogenesis is considered a possible prognostic factor in chronic lymphocytic leukemia (CLL). While there are numerous reports on angiogenic activators in CLL, little is known about clinical significance of angiogenic inhibitors in this disease. The present study evaluated plasma levels of endostatin, a naturally-occurring antiangiogenic cytokine, and its possible relationship to prognostic factors in CLL. *Methods*. Endostatin was quantified using commercially available sandwich ELISA in peripheral blood plasma of 43 patients with never-treated CLL and 26 healthy controls. In ten patients, endostatin levels were measured before and after intensive fludarabine-based therapy.

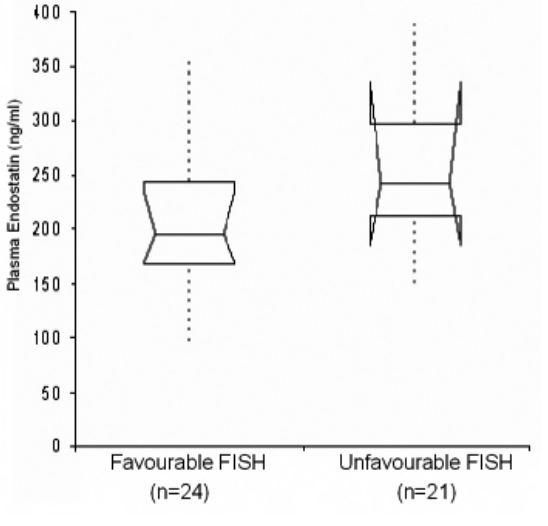

Figure 1.

Results. Endostatin was significantly increased in CLL patients (mean±SD [standard deviation], 226.9±69.0 vs. 171.0±9.1 ng/mL, 95% confidence interval of mean [CI], 205.9-247.8 vs. 152.2-189.8 ng/mL, $p=0.0003$). Patients with unfavourable cytogenetics (i.e., del 11q, del 17p, and/or trisomy 12) had higher endostatin than those with favourable aberrations (no abnormality or del 13q) ($p=0.033$). On the other hand, endostatin levels did not differ between patients stable vs. progressive disease, Rai low vs. intermediate vs. high risk or in IgVH-mutated vs. unmutated patients ($p=0.48$, $p=0.52$, $p=0.44$ and $p=0.47$, respectively). There was no significant difference in progression-free survival of patients with endostatin levels above vs. below median (217.2 ng/mL, $p=0.36$). In ten patients treated with intensive fludarabine-based regimens, there was a trend towards endostatin increase after therapy; however, the difference was not significant ($p=0.13$). *Conclusions*. Plasma endostatin is increased in CLL; in addition, patients with unfavourable genetic abnormalities have significantly higher endostatin levels than those with favourable aberrations. Plasma endostatin may be useful as a part of complex assessment of angiogenesis in CLL. Further studies are warranted to elucidate its role as a prognostic factor in this disease.

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RITUXIMAB IS ACTIVE AGAINST POST-TRANSPLANT CNS LYMPHOMA (PTCNSL).

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We report the case of a symptomatic 74 year old liver transplant female who was diagnosed, after stereotactic biopsy of a left nodular cortical brain lesion, with histologically proven large B-cell post-transplant CNS lymphoma (PTCNSL). The indication for liver transplantation, occurred five years earlier the PTCNSL diagnosis, was cirrhosis induced by hepatitis C virus. During the two months prior to PTCNSL diagnosis, the patient had weight loss, reduction of performance status (as B-symptoms) and in the last weeks sporadically ingravescent partial motor seizure of the right arm. The patient was initially treated with immunosuppression reduction therapy. Two months after, the patient complained of a headache and the brain RMI scan showed lymphoma lesion progression, confirming the inefficacy of the immunosuppression reduction strategy. Once-week systemic infusion treatment with 375 mg/m^2 rituximab was initiated. For hematological toxicity the rituximab dosage was reduced to 75 mg/m^2 once-week infusion. After two months progressive relief of fatigue, headache and significant reduction in size of the brain lymphoma lesion, confirmed by RMI scan, were observed. Fifteen months after, rituximab treatment is still ongoing, the patient is alive in good performance status of 0-1 and the repeated RMI scans confirmed mild continuous brain lymphoma lesion regression. In conclusion no uniform consensus exists regarding optimal treatment options for PTCNSL. The results of this case report provide evidence that systemic infusion with rituximab is active in symptomatic patient with aggressive post-transplant brain lymphoma and may be an important novel treatment option for these patients.

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BORTEZOMIB ASOCIATED TO DEXAMETHASONE IN PATIENTS WITH RELAPSED/ REFRACTORY MULTIPLE MYELOMA

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Background. Bortezomib is a proteasome inhibitor that has been shown to be effective, obtaining durable responses when given as monotherapy for relapsed or refractory multiple myeloma (MM), without severe toxicity. The latest studies prove a 43% of response rates in patients that had received 1-3 prior treatment schedules. *Aim*. To evaluate the efficacy in patients with relapsed or refractory multiple myeloma that received bortezomib plus dexamethasone after two or more prior therapies. We evaluated toxicity and overall survival as well. *Patients and Methods*. We analized 56 patients from various hospitals of East Andalucia with MM treated with standard dose bortezomib (1.3 mg/m^2 given on days 1, 4, 8 and 11 in a 21 day cycle), every 3 weeks plus dexamethasone 40 mg the same days, with a 26 month period follow up (range 1-26). We included 31 males and 25 females with mean age 58,71 (range 37-74). The prognostic markers at time of diagnosis were ß2 microglobuline> 3 in 37,5% of patients, median of plasma cells in bone marrow 48,95%. The median number of prior therapies was 2.3

(range 1-7) and 28,6% of patients had undergone prior autotransplant. *Results*. The response was evaluated according to the EBMT criteria in 48 patients (85,7%) with a media of 6cycles completed of treatment (1-16). The evaluation at 4° cycle was: complete response (CR):8,33%; near complete response (CnR): 20,83%; partial response (PR): 33,3%; minimal response (MR): 10,4%; stable disease (SD): 6,25%; progression: 14,58%. At the end of the treatment (with a media of 6 cycles) 35 patients had been evaluated (62,5%); obtaining the following responses: CR:28,57%; CnR: 14,28%; PR: 14,28%; MR: 8,57%; progression: 8,57%. Overall survival 83,92%. Toxicity profile WHO grade 3-4: thrombocytopenia: 19.6%; neuropathy 12,5%; gastrointestinal: 5,3%; zoster herpex virus infection:14%. In 48% of patients a dose reduction was required. *Conclusion*. Bortezomib is an effective agent with acceptable toxicity for the treatment of patients with relapsed/ refractory MM. The response rates, overall survival and toxicity in our series is similar to data described in previous studies although a longer follow up is needed in order to confirm these results.

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PROGNOSTIC FACTORS OF ANTITHYMOCYTE GLOBULIN FOR APLASTIC ANEMIA

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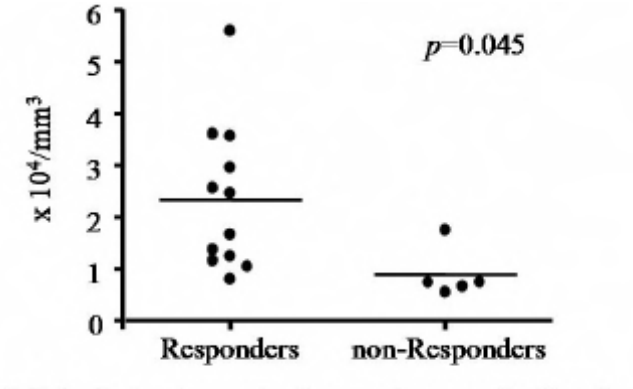
Background. Immunosuppressive treatment with a combination of antithymocyte globulin (ATG) and cyclosporine (CsA) is the most effective treatment for patients with aplastic anemia (AA). Further, it is suitable for patients of advanced age. Several prospective randomized studies have been reported, however, definite prognostic factor associated with response rate has not been cleared. *Aims*. To assess long-term outcomes after immunosuppressive treatment, and analyze the potent prognostic factors. *Methods*. We retrospectively evaluated 24 ATG with CsA treatments (ATG treatments) which include re-treatment of ATG in patients who had not responded to the first ATG treatment or relapsed after the first remission. *Results*. The median age was 66 years, and the median follow-up period was 52 months. The response and relapse rate of ATG treatment were 70.8% and 23.1%, respectively. The response rate of ATG re-treatment was 57.1%. Overall survival and event free survival at 10 years were 66.7% and 50.7%, respectively. Initial reticulocyte count correlated with the response rate of ATG treatment (responders v.s. non-responders = 23331±14201 (±SD) /mm^3 v.s. 8856±4885 /mm^3, p=0.045). Further, the longer duration from diagnosis to ATG treatment (responders v.s. non-responders = 0.615±0.18 v.s. 8.50±4.98 months, p=0.0077) and male correlated with the poor response rate independently. On the other hand, patient age, initial platelet count, initial granulocyte count, initial nuclear cell count of bone marrow, total dose of ATG, and co-administration of granulocyte colony-stimulating factor did not significant influence response rate. Although almost all ATG treatments were tolerable, as the long-term complication, two developed monosomy 7 clonal abnormality. *Conclusions*. These results suggest that ATG treatment can achieve a high response rate and long-term survival among adult patients with AA. Initial reticulocyte count, the duration from diagnosis to ATG treatment, and sex could be the potent prognostic factors with ATG treatment. However, we have to pay attention to the development of the clonal diseases.



Initial reticulocyte counts of responders was significantly higher than that of the non-responders (p=0.045).

Figure 1.

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THE ANTILEUKEMIC ACTIVITY OF THALIDOMIDE (THAL) IN COMBINATION WITH FLUDARABINE (F) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Thalidomide (THAL) is an immunomodulatory agent with pleiotropic activities. It may enhanced proapoptic activity of fludarabine (F) with the resultant improvement of clinical responses. We report the interim findings of the efficacy and safety of THAL+F combined therapy in B-CLL patients. *Methods*. 31 patients (pts) were enrolled in this study. The median age was 62 yrs (range 43-75). 14 of them (10M, 4F) were newly diagnosed and 17 (7M, 10F) pts were refractory or relapsed. Of these 3 had low risk, 20 pts intermediate risk, 8 pts high risk disease acc to modified Rai staging, 71% of pts had elevated serum β-2 microglobulin. The increased Zap70 and/or CD38 expression had 64% and/or 28% of pts respectively. 16 pts (55%) had short LDT < 6 months. 5 pts were refractory to F, 6 pts to alkylating agents and 6 pts were relapsed. Median prior lines of therapy were 3, range 1-6. THAL 100 mg po, alone was given for the first 7 days of cycle 1 and continued a la longue for 6 months, F 25 mg/m^2 iv or 40 mg/m^2 po, was given for 5 days every 28 days for up to 6 cycles, starting on the seventh day after initiating THAL. Acetylsalicylic acid 75 mg was used for preventation of venous thromboembolism. *Results*. Median duration of follow-up was 6months (range 1-14). Pts completed 1- 6 cycles, mean 5. Monotherapy of THAL decreased mean 30% (range 3-57) of white blood cells (WBC) in 26 pts. Directly after 1 cycle of therapy 31 pts showed subsequent reduction in WBC. 22 pts have received treatment for at least 3 months and are therefore evaluable for clinical response acc to NCI-WG criteria. Overall response rate among evaluable pts was 91% with CR in 18% (n=4) and PR in 72% (n=16) of the pts. 2 heavily pretreated pts attained stable disease after 2 cycles but therapy was stopped due to toxicity. 8 pts finshed treatment. The median duration of progression-free survival was 4 months (range1-7). 4 pts were inevaluable (unable to complete 2 treatment cycles; either for toxicity or progression), 5 pts are too early for response evaluation. Toxicity: All 31 pts are evaluable for toxicity. Constipation and fatigue were noted in nearly all pts. A tumor flare reaction developed in 23% of pts during the first week of treatment with THAL. Infections occured in 52% of pts, severe > G3 were observed in 4 pts. The main hematological toxicites were > G3 neutropenia (26%) and AIHA (13%). Correlative studies: Levels of TNF-α and IL-10 were assessed D0, D7 and D12. T regulatory cells were identified as $CD4^+CD25^{high}FOXP3^+$. This data will be presented at the meeting. *Conclusions*. Monotherapy of THAL and in combination with F is active not only as a first line therapy but also as a treatment in heavily pretreated pts with B-CLL, with tolerable toxicity. These results warrant further investigation of larger group of pts .

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PALPEBRAL OEDEMA REVEALING ORBITAL INTRAMUSCULAR OEDEMA AND ADIPOUS PALPEBRAL INFILTRATION IN CML PATIENTS TREATED WITH IMATINIB MESYLATE

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Background. Imatinib Mesylate is now the first line therapy for patients (pts) with chronic phase chronic myelogenous leukaemia (CP CML). In most pts side effects are moderate to mild and easily corrected by appropriate medications. Fluid retention and oedema are common and occur in 50% of patients. In most cases, diuretics are efficient. Periorbital oedema is the most frequent manifestation of fluid retention and appears to be dose related. We report 4 cases of palpebral oedema with additional ophtalmological signs such as retraction of the upper lid and slight exophtalmia. *Patients and Methods*. CP CML pts treated with imatinib 300 to 600 mg per day at Saint-Louis hospital were included in the study if they presented major periorbital oedema or oedema with additional ophtalmological manifestations. Patients were then referred to the ophthalmologist for clinical evaluation. MRI was proposed to all patients at the National Ophtalmological Center, Hôpital des Quinze-Vingts. *Results*. Four pts out of 120 were explored. They received imatinib at the dose of 300 to 600 mg per day during a median of 31 months. Oedemas were noted after 12 months of therapy (1-19 months) and were associated with generalized oedema and weight gain in 2 cases. Patients with generalized oedema developed the palpebral complication earlier compare

to patients with isolated palpebral infiltration. Ophtalmological examination revealed grade 3 palpebral infiltration without abnormal ocular motility in all cases. Other ocular signs were noted such as clinical exophtalmia and upper lid retraction both suggesting Basedow's disease features. These signs were confirmed with MRI showing a grade 1-2 exophtalmia with adipous hypertrophy, a normal aspect of the muscles but an evident palpebral modification with oedema and adipous hypertrophy both with WT1 hyper signal and hyper WT2 signal. Endocrinological investigations including TSH dosage, T3, T4 and auto antibodies against thyroglobulin were performed and ruled out the hypothesis of endocrinopathy. *Discussions and Conclusions*. We point out this particular complication of imatinib that mimics endocrinological features. Diuretics were efficient in reducing generalized oedema in the 2 patients concerned,but did not improve the palpebral infiltration in any patient. The dose of imatinib was reduced in 3 pts. We then observed a significant decrease of palpebral infiltration in those patients. Our observations strongly suggest that imatinib is able to induce muscular infiltrations and adipous hypertrophy noted in MRI imaging. Our hypothesis that imatinib could interfere with lipogenesis warrant further studies.

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IGHV3-21-EXPRESSED CLL CASES IN UKRAINE

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Neoplastic B-lymphocytes in patients with chronic lymphocytic leukemia (CLL) expressed restricted set of immunoglobulin variable heavy chain (IGHV) genes which may determine the clinical course of disease. First of all, an overexpression of IGHV3-21 gene was found in Scandinavian CLL patients with progressive clinical course and poor survival. The aim of our work was to check these data in Slavianian population (Ukraine). IGHV genes were studied in 168 CLL patients with from Ukraine using polymerase chain rection and direct sequence analysis. In a total, 165 in-frame rearrangements were amplified. The most frequent IGHV gene was IGHV1-69, which was identified in 36 cases (21.8%); followed by IGHV4-34 (11 cases; 6.7%), and IGHV3-21 (9 cases; 5.4%). Using the 98% cut off for homology to germ line, 47 (28.5%) of all in-frame cases were classified as mutated, and 118 cases were identified as unmutated (71.5%). IGHV3-21 was the 4th among the unmutated cases (5 cases; 4.3%) and the 3rd among the mutated cases (4 cases; 8.5%). Only 2 IGHV3-21-expressed CLL cases belonged to common-HCDR3 subset (mutated, IGHV3-21/IGHD-/IGHJ6, DANGMDV and DMNAMDV HCDR3 sequences, IGLV3-21*02/IGLJ3*01, QVWDSSSDHPWV LVDR3 sequence in both cases). Others 7 IGHV3-21-expressed cases belonged to nonhomogeneous-HCDR3 subset. By the frequency of IGHV3-21 expression and the ratio of common/nonhomogeneous cases our cohort was closer to Mediterranean cohort described by Ghia, 2005 (2.9% IGHV3-21-positive cases, range 0.86-4.12%; 7/9 common/nonhomogeneous cases) than Scandinavian cohort described by Tobin, 2003 (11.7% IGHV3-21-posivite cases, 21/10 common/nonhomogeneous cases). In addition, two our cases had homology with CLL cases from Mediterranean cohort: case F9 (HCDR3 DSDYDFWSGSWGYYGMDV) displayed homology with CLL case DQ987781 (HCDR3 DGDYDFWSGQWGYYGMDV), and case E89 (HCDR3 NRYTEYCSSTSCHPSYYYYYGMDV) displayed homology with Greece CLL case Gre6 (HCDR3 DRLLGYCSSTSCWDSYYYYYGMDV). Median overall survival in the whole CLL group was 104 months (among unmutated cases it was not reached and was equal to 90 months in mutated subgroup). Median overall survival for IGHV3-21-expressed cases was not reached, 3 dead IGHV3-21-expressed patients had unmutated gene with nonhomogeneous HCDR3 (overall survival 8, 43, and 73 months). Both IGHV3-21-expressed cases with common HCDR3 are alive (272 and 123 months) and had quite long progression-free period (183 and 96 months) during which they did not need treatment. Such data are differing from described earlier aggressive clinical course of common HCDR3 IGHV3-21-espressed CLL cases. It could be assumed, that possible potential antigenic stimulation via common HCDR3 may be under influence of environmental factors in different geographic regions.

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ZOSTER-RELATED PAIN IN HAEMATOLOGICAL MALIGNANCIES: DURABLE PAIN RELIEF BY OXYCODONE

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Background. Herpes Zoster Virus (HZV) outbreak is a significant cause of morbidity in the setting of blood-related malignancies, occurring mostly among patients affected by lymphoproliferative disorders (LPD) and in those submitted to haematopoietic stem cell transplantation (HSCT), among which HZV reactivation is reported between 15-45% in the autologous setting; one-third of these HZV patients developed post herpetic neuralgia (PHN). Moreover, in patients submitted to allogeneic HSCT, HZV reactivation is reported ranging from 41 to 59%. Early treatment of acute zoster pain (AZP) can reduce the incidence of post herpetic neuralgia (PHN). We treated with oxycodone 9 consecutive HZV-pain patients unresponsive to several agents, including anticonvulsants and analgesics. *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 in a thoracic dermatomal early treated with acyclovir and gabapentin. Given the persistence of neuropathic complaints, after three months gabapentin was replaced by high doses of pregabalin that the patient received for six months 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 a stably maintained pain relief. Patients 3 was affected by acute myeloblastic leukaemia and presented PHN afflicting the trigeminal region and unresponsive to pregabalin and tramadol, that was replaced by oxycodone in escalating dose until an acceptable pain relief. Patients 4 to 9 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 7 (1-18) months. *Conclusions*. Opioids suppress the central and the primary afferent nociceptors response; thus they should be most useful when PHN pain is maintained by input from dysfunctional afferents. Moreover, convincing evidences of their provided benefits in this setting have been reported by controlled studies and metanalysis. In particular, oxycodone and tramadol were reported as effective to relieve neuropatic pain. Although the short follow-up, the presented experience suggests that: 1) an opioid should be taken into account in patients with 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, an opioid provided of beneficial effect in relieving neuropathic pain, can represent a suitable option.

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A RAPID ASSAY OF CYTOSINE ARABINOSIDE UPTAKE AND METABOLISM BY ACUTE MYELOBLASTIC LEUKAEMIC CELLS USING A BIOLUMINESCENT BACTERIAL BIOSENSOR

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Acute myeloblastic leukaemia (AML) is a heterogeneous group of haematological disorders resulting from the malignant transformation of myeloid precursor cells. This leads to the proliferation of immature and undifferentiated cells in the blood and bone marrow. Effective treatment of AML is still challenging and the clinical outcome is disappointing and unpredictable.Only 70% of newly diagnosed patients receiving standard regimens with Cytosine Arabinoside (Ara-C) respond to treatment. Furthermore,a large proportion of these patients fail to achieve long-term remission and develop resistance to subsequent therapy. The nucleoside analogue Ara-C is one of the most active anti-cancer agents and has been the mainstay element of treatment used in AML for over

three decades. *In vivo*, Ara-C is transported into the cell via the specific nucleoside transporter hENT1 and rapidly phosphorylated by deoxycytidine kinase (dCK) into its monophosphate form Ara-CMP. This is further phosphorylated by nucleoside kinases into its tri-phosphate active form Ara-CTP. Drug inactivation can result from Ara-C conversion into Ara-uracil by cytidine deaminase (cdd) or from dephosphorylation of Ara-CMP by cytoplasmic nucleotidases. The anti-proliferative and cytotoxic effects of Ara-CTP are due to its ability to interfere withwith DNA polymerase and to incorporate into DNA strands leading to chain termination and DNA synthesis arrest. *In vitro* assessment of Ara-C efficacy has traditionally involved measurement of cell death or S-phase activity following AML cell exposure to Ara-C or the use of clonogenic proliferative assays. These methods are unstandardised, time consuming, expensive and not suitable for routine screening. Bioluminescence refers to the production of visible light in living organisms due to the oxidation of organic compounds (luciferins) in the presence of molecular oxygen and energy (NADH) catalysed by the enzyme luciferase. Only metabolically active bacteria produce light but the bioluminescent phenotype can easily be conferred to most bacteria by introducing and expressing the luxCDABE operon, isolated form Photorhabdus luminescens, under the control of constitutive promotors. Ara-C has no effect on E.coli as it lacks dCK and deaminates Ara-C into Ara-uracil through the activity of cdd. A cdd deficient strain of E. coli (S phage 5218) has been cloned which,upon expression of the human dCK gene exhibits reduced relative growth in the presence of Ara-C in basic minimal medium. The Ara-C effect on growth was completely abolished when assayed in the absence of the dCK inducer IPTG, indicating that human cDK expression in the bacteria leads to Ara-CTP incorporation into bacterial DNA. In this study we report the construction of a cdd-deficient mutant E. coli MG1655 strain with enhanced sensitivity towards Ara-C and its use as a bacterial biosensor in a rapid assay to determine Ara-C uptake and phosphorylation by human AML cells. The assay was assessed in cell lines and clinical AML specimens from eight patients who had given written informed consent. Intracellular concentrations of Ara-C in the clinical range between 25 and 100 uM were detectable in less than 8 hours and correlated with the 3 day cytotoxicity test for Ara-C sensitivity. In preliminary tests with the eight clinical samples, the biosensor assay was able, within 8 hours to predict the clinical outcome in all cases. As a significant proportion of AML patients fail to respond to Ara-C, this assay may enable response prediction before patients undergo chemotherapy and possibly allow the targetted treatment of highly sensitive patients with reduced doses of Ara-C.

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IN VITRO INDUCTION OF A DENDRITIC CELL PHENOTYPE IN PRIMARY HUMAN ACUTE MYELOGENOUS LEUKEMIA (AML) BLASTS: THE CHEMOKINE RELEASE PROFILE OF THE AML DENDRITIC CELLS DEPENDS ON THE EXPERIMENTAL PROTOCOL

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Background. T cell targeting immunotherapy is now considered in acute myelogenous leukemia (AML). A dendritic cell (DC) phenotype can be induced in primary AML cells, and one possible therapeutic approach is therefore to use such cells for *in vivo* vaccination of patients or *ex vivo* priming of leukemia-reactive T cells in stem cell grafts. However, the release of T cell chemotactic chemokines by AML-DC has not been characterized previously. *Aims*. To investigate the release of a wide range of T cell chemotactic chemokines by *in vitro* induced AML-DC. *Methods*. Primary AML cells were cultured *in vitro* according to four protocols that previously have been characterized in detail: (i) GM-CSF + IL4 days 1-14 and TNF-α for days 6-14; (ii) GM-CSF + IL4 + TNF-α + FLT3-L for eight days; (iii) GM-CSF + IL4 +TNF-α +FLT3-L + SCF + TGFβ1 for eight days; and (iv) 25 Gy γ-irradiation combined with culture with GM-CSF + SCF + IL3 for four days. Chemokine release was determined after induction of the AML-DC phenotype. *Results*. AML cells cultured according to the DC protocols showed altered chemokine release profiles. Firstly, for the GM-CSF/IL4/TNF-α protocol AML-DC showed increased release of CCL2, CCL4, CCL5, CCL17 and CCL22, whereas the release of CXCL8 and CXCL10 was not altered. Secondly, increased CCL17 levels were observed for all four protocls, whereas the increased CCL22 release reached statistical significance only for those three protocols that did not include irradiation. Increased levels of CCL2, CCL3, CCL4, CCL5 and CXCL8 were only observed for some of the protocols, but decreased levels were not observed for any chemokine. The primary cells showed a wide variation in their chemokine release, and a wide variation between patients was also observed after differentiation. *Summary/Conclusions*. Induction of a dendritic cell phenotype in primary AML cells causes a modulation of the chemokine release profile with increased levels of several T cell chemotactic chemokines.

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COMPLETE REMISSION OF SMOLDERING MYELOMA IN AN HIV PATIENT AFTER HIGHLY ANTIRETROVIRAL THERAPY

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Background. A much higher incidence of monoclonal gammopathy (MG) has been reported among young HIV-infected patients. Despite MG is often considered an asymptomatic process in immunocompetent individuals, it may herald more serious diseases such as multiple myeloma, light-chain amyloidosis and other B-cell related malignancies. Currently, there is scarce knowledge about the natural history of MG in HIV infection. We report a case of an HIV-patient fulfilling multiple myeloma criteria (WHO), which regressed to a polyclonal hypergammaglobulinemia with antiretroviral therapy (HAART). *Case 1*. A 27-years-old Black woman was admitted, complaining of asthenia and arthralgias. A month before admission, she was diagnosed with HIV infection. Her initially CD4 cell count was 215 cell/mL and her viral load 7100 copies/mL. On admission, physical examination was normal. Initial laboratory tests revealed: creatinine 0.95 mg/dL (normal value [nv] 0.6-1.4 mg/dL), serum total protein 10 g/dL (nv 6.4-8.3 g/dL), albumin 3.4 g/dL (nv 3.8-5.1 g/dL), hemoglobin 96 g/L, white cell count 1.9×10^9/L, platelets 228×10^9/L, β2-microglobuline 3.1 mg/L (nc 0-2.5 mg/L). Serum immunoelectrophoresis and immunofixation detected M-protein component of 45 g/L and a monoclonal IgG-kappa of 66 g/dL. Antibodies for HCV, HBV, EBV and herpes virus 1 and 2 were negative. In order to rule out a multiple myeloma in an HIV-patient, a bone marrow exam was performed which revealed an atypical plasma cell infiltrate (14%) of bone marrow cellularity. The plasma cell immunophenotype showed a $CD138^+$, $CD38^+$, $CD19^+$, $CD56^+$ and $CD10^+$ aberrant population. Moreover, Bence Jones proteinuria in the range of 126 mg/dL was demonstrated. Biopsy for amyloid was negative. Skeletal exam, thorax and abdomen CT scan were normal. A diagnosis of smoldering myeloma was done. After one and a half year of HAART, her CD4 cell count reached 342 cells/mL and her HIV viral load was undetectable. During this treatment period, the M-protein component not only progressively decreased but also the serum immunoelectrophoresis showed a polyclonal hypergammaglobulinemia, with repeateadly negative serum and urine immunofixation. Plasma cell infiltrate in bone marrow also decreased to 5% (Figure 1).

Figure 1. Values of monoclonal component (MC), IgC quantification and CD4 cells during the patient follow up.

Conclusions. Plasma cell disorders are common in HIV-young patients. These disorders range from benign polyclonal hypergammaglobulinemia to MGUS and malignant plasma cell dyscrasias, including multiple myeloma, amyloidosis and plasma cell leukaemia. In the HAART era, there is uncertainty regarding the incidence and the natural history of M-proteins in HIV-infected patients. The leading hypothesis is that chronic antigenic stimulation of HIV or another coexisting infections leads to B-cell hyperplasia. This B-cell dysregulation in the context of impaired T-cell response, as well as an altered cytokine milieu, could contribute to the appearance of a malignant clone. Although there is limited experience, some reports suggest that HAART can decrease serum monoclonal protein. Our patient fulfilled multiple myeloma criteria that disappeared with HAART. In our knowledge, this is the first case reported of

smoldering myeloma that reaches complete remission after HAART. According to this clinical observation, if plasma cell dyscrasia is diagnosed in an HIV-patient, physicians should be aware of the possible benefit of HAART, and reevaluate the disease after immune restoration and viral load control.

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TREATMENT OF CYTOMEGALOVIRUS REACTIVATION DURING THERAPY WITH ALEMTUZUMAB IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA A DILEMMA TO SOLVE

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Alemtuzumab (Campath), monoclonal antibody, anti-CD52, is a new drug for a treatment of chronic lymphocytic leukemia (CLL), that has an importance as monotherapy and also in combination with chemotherapy. Among side effects of alemtuzumab reactivation of cytomegalovirus (CMV) and its therapeutic management seems to be an important problem. Therapeutic standard in reactivation of CMV is to stop the treatment with alemtuzumab and to start the treatment with ganciclovir until CMV negativity. Here it is presented own experience concerning CMV reactivation in ten patients with progressive CLL, treated with alemtuzumab as a first-line treatment. They were three women and seven men, aged 53-75 years (median age -59.7years). Two of them were in stage I according Rai classiffication, five in stage II, two in stage III and one in stage IV. Median time from diagnosis to treatment varied between 1 month to 101 months (median time-19.7months).Absolute lymphocyte count before treatement was 56.7G/l to 228.0G/l (median count-107.0G/).All patients were CMV seropositive before treatment with alemtuzumab and anti-CMV IgG titre was 1:230 to 1:49 000 (median '1:16 653).The patients were reported to have negative anti-CMV IgM and also serum CMV-DNA assay by PCR method was below 500 copies/mL. Patients received alemtuzumab at a dose 30mg I.V. three times weekly during 12 weeks. Prophylaxis of infection consisted with aciclovir at a dose 800 mg daily and co-trimoxazole at a dose 1920 mg three times weekly during treatment period and two months after stopping the therapy. Serum quantitive CMV-DNA was estimated every week using PCR method and patients were underwent physical examination 3 times weekly. During treatment period in eight patients (80%) reactivation of CMV occurred, in one case symptoms of CMV disease was observed (fever is the only symptom) and in one patient neither reactivation nor CMV disease occurred. The CMV reactivation appeared between 3-8 weeks of treatment . Serum CMV-DNA titre was; 700 to 19 000 copies/mL: only in one patient it was 380 000 copies/mL (this patient had symptomatic CMV disease and received the treatment with ganciclovir). We took into consideration the treatment with ganciclovir in all patients but the decision depended on the presence of CMV symptoms, titre of serum CMV-DNA during reactivation and dynamics of increasing CMV-DNA titres which were estimated every week. Two patients received the treatment with ganciclovir; one of them had CMV disease, seven patients despite positivity of CMV-DNA had not received any treatment. None of these seven patients had symptoms of CMV disease. In all these patients during 1 to 4 weeks spontaneous decreasing of CMV-DNA were observed. In five patients with a low titre of serum CMV-DNA, without increasing this titre, the treatment with alemtuzumab was not interrupted. It is concluded, that in the case of CMV reactivation in CLL patients treated with alemtuzumab, without symptomatic CMV disease, very careful clinical observation , every-week CMV-DNA assay and prophylaxis with aciclovir is a sufficient management. In other patients with CMV reactivation, particulary exposed on CMV disease (even slight symptoms of CMV disease, high rate of CMV-DNA titre observed during reactivation and high rate of anti-CMV IgG before the treatment) the therapy with ganciclovir is recommended.

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MOLECULAR SCREENING OF PDGFRA AND PDGFRB GENES IN KIT AND FLT3 NEGATIVE CORE BINDING FACTOR LEUKEMIAS

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Mutations involving KIT and FLT3 genes, encoding tyrosine kinase (TK) membrane receptors, are frequently detected in core binding factor leukemia (CBFL) adult patients and are reported as mutually exclusive. PDFGRA and PDGFRB encode class III TK receptors and are involved both in physiological processes, such as fibrosis, and in the pathogenesis of haematological and solid tumours. Mutations in PDGFRA are found in gastrointestinal stromal tumours (GIST), rarely in synovial sarcomas (SSs) and in malignant peripheral nerve sheath tumours (MPNST), whereas FIP1L1-PDGFRA fusion product occurs in systemic mastocytosis associated with eosinophilia, in idiopathic hypereosinophilic syndrome, chronic eosinophilic leukemia and in polycythemia vera patients. Many different PDGFRB chimeras are described in BCR-ABL negative chronic myeloproliferative disorders. In order to detect PDGFR mutations in CBFL, we screened 35 KIT and FLT3 negative patients by specific SSCP analysis and direct sequencing. For analysis of the juxtamembrane and TK domains of PDGFRA, exons 9, 11-15, 17-20 were amplified. We studied exons 12 and 18 for analysis of the TK domain of PDGFRB. PCR were loaded on non denaturing gradient gels (5-20%) at two different temperature conditions (12°C and 23°C) for 20 hours. Samples displaying abnormal migration patterns underwent direct sequencing with forward and reverse primers. Three types of single-nucleotide variations (SNP) were detected in PDGFRA gene: in exon 13 (rs10028020-SNP ID) 5 out of 35 patients showed a GCG>GCA change in heterozygous form; in exon 18 (rs2228230-SNP ID) 5/35 patients displayed a GTC>GTT change in heterozygous form and in exon 12 (rs1873778-SNP ID) 4/35 patients showed a CCA>CCG change in homozygous form. Moreover, exon 13 and exon 18 polymorphisms were both present in 3 patients. All three SNPs in PDGFRA gene were previously described. No molecular alteration was detected in PDGFRB gene. In conclusion, no pathogenic mutation in PDGFR genes was detected among our KIT and FLT3 negative CBFL patients and further molecular investigations will be needed to better clarify their genetic characteristics.

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ASSOCIATION OF CD38 EXPRESSION AND DIAGNOSTIC IMMUNOPHENOTYPIC SCORE IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background-aims. The typical B-cell chronic lymphocytic leukaemia (B-CLL) phenotype is $CD5^+$, $CD23^+$, $FMC7^-$, dim expression of surface Ig (sIg) and weak/absent expression of membrane CD22 and CD79b. Because there is no specific marker for B-CLL, a composite phenotype considering the above markers compounded into a scoring system, helps to distinguish CLL from the other B-cell malignancies. According to this system, 92% of B-CLL cases score 4 or 5, 6% score 3 and 2% score 1 or 2. CD38 is a membrane protein that marks cellular activation and it is now considered as an independent prognostic marker of outcome. We study the possible relationships between a prognostic marker (CD38) and the immunophenotypic score-consisting antigens. *Patients and Methods*. From 2000 to 2006, 176 previously untreated B-CLL patients [M/F: 99/77, median age: 68 (36-87) years] were diagnosed at our centre. The lymphocyte morphology was typical in 171 cases (97%) and the majority of the patients [160/176, (91%)] were classified as Rai stage 0-2. Three-color flow cytometric analysis was performed on their blood samples and the reactivity with CD2, CD5, CD19, CD20, CD22, CD23, CD25, CD38, CD79b, FMC7, and immunoglobulin kappa and lambda light chains, was analyzed. Positivity for antigen expression was set at 30% while two cut-off points (7% and 30%) were used for CD38 expression. Mann Whitney U test and Spearman's rho test were used for

statistical analysis. *Results*. Seventy-seven patients (44%) had an immunophenotypic score of 5, 67 patients (38%) scored 4 and 32 patients (18%) were diagnosed with a CLL score of 3. Sixty-seven patients (38%) showed CD38 cellular expression in 30% B-lymphocytes or more and 126 patients (72%) were found CD38+ if the cut-off was 7%. All subgroups of CD38+ patients showed a significantly lower mean immunophenotypic score at presentation than the respective CD38- patients (4.183±0.76 vs. 4.44±0.67, p: 0.043 for CD38 positivity ≥7% and 4.015±0.83 vs. 4.37±0.80, p: 0.005 if the threshold for CD38 expression was 30%). The CD38 cellular expression was negatively associated with CD20 (rho:-0.172, p:0.024) and CD5 (rho:-0.294, $p<0.001$) antigens but did not correlate with lymphocyte activation markers (CD23 and CD25) nor atypical CLL markers (FMC7, CD79b and CD22) expression. Subgroups, according to CD38 expression, did not differ for gender, age, lymphocyte morphology, WBC and lymphocyte count, Hb, platelets and advanced disease stage. *Conclusions*. According to our findings, CD38+ patients seemed to have a significantly lower immunophenotypic score at presentation that the CD38- patients, irrespectively of the cut-off limit. CD38 expression was not correlated with other antigens of prognostic significance such as FMC7 and CD79b.

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CYCLOSPORIN A AND DEXAMETHASONE INDUCE APOPTOSIS IN MALIGNANT CELLS OF PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (B-CLL) is a malignant disorder characterized by the accumulation of leukemic cells in the G0-G1 phase of the cell cycle and expressing high levels of the anti-apoptotic protein Bcl-2. Leukemic cell apoptosis can be induced by glucocorticoids and other anti-tumor drugs. *Aim*. Since in some patients we observed that the treatment of autoimmune complications with Cyclosporine A (CsA) resulted in an improvement not only of the autoimmune phenomena, but also of the leukemic process, the aim of this study was to investigate the *in vitro* cytotoxicity of CsA as compared to Dexamethasone (Dex) on leukemic cells. *Methods*. In the present study we evaluated the *in vitro* cytotoxicity of CsA as compared to Dexamethasone (Dex) on leukemic cells obtained from 32 patients with B-CLL. *Results*. Leukemic cells obtained from 32 B-CLL patients showed a heterogeneous pattern of spontaneous apoptosis at 24 hr interval and this pattern permitted to identify: Group 1 (14/32) with high (>20%) apoptotic rate and Group 2 (18/32) with low cell death. A few cell suspensions did not show any spontaneous cell death. Cytofluorimetric analysis confirmed that the observed cytotoxicity was due to apoptosis. CsA and Dex increased cell death in both groups with a different timing by an apoptotic mechanism that does not involve Bcl-2. In fact, while CsA already induced apoptosis at 4 hrs of *in vitro* incubation, the activity of Dex was more evident at 24 hrs. Furthermore, in Group 2, CsA-induced apoptosis was significant higher than that observed with Dex both at 4 and 24 hrs. *Conclusions*. The data herein provided indicate that CsA has a significant pro-apoptotic activity in B-CLL. Our observations might be taken into account to consider new therapeutic strategies in B-CLL.

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TREATMENT OF RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN CML PATIENTS:IMATINIB ONLY VS IMATINIB&DLI

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Background/Aim. Donor lymphocyte infusion (DLI) is the gold standard for relapse after alloHSCT. The role of single agent or combined application of imatinib,which is highly effective for induction of molecular remission in CML patients, in post alloHSCT relapse is still an open question. *Methods*. In this single center study, we retrospectively analyzed molecular response and graft functions of relapsed CML patients after transplantation, who received imatinib only (n=10), Imatinib+DLI (n=9). The median age of patients in the imatinib group (group 1) was 29 (range,19-54) , F/M:3/7. Nine patients were in CP1 and one was in CP2 at the time of transplantation. In the combination group (group 2), the median age was 41 years (20-54) , F/M:3/6. In group 2 the status of patients at transplantation was CP1:4, AP: 3 and BP:1. Three patients received reduced intensity and the remaining myeloablative conditioning. Molecular remission (MR) was defined as; complete negativity of RQ-PCR (<3 log). The great majority of the patients achieving MR proved to be in complete chimeric (CC) status. *Results.* After 6 months of follow-up; 9 of 10 patients treated with only imatinib (90%) achieved MR at median 3(2-5)months.Only one patient remained refractory to treatment and died because of pneumocystis carinii pneumonia. Two patients relapsed after 6 months, received DLI, one of them died of DLI induced GVHD, the other one obtained MR. After 12 months of follow-up, 3 of 7 patients relapsed and 2 had remission after addition of DLI to imatinib. At the end of 12 months in group 1 only four patients remained in sustained MR with Imatinib only (40%) and three after combination with DLI (70%). In group 2, 4of 9 patients were treated with upfront DLI plus imatinib and 5 of 9 patients with imatinib followed by DLI up to 6 months. After 6 months of follow up; 5 patients remained refractory but 4 patients achieved MR (44%). At the end of 12 months; 3 of the 4 patients were still in molecular remission (33%). Two patients died of progressive disease, 3 patients remained refractory (recurrent relapse) and one patient experienced molecular relapse. *Conclusion*. Our single center experience has shown that MR and CC status is achievable after imatinib in relapsed CML patients after alloHSCT but the response is not durable. DLI synergized with imatinib for induction of MR but the cumulative high risk CML patients in group 2 caused to low remission rates. DLI only should be the standard approach in CML patients with molecular relapse.

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APPROACHES TO THERAPY OF PATIENTS WITH MULTIPLE MYELOMA DEPENDING ON IMMUNOLOGIC VARIANTS OF DISEASE

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Efficacy of VAD (vincristine, adryamycine, dexamethazone) as first line therapy (first group, n=42, age from 43 to 80 years) and VD (velcade (bortezomib) plus dexamethazone) as second line therapy (second group, n=20, age from 43 to 80 years) was evaluated in patients (pts) with II-III stages multiple myeloma (MM). First group consisted of 10 pts with MM A (IgA), 28 pts with MM G (IgG), 3 pts with MM Bens Jones (BJ protein), 1 pt with MM E (IgE), 1 pt with MM M (IgM) and received 4-6 course standard VAD therapy. In case of disease progression during first line therapy or progression after phase plateau patients received as second line therapy velcade 1,3 mg/m^2 at 1,4,8,11 days and dexametazone 20 mg at 1,2, 4,5, 8,9, 11,12 days every over 10 days (6-9 courses). This group consisted of 5 pts with MM A, 11 pts with MM G, 1 pt with MM E, 1 pt with MM M, 3 pts with MM BJ. After VAD therapy overall response (OR) in pts with MM A was received in 20% of cases (1- near CR, 1- PR), in pts with MM G-in 64% (6-CR, 2-nearCR, 7-PR, 3-minimal response) accordingly, ($p<0,05$); stabilization of disease(SD) was achieved in 3 (30%) and 3 (11%) pts, accordingly; progression disease(PD) was revealed in 5(50%) pts and 7 (25%) pts accordingly. Thus VAD therapy in pts with MM A was less successful than in pts with MM G. After second line VD therapy 5 (100%) pts with MM A we achieved CR. 6(55%) from 11 pts with MM G had OR on VD therapy (1-CR, 3-near CR, 1-PR, 1-mR). SD and PD were revealed in 2 (18%) and 3(27%) pts with MM G accordingly. These preliminary results indicated that MM A have high sensitiveness to velcade. I pt with MM A had a relapse of solitary bone destruction after 3 months, but 4 pts stayed in CR during 1 year observation on support therapy by VD (I time per 3 months). Adverse events of VD therapy were acceptable: peripheral neuropathy (50%), herpes infection (15%), diarrhea (15%), nausea (10%), decreased platelet count (10%), paralytic ileus (5%). Such prognostic factors as type of light-chain, β2-microglobulin, level of heat shock protein 70, LHD, albumin didn't influence the efficacy of therapy by velcade. We assume that therapy velcade plus dexamethazone should be considered as first line therapy in patients with MM with secretion of IgA.

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SURVIVAL AND COMPLICATIONS OF TRANSFUSION DEPENDENT β-THALASSEMIA MAJOR IN LEBANON; A DECADES EXPERIENCE

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Background. β-thalassemia major (TM) is a debilitating disease with a considerable prevalence in Lebanon. While the prognosis of patients affected by β-thalassemia major has improved after the introduction of regular blood transfusion regimens, complications and mortality related to iron overload started to emerge. With the introduction of desferrioxamine in the 1970s, the prospects of the disease improved. The chronic nature of both the illness and its treatment modalities have lead to the creation of the Chronic Care Center (CCC) in the year 1994, an institution where patient care and information is centralized. *Aims*. Since its inauguration, the CCC has significantly contributed to decreasing the burden of β-thalassemia, as well as spreading awareness about this disease. The present article deals primarily with our experience to this day, specifically pertaining to the mortality of TM and development of complications among the patients. *Methods*. All TM patients (218) included in this study are regularly followed up at CCC with 30 patients who were lost to follow-up being censored. Patients have been divided into three groups according to the following birth cohorts: patients born in 1970-1983, 1984-1993, and patients born in 1994 and beyond. Data collected, through chart review, included: year of birth, age at diagnosis, gender, consanguinity status, date and cause of death, as well as date of appearance of any of the following complications: hypothyroidism requiring replacement, hypogonadism, infection with the hepatitis C virus, HIV infection, or thrombotic events. *Results*. A total of 15 deaths was recorded, nine (60%) of which were due to cardiac causes (Table 1). Heart failure affects 6.5% of patients (n=14). Hypogonadism was shown to affect 27.9% of patients (n=60) and hypothyroidism was present in 20.9% (n=45). Fourteen percent (n=30) of the patients have Hepatitis C virus (HCV) infection. Though not statistically significant (p=0.555), complication free survival period was noted to be slightly better among patients with mean serum ferritin levels below 2500 ng/mL as compared to patients with higher levels. Patients born in 1994 and beyond were found to have a significantly more extended complication-free survival period. *Discussions*. The centralization of care and the more consistent follow-up at the CCC led to an increase in the complication-free survival period. Patients are being diagnosed at an earlier age and chelation therapy is being initiated considerably earlier (7.20±5.59 years of age for patients born from 1970 to 1984, compared to 1.07±1.47 years of age for patients born after 1994). Complications due to iron overload still persist however, and the non-compliance to desferrioxamine regimens is thought to be the main reason for this. *Conclusions*. The advent and introduction of new oral iron chelators as well as better iron overload quantitation methods will most likely lead to new findings and decrease in the sequale of the disease, thus a follow up study will be required to examine their impact and the new survival trends.

Table 1. Causes of Death.

Cause	N	%
Heart Failure	9	60.0
Infectious Cause	5	33.3
Unknown	1	6.7

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MONITORING ERYTHROCYTES CREATINE, DENSITY AND DEFORMABILITY IN DETECTING PRECLINICAL ACTIVITY OF HEMOLYSIS IN THERAPY OF AUTOIMMUNE HEMOLYTIC ANEMIA

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Autoimmune hemolytic anemias (AIHA) are acquired diseases, characterized by selective destruction of erythrocytes by autoantibodies. The reticulocyte count, creatine level and deformability of erythrocytes are considered to be quantitave indicators of hemolytic process, which can show preclinical hemolytic activity, but complex investigation of hemolysis by different indices hasn , t been done yet. In 1967 Griffith suggested that creatine level of erythrocyte reflects the mean age of erythrocyte population. *Aim*. To detect minimal hemolytical activity with the main and additional diagnostical tests of hemolytic process (hemoglobin -Hb, reticulocyte - Rt, bilirubin, creatine, density, deformability of erythrocyte which can help to estimate the efficacy of treatment and prevent the development of severe hemolytic crises. *Materials and methods*. 47 patient with autoimmune hemolytic anemia (AIHA) were investigated: 18 male, 29 female, 22-68 y.o. *Results*. During hemolytic crises the hemoglobin level dropped (58,2±15,5 g/l), reticulocyte count increased up to 10,6±2,8%, bilirubin level also increased to 47,4±24,7 (N-0- 20 mkm/l); mean level of creatine in erythrocytes was 5 time above normal value (28,7±13,0 mg/dL, N - 5,7±1,5 mg/dL), density and deformability of erythrocyte were also elevated (d l 16,8±5,4% N - 0,3±0,3%; d t 20,6±3,4%, N - 0,6±0,3%). 26 patient with chronic AIHA received prednisolone 1-2 mg/kg. After 1 month therapy Hb, Rt, bilirubin normalized, but creatine and deformability of erythrocyte still normal ranges after 2 month remission. Patients with partial remission were characterized by normal level of hemoglobin, high content of reticulocytes; but at the same time creatine, density and deformability of erythrocyte exceeded normal values and didn , t reach normal parametres. Patients with acute form AIHA (n=15) were treated with high dose methylprednisolon for 3 days, 4-5 cycles. Hb, Rt, bilirubin normalized after 4 cycles of pulse therapy. After Hb, Rt, Bi had been normalized, creatine of erythrocytes was decreasing slowly (11,4 mg/dL) and normalized within 3-4 month. In remission (n=5) all values were normal. Three patients with resistant form AIHA were treated with Rituximab (375 mg/m^2). Three month later all parameters of hemolytic activity (main and additional) normalized . Their remission duration is 5, 9, 19 month. During remission, monitoring all parameters have been kept normal. In relapse erythrocyte creatine levels were found to be rised at first as well as erythrocyte deformability. Monitoring parameters of one patient after the first course of Rituximab (RTX) had shown slowly increasing creatine level and deformability of erythrocyte, with stable level of hemoglobin and reticulocytes. At primary period of hemolyse (without clinical manifestation) RTX was applied again. It can prevent the development of severe hemolytic crises. Complete remission having been observed for 3 years. We observed high correlation between reticulocyte count, creatine and deformability erythrocytes (r=0,86, r=0,78 p<0,05), and negative correlation between hemoglobin and creatine erythrocyte (r=0,76, p<0,05). *Conclusions*. The monitoring of additional parameters (creatine, density and deformability of erythrocytes) is of significant importance to diagnosis of minimal hemolysis and can help to prevent it, when started.

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DIFFERENT EFFECT OF THE HEAT SHOCK PROTEIN 90 INHIBITOR ON APOPTOTIC CELL DEATH IN LEUKEMIA CELL LINES IS ASSOCIATED WITH AKT

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Background. Heat shock protein (HSP) 90 complex mediate the maturation and stability of proteins such as AKT, mitogen activating protein kinase (MAPK), and p53. HSPs were shown highly expressed in leukemia and HSP 90 antagonists represent a novel anti-leukemic treatment. *Aims*. In this study we examined the cell death in KG-1, an acute myeloid leukemia cell line, and in K562, a chronic myeloid leukemia in blast crisis cell line, which is induced by a HSP 90 inhibitor 17-allylamino-17-demethoxy-geldanamycin (17-AAG). *Methods*. KG-1 and K562 cell lines were cultured for 4 days in addition of 17-AAG and LY294002 or PD98059. Cells numbers were measured by MTT assay. At each time point FACS analysis for annexin V/propidium iodide (PI) staining was done to measure apoptotic cell death. The expression and phosphorylation pattern of AKT was analyzed by western blotting.

Results. Though there was no recognizable decrease of cell number in KG-1 through broad range of 17-AAG concentrations (0.02 to 2.5 uM), dose-dependent decrease of cell number was prominent in K562. Cell death in KG-1 and K562 was mainly mediated by apoptosis and the pattern of annexin V/PI staining at 72 hours, not 24 and 48 hours, was consistent with the degree of decrease in cell proliferation measured at 96 hours. AKT and p-AKT level in K562 were reversely correlated with the concentration of 17-AAG, but not in KG-1. To verify the role of AKT in KG-1 which was not sensitive to 17-AAG, cells were cultured with PI3K inhibitor LY294002 or ERK1/2 inhibitor PD98059. Not PD98059 but LY294002 caused severe apoptotic cell death accompanied with the decrease of AKT and p-AKT. *Summary/conclusions*. HSP 90 inhibitor 17-AAG affected on apoptotic cell death and AKT according to the type and origin of leukemic cells differently and treatment of leukemia with HSP 90 inhibitor should be selectively applied with caution. Inhibition of AKT could be a main target when leukemia is not sensitive to HSP 90 inhibitor.

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ANALYSIS OF GENE EXPRESSION PATTERN IN BONE MARROW AND LIVER OF MICE DEVELOPED MYELOPROLIFERATIVE DISEASE AFTER LONG-TERM G-CSF TREATMENT

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Background. At the present time there is a strong need for animal models to study the mechanisms of leukemogenesis. We obtained transplantable leukemia after treatment of (CBAxC57Bl6) F1 12-16 week-old mice with low (25 mcg/kg) G-CSF doses. G-CSF was injected 4 days successively once a month. MPD-like myeloid leukemia with histiocytic sarcoma occurred in one out of 20 mice after 3d G-CSF course. Bone marrow and liver cells of the mouse are fully transplantable, recipients become moribund within 17-32 days since cells injection. All ill animals had enlarged liver (M 4,1±0,1 g versus normal 1,44±0,03 g) and spleen (M 304,9±15,5 mg versus normal 93,1±1,8 mg). The developed leukemia was not of virus origin that was proved by three independent methods. *Aims*. To analyze gene expression pattern in leukemic mice for elucidation of mechanisms that have led to the development of leukemia. *Methods*. Subtracted cDNA library of differentially expressed sequences was constructed from bone marrow cells of normal and leukemic mice using Suppression Subtractive Hybridization (SSH). Leukemia- and normal-specific cDNAs were sequenced and associated with known genes. Expression level of several leukemia-specific genes was further studied by RT (reverse transcription)-PCR in bone marrow and liver of leukemic mice. *Results*. 3 out of 48 leukemia-specific sequences turned out to be subunits of ATP synthase, each of the following up-regulated genes: IL1-r2 (CD121b), CathK and ARF-BP1, ATPase subunits of 26S protease was identified twice. Up-regulated expression of these genes was proved by RT-PCR. Clinically ill mice showed a moderate extent of anemia and reticulocytosis accompanied by extensive suppression of ,-globin expression (8 out of 10 down-regulated genes). The expression level of c-Abl and G-CSF doubled in bone marrow of leukemic mice compared to the normal bone marrow. The expression level of genes regulating cell proliferation did not change dramatically-only C-Myc expression had increased 3-fold, however concentration of early hematopoietic precursor cells (LTC-IC) had decreased about 5-fold (0,75 versus 3.32 per 105 cells in healthy mice). The pronounced changes had been revealed in expression of Mpo gene (3,4-fold increase). The liver of ill mice consisted of undifferentiated cells. Increasing of CD45 expression up to 11-fold simultaneously with substitution of liver parenchyma by tumor cells points to hematopoietic origin of invading cells. Oligopotent hematopoietic precursor cells (CFU-C) were also revealed in affected liver (52,5±7,7 per 105 cells).There were mild changes in G-CSF expression in the liver cells of leukemic mice, whereas the expression of Csf3r (CD114) increased 18-fold compared to the normal liver cells. The expression of Csf1r (CD115) increased 20-fold, fibronectin and granulin increased 5-fold and Ly6E increased 3-fold compared to the leukemic bone marrow indicating highly malignant phenotype of cells invading liver. The expression of antiapoptotic genes was elevated up to 4-fold for bcl-2 and 2-fold for cIAP2. *Summary/Conclusions.* The G-CSF treatment may lead to the development of myeloid leukemia with high ability to invade liver tissue and dramatically changed gene expression pattern.

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APOPTOSIS REGULATION AND THE ROLE OF VEGF IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the western world with an incidence of 3.36/100,000 in European males. It is characterised by a clonal growth of long lived, slowly proliferating mature B lymphoid cells in the bone marrow (BM), peripheral blood (PB) and lymphoid tissues. CLL has an extremely variable prognosis at presentation: a third of cases require chemotherapy from the start, a third of cases have an extremely indolent course and never require active therapy and a third of cases progress over time and eventually require some form of chemotherapy. Although CLL generally responds to initial chemotherapy, it remains an incurable disease. B-CLL cells have a long survival owing to alterations in the normal pathways of apoptosis. It has been shown that B-CLL cells rapidly undergo apoptosis during in-vitro culture indicating that signals from the microenvironment are of vital importance in maintaining resistance to apoptosis. Recent studies indicate that vascular endothelial growth factor (VEGF) and it's receptors may have an important role to play in CLL cell survival. The cytokine IL-4 is also known to have a pro-survival role for CLL cells. The aim of our study was to analyse survival of purified CLL cells cultured in serum free and serum supplemented conditions with the addition of VEGF and IL-4 and also analyse VEGF patient plasma levels. Survival of purified CLL cells cultured in serum free and serum supplemented conditions was assessed by Annexin V/Propidium Iodide staining followed by flow cytometry analysis. Patient plasma VEGF levels were assessed by ELISA. Informed consent was obtained from patients. Addition of VEGF to serum free media did not result in increased cell viability whereas IL-4 consistently increased cell viability. Addition of VEGF to serum supplemented media resulted in a significant increase in cell viability in a subset of patients (6 out of 17). Analysis of VEGF levels in patient plasma samples (23) showed a correlation between increased levels of VEGF and high white blood cell/lymphocyte counts ($p<0.05$). CD38 expression, a poor prognostic indicator, was also assessed. Approximately 30% of patients analysed were $CD38^+$ but at this time no clear correlation with other clinical data has been observed. Our data has shown that addition of exogenous VEGF to CLL cells cultured in serum free media does not increase the survival of these cells. Addition of VEGF to CLL cells cultured in serum supplemented media increased cell survival in certain samples indicating VEGF may act synergistically with factors present in serum to increase cell survival. The correlation between increased levels of VEGF and high white blood cell/lymphocyte counts also suggests a role for VEGF in CLL disease progression.

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IDENTIFICATION OF HEME OXYGENASE-1 (HO-1 = HSP32) AS A NOVEL THERAPEUTIC TARGET IN CANINE MASTOCYTOMA CELLS

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Background. Mastocytomas are among the most frequent neoplasms in canines. Aggressive mastocytomas usually are resistant against conventional therapy and the prognosis is grave. Therefore, current research focuses on new targets in neoplastic mast cells (MC) and the development of targeted drugs. Heat shock protein 32 (Hsp32), also known as heme oxygenase-1 (HO-1), is a survival-enhancing molecule that is expressed in various neoplastic cells. *Methods*. We examined the expression of Hsp32 (HO-1) in primary canine MC as well as in the canine mastocytoma cell line C2. Expression of the Hsp32 protein was examined by immunocytochemistry and Western blotting, and expression of Hsp32 mRNA by RT-PCR. To define the functional role of Hsp32, two novel Hsp32-targeting drugs, pegylated zinc-protoporphyrin (PEG-ZnPP) and styrene maleic acid-micelle-encapsulated ZnPP (SMA-ZnPP), were applied. *Results*. As assessed by immunocytochemistry and Western blotting, primary neoplastic MC and C2 cells were found to express

the Hsp32 protein in a constitutive manner. Moreover, we were able to show that C2 cells express Hsp32 mRNA. Exposure of C2 cells to hemin (10μM) resulted in an upregulation of expression of Hsp32. Both Hsp32-targeting drugs, PEG-ZnPP and SMA-ZnPP, were found to inhibit the proliferation of C2 cells and of neoplastic MC. The growth-inhibitory effects of PEG-ZnPP and SMA-ZnPP were time- and dose-dependent (IC50: 1-20 μM), and associated with apoptosis. *Conclusions*. Hsp32/HO-1 is an important survival factor and interesting new target in neoplastic canine MC. Clinical trials with Hsp32-targeted drugs are now warranted to define the *in vivo* antineoplastic efficacy of these new drugs.

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HAEMOSTASIS DISORDER IN MONOCLONAL GAMMOPATHIES

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Background. It is observed that patients with monoclonal gammopathy frequently have haemostatic disorder which can be associated with bleeding. Haemostatic disorder arising mechanism is not completely explained, but it is most frequently believed to be in connection with paraprotein. *Aims*. The aim of this study was to prove the incidence of haemostatic disorder at patients with monoclonal gammopathies and role of paraprotein in its arising by investigation with platelets function and coagulation tests. The level of bone marrow infiltration with pathological plasmocytes, level of paraprotein in serum and levels of biochemistry parameters, such as total proteins, BUN, creatinin, β2-microglobulin (β2-MG), C reactive protein (CRP) and calcium ions ($Ca2^{++}$), at platelets function were estimated too. *Methods*. In this study were included 48 patients with monoclonal gammopathies, who were treated in Institute of haematology, Clinical Centre of Serbia, in the period of 2003-2005. Coagulation tests and investigation of platelets adhesion on glass pearls, platelets aggregation induced with ADP, collagen, epinephrine and ristocetin were done initially. At patients with haemostatic disorder, investigation was repeated after disappearing of paraprotein. Concentration of paraprotein in serum was measured by 1% agarose gel electrophoresis of serum's proteins. The percentage of bone marrow infiltration with plasmocytes was being determined, as well as biochemistry parameters in patients' blood: BUN, creatinin, total proteins, β2-MG, CRP and $Ca2^{++}$. Afterwards, their effects on the platelets dysfunction were investigated. *Results*. Platelets adhesion was disturbed at one half of patients, and platelets aggregation at one third. Platelets aggregation was normalized together with disappearing of paraprotein during a treatment. When patients attained remission, their platelets aggregation by ADP, collagen and epinephrine, improved significantly ($p<0.05$). A significant negative correlation was proved between serum level of the following parameters: total proteins and disturbance of platelets aggregation by ristocetin ($p<0.01$); creatinin and disturbance platelets aggregation by ADP and collagen ($p<0.05$); β2-MG and disturbance of platelets aggregation by ADP ($p<0.05$); CRP and disturbance of platelets aggregation ($p<0.05$); paraprotein and disturbance of platelets aggregation by ristocetin ($p<0.01$). Paraprotein of IgG type caused significantly more frequent the disturbance of platelets aggregation by ristocetin compared to IgA type ($p<0.05$). A significant negative correlation was proved between the percentage of bone marrow infiltration with plasmocytes and platelets adhesion ($p<0.05$). *Conclusions*. These investigations have proved that paraprotein leads to haemostatic disorder at patients with monoclonal gammopathies, because when it disappears from circulation during a treatment, the haemostasis normalizes. The level of paraprotein in patients' serum has had significant effect on disturbance of platelets aggregation. The importance of the level of the following biochemistry parameters was proved: creatinin, total proteins, β2-MG and CRP on disturbance at platelets aggregation, as well as the importance of percentage of bone marrow infiltration with plasmocytes on the platelets adhesion. Further investigation is necessary.

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DIVERGENCE OF RELATIVE BCR-ABL EXPRESSION LEVEL IN CML PATIENTS DURING 5 YEAR PERIOD OF IMATINIB TREATMENT

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Background. Quantitative evaluation of BCR-ABL gene expression in CML patients by means of RQ PCR has considered nowadays a valuable method to monitor minimal residual disease, to characterize treatment response, to predict relapse long before clinical manifestation. It has become particularly evident due to involvement of imatinib and other targeted anti tumor drugs into clinical practice. *Aim*. To estimate deviations in BCR-ABL gene expression level in CML patients treated by imatinib in different terms of follow up throughout 5 years of treatment. *Methods*. RQ PCR analysis in CML patients was performed according to TaqMan technique. The gene encoding β-2-microglobuline (β2m) was used as an internal reference control. BCR-ABL relative gene expression data (reB-A) was calculated as an equation bcr-abl/$\beta2m*10^7$. Sets of BCR-ABL and β2m plasmid dilutions with known concentrations were used as external controls. 386 blood samples were obtained from CML patients treated with imatinib (400 mg). Duration of treatment varied from 2 to 62 months. The magnitudes of treatment duration were arranged into duration intervals of every 6 months and frequency analysis was performed. Samples within every interval were subdivided onto 3 groups according to the value of reB-A. 1st group included samples with low reB-A values (0 to 100). The other one contained samples to be between 101 and 1000 (medium). The last one included samples with high reB-A (1001 and higher). We calculated then the% proportion of each group within every interval. Median and mean values were also calculated. *Results*. It was shown that proportion of 1st group samples with low reB-A increased from 26% up to 80% within duration intervals including 2 to 42 months. Starting from duration interval corresponding to 48 months of treatment, the proportion of the 1st group gradually reduced and up to interval of 60 months it succeeded only 43,5%. The proportion of samples with high reB-A (3rd group) decreased from 52% to 1% during intervals of 6 to 18 months, then it increased up to 20% within duration interval of 30 months, then decreased a little again within interval of 36 months and after that it gradually increased up to 47,8% until interval of 60 months. The proportion of samples with medium reB-A varied having lowest value within the interval of 30 months (0%) and 42 months (5%). Median value of reB-A decreased from 1810 to 27 during intervals corresponding to 6-36 months, then it steadily increased up to 1583 until an interval of 60 months. There were 2 spikes of mean value at the intervals of 24 and 30 months (11835 and 18743, respectively). *Conclusions*. It was shown that during rather long period of imatinib therapy there was a considerable divergence of BCR-ABL expression level in CML patients. The trend to increasing of BCR-ABL expression level in CML patients after 18 months of therapy may be explained by gradually developing of imatinib resistance.

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RESULTS OF IMATINIB THERAPY IN CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA PATIENTS: ANALYSIS OF A MULTICENTER NONRANDOMISED TRIAL FROM RUSSIAN CML STUDY GROUP

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Imatinib(IM) is preferred therapy for chronic phase(CP) chronic myelogenous leukemia(CML) patients. Despite impressive results, resistance to IM has observed in certain number of patients, especially in late chronic phase. Definite clinical and molecular mechanisms of resistance are yet unknown. In our nonrandomised multicentre study we analysed several clinical factors, which may affect on IM efficacy. 338 (64 newly diagnosed and 274 in late chronic phase) patients were included. All patients gave written informed consent. Median follow up for newly diagnosed and late CP patients was 21mos(4-60 mos) and 33 mos(6-66 mos), accordingly. Probability of 5-year overall survival was 89%, 98% and 88% for overall, newly diagnosed and in late CP CML patients, accordingly. Remarkably, only 1,6% of newly diagnosed and 11% in late CP patients transformed into accelerated(AP) or blastic phase(BP). 6% and 25% of patients, accordingly, with or without complete hematologic response(CHR) by 3 mos transformed to AP/BP. Achievement of complete cytogenetic response(CCyR) at any time of IM therapy predicted the risk of disease progression to AC or BP. Thus,

2% and 19% of patients with or without CCyR has progressed to AP or BP. Probability of achievement of CCyR by 48 mos were very high in both groups. Expectedly, it was higher in newly diagnosed(96%), than in late CP patients (88%). Time to CHR and major cytogenetic response(MCyR) influenced on the rate of CCyR. CHR by 3mos and MCyR by 6mos were the best predictable factors for probability of CCyR. 81% and only 25% patients with or without CHR by 3 mos, 88% and 67% patients with or without MCyR by 6mos, accordingly, obtain CCyR. Additionally, in the absence of MCyR by 24mos, probability of CCyR achievement was down nearly to zero. In addition, splenomegaly, trombocytosis, low hemoglobin level and presence of blast cells in blood before IM therapy were poor prognostic factors in late CP patients. Probability of CCyR was 44% vs. 80%, 47% vs. 74%, 50% vs. 70% and 33% vs. 75%, accordingly, in patients with or without splenomegaly, trombocytosis, low hemoglobin level and presence of blast cells in blood. Although we did not reveal any negative prognostic factors in newly diagnosed patients, it is quite likely to be consequence of the small number of analysed patients. *Conclusions*. IM induced high rate CHR and CCyR in both newly diagnosed and late CP CML patients. Nearly all patients with CCyR do not progress to AP or BP. Splenomegaly, trombocytosis, low hemoglobin level and presence of blast cells in blood before and absence of CHR by 3 mos and McyR by 6 mos during IM therapy were negative prognostic sings only in late CP CML patients.

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WILMS' TUMOR AND NUCLEAR FACTOR-KAPPA B PROTEIN EXPRESSION IN *DE NOVO* ADULT ACUTE MYELOID LEUKEMIA

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Wilms' tumor protein (WT1) is a widely accepted marker for poorly differentiated types of acute myeloid leukemia (AML). The prognostic value of WT1 remains not completely established and do not have a unidirectional significance for the disease progression. Moreover, it is repeatedly reported that WT1 level may change during therapy and disease progression. The nuclear factor- κB (NF-κB) is a transcriptional factor, which is usually localized in the cell cytoplasm as an inactive heterotrimer, consisting of three subunits: 50, 65 and IκBα. The expression and nuclear localization of the p65 NF-κB subunit is frequently found in malignantly transformed cells. Furthermore, it is associated with resistance to different chemotherapeutic agents. The aim of our study was to determine the expression of WT1 and NF-ÎκB at diagnosis and after therapy in patients with *de novo* AML. A group of 16 patients was analyzed. The expression of WT1 and NF-κB was detected by western immunoblotting. The presence of WT1 was found in 68,7% (11/16), whereas NF-κB expression was recorded in 81,2% (13/16) of the patients. A concomitant expression of WT1 and NF-κB was found in 56,2% (9/16). NF-κB expression alone was found in 25% (4/16), whereas WT1 expression alone was shown in 12,5% (2/16). A small part of 6.2% (1/16) of the patients had neither WT1 nor p65 NF-κB proteins. Complete remissions were obtained in 11,1% (1/9) of the patients with simultaneous WT1 and NF-κB expression. Taken together our data concerning WT1 and NF-κB expression profile as well as clinical outcome indicate that the expression of WT1 and NF-κB is a common feature of the AML disease. Moreover, these transcription factors do have an important but not completely understood role as prognostic factors and indicators of the expected therapy outcome. According to our findings the simultaneous presence of WT1 and NF-κB is related to poor therapeutic answer and shorter remission durations. Our data are in line with the gene-expression profiling strategy for outcome prediction in AML. A continuous and longer follow up as well as enlargement of the patients' number are needed to definitely establish the predictive role of WT1 and NF-κB in adult AML.

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THE RESULTS OF COMBINED TREATMENT OF EARLY CHRONIC PHASE OF PH-POSITIVE CHRONIC MYELOID LEUKEMIA WITH IMATINIB, CITOSIN ARABINOSID AND ?-INTERFERON

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In order to prevent refractoriness to imatinib and to achieve fast cytogenetic and mo-lecular response in CML early chronic phase, we designed a combined treatment, that would promote early achievement of durable cytogenetic and molecular response. The induction therapy included imatinib 400 mg daily PO and cytosine arabinoside (Ara-C) 10 mg/m^2 s/c bid for 10 days every month until the complete cytogenetic response (CCyR) was achieved. After that Ara-C was replaced by interferon-·2b (PEG-Intron 50 mcg weekly s/c). The results were estimated by conventional cytogenetics and quantitative RT-PCR. The study group included 5 males and 10 females, aged from 21 to 52 years. Accord-ing to Socal criteria, 8 patients (54%) belonged to low, 2 (13%) to intermediate and 5 (33%) to high risk group. Median overall treatment duration was 29 (6'41) months, imatinib + Ara-C--6 (3-14) months and imatinib + interferon-24 (7-37) months. After 3 months of treatment the complete hematological response was achieved in all patients and is persisting till now (27-38 months). Major cytogenetic response (MCyR) at 3 months was found in 67% patients (in 40%-CCyR), 2 patients (13%) had no signs of cytogenetic response. At 6 months 86% patients have achieved MCyR (CCyR-72%), at 12 months CCyR had 92% patients. Only one patient didn't obtain any response and needed imatinib dose escalation to 800 mg daily. At 24 months 10 patients has stable CCyR, in 4 patients the treatment was stopped (in 2 patients due to toxicity, in one-for social reasons, and one patient has developed secondary refrac-toriness). At the beginning of interferon + imatinib therapy 55% patients had major molecular response (MMolR). At 12 months it was achieved in 70% cases (and complete mo-lecular response (CMolR)-in 60%). After 24 months of treatment 70% patients had CMolR. At 36 months only 3 patients (20%) were treated and had MMolR. The hematological toxicity grade 1'2 was observed during treatment in 80% patients and grade 3, that needed temporary treatment interruption-in 33%. Hematological toxicity grade 4 was not observed. Non-hematological toxicity was observed in 73% patients on imatinib + Ara-C: in 67%-grade 1'2, in 13%-grade 3 and no one had grade 4. It was also observed in 75% patients on imatinib + interferon (68%-grade 1'2, 59%-grade 3, 8%-grade 4). Severe non-hematological toxicity (hepatotoxicity, depression) forced to cancel interferon in 9 (60%) patients. The successive use of imatinib + Ara-C and imatinib + interferon combinations al-lowed more rapid achievement of molecular and durable cytogenetic response in comparison with imatinib alone, but at 12 months the response rates became equal, while prominent non-hematological toxicity forced the transition of 40% patients to monotherapy with imatinib.

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CLINICAL AND BIOLOGICAL ASPECTS OF INFANT ACUTE MYELOID LEUKEMIA (0 - 1 YEAR OLD) : A SINGLE FRENCH INSTITUTION EXPERIENCE

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Background and Aims. Infant acute myeloid leukemia (AML) accounts for 6 to 14% of the AML of childhood. The prognosis; clinical and biological characteristics of infant leukaemia differ from those of leukemia in children older than 1 year. The most common characteristics are high incidence of acute monoblastic or myelomonoblastic leukemia with hyperleukocytosis and extramedullary involvement. The MLL gene rearrangements occur at a high frequency (60%). In order to characterize infant AML in our institution, we reviewed the clinical, biological features and outcome of 16 infant cases (19% of AML) diagnosed during the past ten years. *Methods*. We studied the charts of infants (0 to 1 year old) in whom AML was diagnosed from January 1996 to December 2006 at the Pediatric Hematology Department of Debrousse Hospital in Lyon. Fifteen patients presented with *de novo* AML and one patient had secondary AML. Children with genetic disorders such as Down Syndrome; Noonan Syndrome; or with immune deficiency disease were excluded of the study. A particular attention was paid to dysmyelopoiesis in bone marrow and a detailed morphologic review was performed by two cytologists. *Results*. Thirteen infants had AML, two had extramedullary myeloid tumours (skin) and one had a bilineal acute leukaemia. The age of patients at diagnosis ranged from birth to 10 months. Median age was 5.4 months. There was a male prevalence with a sex-ratio of 1.3. Six of 16 AML patients presented with cutaneous manifestations. Central nervous system was involved in 2 cases and testis in 1 case. Most of the patients demonstrated hepatosplenomegaly. Hyperleukocytosis was seen in 5 patients. Anemia and thrombocytopenia were seen in 13 patients. Severe thrombocytopenia of less than 50 $\times 10^9$/L was seen in 8 patients. According to the FAB classification, morphological analysis revealed M4 for 3 patients, M5 for 8 patients, M7 for 2 patients, unclassifiable AML for 2 patients and 1 bilineal leukaemia of B lymphoid and monocytic lineage. For cytological study, dysplasia of marrow cells was

assessed in granulocytic, erythroid and megakaryocytic lineages. Moderate dysmyelopoiesis was identified in 6 patients with a predominance of dysgranulopoiesis. Five patients showed normal karyotypes. Hyperdiploidy (62 chromosomes) was found in one patient. Monosomy 7 and t(1;22) (p13;q13) were found respectively in one patient. MLL gene rearrangements were seen in 5 of 10 patients (50%). After diagnosis, patients were enrolled in different chemotherapy protocols: Interfant 99 and 06, EORTC-CLCG 58921, ELAM 02. Complete remission was achieved in all infants. Seven patients received bone marrow transplantation, 5 in first remission. Four patients relapsed and three of them died because of infectious complications or leukemia progression. One patient with congenital leukemia cutis showed favorable evolution with spontaneous regression of cutaneous disease. Twelve patients are still alive without leukaemia. *Conclusions*. Our first results confirm reports in the literature and must be confirmed by a large prospective study. Indeed a national collaborative study should be interesting and could permit to collect a large number of childhood patients with the goal of characterizing infant leukaemia in France.

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PLATELET MEMBRANE GLYCOPROTEIDS IN THROMBOCYTOPATHIES

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Hereditary thrombocytopathies are the most frequent cause of hemorrhagic syndrome in children. Preliminary diagnosis may be established on the basis of functional platelet disorders and anamnesis, immunological and biochemical investigations showing deficit or qualitative defect of the platelet membrane protein, allow make a definitive diagnosis. The method of cytoflowmetry assess various hereditary platelet disorders, including unique, associated with deficit of membrane glycoproteids (GP). *Aims*. Assessment of platelet membrane glycoproteids in thrombocytopathies. *Methods*. We have conducted a platelet antigen typing in 50 patients with hereditary thrombocytopathies by cytoflowmetry using monoclonal antibodies against membrane GP CD31 (GP Ia/IIa), CD42 (GPIb/IX/V), CD41/CD61 (GPIIb/IIIa) and CD62P (P-selectin). 25 healthy children were included into control group. Expression of these antigens was assessed in the surfaces of the resting and ADP-activated platelets. *Results*. Deficit of the GPIIb/IIIa was revealed in 18 (36,5%) patients which was typical for Glanzman's thrombastenia (TG). These patients have had decreased expression of CD41/ëD61, directed against GPIIb/IIIa. According on level of antibody-platelet binding TG type I was established in 11 patients and type II in 7. In patients with TG type I level of the specific binding was in resting platelets 1,84±0,46*103 (p<0,001), and in activated platelets 0,43±0,25*103 (p<0,001), in control group binding was 50±5,15*103 and 79,08±11,5*103 molecule per platelet. In patients with TG type II level of binding was higher -17,25±7,58*103 (p<0,01) in resting platelets and in activated platelets. We have revealed two patients with Bernard-Sulier syndrome (BSS) in whom complete absence of the specific binding was established. Levels of GPIb were assessed by CD42 expression in the complex of membrane GP Ib/IX/V. In the resting platelets expression of this antigen was 1,5±0,26*103 (p<0,001 and 0,7±0,13*103 (p<0,001) in ADP-activated platelets in comparison with 34,2±4,6*103 and 20,5±4,03*103 in the control group. In 24(49%) patients P-selectine deficit was detected. It was absent in the resting platelets: 0,29±0,09*103 and in activated platelets: 0,24±0,5*103 (p<0,01), in comparison with control group: 8,7±1,4*103. Expression of the other membrane receptors binding revealed an increasing of CD42 and CD41/CD61 expression to 38±1,2*103 and 63±2,4*103 in comparison with control group. Possibly, an increased antibody binding and increased expression of GPIIb/IIIa and GPIb/IX/V in platelets follow the platelet size increasing. Collagen receptor deficit was revealed in 6 (10,5%) patients. Decreasing of the specific antigen GP Ia/IIa in the various types of platelets (monoclonal antibodies to CD31) was established: 1,17±0,43*103 (p<0,001) in the resting platelets and 1,23±0,65*103 (p<0,001) in activated platelets in comparison with control group: 30,7±4,03*103 26,4±4,2*103 respectively. *Conclusions*. Thus, definitive diagnosis of hereditary thrombocytopathies and those types may be established after immunological investigations using monoclonal antibodies against membrane glycoproteid complexes. Computed cytoflowmetry reveals structural defects of the resting (non-stimulated platelets) and activated platelets and may be standard method in diagnosis of hereditary thrombocytopathies.

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INCIDENCE OF AVASCULAR BONE NECROSIS AS A COMPLICATION OF LEUKEMIA IN CHILDHOOD - A CENTER EXPERIENCE

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Aim. In our study we retrospectively analyzed the cases of avascular necrosis of the bone (AVN) among children treated for leukemia in one institution during 11 years period. *Patients and Methods*: Between 1995 and 2006 two hundred and forty four patients, aged from 6 months to 18 years were treated for leukemia in our department. 201 were diagnosed as having acute lymphoblastic leukemia (ALL), 37-acute myeloblastic leukemia (AML) and 6-chronic myeloblastic leukemia (CML). Based on medical records we analyzed the incidence of avascular bone necrosis among this group. *Results*. Nine cases of AVN was detected in our group (3,7%): 6 ALL patients (3 girls and 3 boys), one girl with AML and two girls with CML. Avascular necrosis of femoral head occurred in 6 children (4 with ALL 1 with AML and 1 CML), tibia necrosis affected one girl with ALL. One girl with CML presented symptoms of necrosis distant part of femur and finally humerus head necrosis was suspected in one boy with ALL. Median interval from ALL diagnosis to AVN occurrence was 15 months. In the girls with CML, the AVN occurred 38 and 36 months following initial diagnosis and 12 and 8 months post allogeneic bone marrow transplantation (BMT) respectively. Treatment of AVN supervised by orthopedist included firstly the rest in bed, relaxation of the hip and subsequently rehabilitation without surgery. This strategy was successful in four children with ALL since the function of affected limb now is satisfactorily. However the patients may need joint replacement in the future. *Conclusion*. Incidence of avascular bone necrosis in children with acute leukemia is less frequent in our material then reported in literature. Treatment without surgery may be sufficient for limb function recovery but long term outcome remains unclear.

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HOW MANY RADIOLOGY IMMAGING IN LYMPHOMA PATIENTS DO WE NEED? (A SINGLE CENTER EXPERIENCE)

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Introduction. Computerized tomography (CT) is one of the most sensitive examination for staging and monitoring lymphoma patients. Unfortunatelly, it is also one of the most highly radiation-exposure examinations in radiology. In the past few years some question arose: 1. How many exposures from CT receive the patient during lymphoma staging, treatment and later controls? 2.What is the incidence of probable secondary malignancies resulting from CT exposure in NHL patients? 3. Are there possibilities to avoid negative impact of CT? 4. What methods could be used as alternative? Since now there are no conclusive data on those topics. *Purpose*. Aim of our study was to determine overage radiation dose from CT scans in lymphoma patients, estimate risk of secondary malignancies and evaluate correlation among secondary malignancies, radiation dose, age, sex, anemia and lymphoma type. *Patients and Methods*. A total of 114 patients with Hodgkin and non Hodgkin Lymphoma refered to Hematologist in Clinical Hospital Center Rijeka, Croatia during ten years were evaluated for CT radiation dose during staging and reevaluation . Typical effective dose were measured in millisieverts (mSv) and was expressed as equivalent number of chest X rays (PA film) for equivalent effective dose (based on the assumption of an average *effective dose* from chest x ray (PA film) of 0.02 mSv). Time period for equivalent effective dose from natural background radiation based on the assumption of an average *effective dose* from natural background radiation of 3 mSv per year in the United States.Typical effective dose for the CT of the thorax and abdomen were approximately 10 mSv each, and CT of the head 2 mSv according to previous researches. The patients were followed up (minimume one year /max. 10 y) for secondary maligancies. Correlations of lymphoma type, radiation dose , age, sex, and secondary malignant disease were statistically analysed using β-ponders and correlation analysis. *Results*. We analysed retrospectively data of 114 pts with Hodgkin and non Hodgkin lymphoma. Approximately 35% have had at least three total body scans (CT abdomen+ CT thorax) .12,2% had also head CT. We made approximatelly 6,2 CT scans in each patient , and total radiation dose ranged from about 10 mSv to 126 mSv (median 62,3 mSv). Among those patients 11 had secondary malignancies (eight of them

were older than 65 y) which can be compared to the natural incidence of fatal cancer in our population. *Conclusion*. Previous researches revealed that the risk of radiation induced cancer is much smaller than the natural risk of cancer. Our results showed that increased numbers of CT screening procedures has not been correlated with increased incidence of secondary malignancies. Age was only predictive risk indicator (p<0,001) for developing cancer but not more than natural incidence for older age in our population.

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THE V34L POLYMORPHISM OF FACTOR XIII IS NOT A RISK FOR DEEP VENOUS THROMBOSIS IN THE LEBANESE POPULATION

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Background. Several studies have investigated the association of factor XIII Val34Leu polymorphism with risk of venous thromboembolism. Varying results on the effect of this polymorphism on venous thrombotic risk have been reported. *Aims*. The aim of this study was to investigate the role of factor XIII Val34Leu polymorphism in Lebanese patients with deep venous thrombosis (DVT). *Methods*. The sample consisted of 65 Lebanese patients with deep venous thrombosis and 125 asymptomatic healthy subjects with no history of venous thromboembolism. The controls were not family members of the patients. The DVT patients were admitted between March 2003 and December 2005 to the American University of Beirut Medical Center where the diagnosis was confirmed by venous Doppler ultrasonography. Patients with positive lupus anticoagulant testing or with circumstantial predisposing factor (e.g. surgery, malignancy, pregnancy or use of oral contraceptive pills) were excluded from this study. The DNA of patients and controls was extracted and stored at -80°C for later use. Testing for Factor XIII gene mutations was done using the Reverse Hybridization StripAssay (CVD StripAssay, ViennaLab, Austria). Extraction, PCR amplification, and Hybridization steps were all followed upon the recommendations of the manufacturer. *Results*. The average ages for the DVT and control groups were 45.5±18.7 and 35.4±18.6 years respectively. Nine patients had a previous episode of DVT. Thirteen patients (20%) and 27 controls (21.6%) had heterozygous factor XIII Val34Leu genotype while one patient (1.5%) and 4 controls (3.2%) had the homozygous genotype. There was no significant association between factor XIII mutation and having DVT (p= 0.76). *Conclusions*. Our preliminary findings show that Val34Leu polymorphism in factor XIII gene is not associated with DVT in the Lebanese population. Further larger studies are needed to be conducted in the Lebanese population in order to verify the clinical importance of this finding.

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REACTIVATION OF HERPES VIRUS-6 IN A CHRONIC MYELOYD LEUKEMIA (CML) PATIENT DURING TREATMENT WITH DASATINIB

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T lymphocyte activation is controlled by the T cell antigen receptor (TCR) in combination with additional signals triggered by accessory molecules present on the surface of the antigen-presenting cells (APC). The earliest biochemical response elicited by the TCR is the activation of different protein tyrosine kinases (PTK). Two PTKs associated with the TCR/CD3 complex the src family kinase p59fyn and a 70-kD PTK termed ZAP-70 and a second src family PTK, p56lck are critical for TCR function. Dasatinib (BMS-354825) is a dual Src- and ABL- kinase inhibitor approximately 300-fold more potent than Imatinib, that has recently demonstrated to be active for the treatment of patients with Imatinib resistant Ph^+ Chronic Myelogeneous Leukemia (CML) and Acute Lymphoblastic Leukemia (ALL). Several studies have reported that treatment with Imatinib can induce an inhibition of the T-cell mediated immune response. Imatinib inhibits T cell receptor-mediated T cell proliferation and activation in a dose-dependent manner and reduces tyrosine phosphorylation of ZAP-70 and LAT in response to activation through TCR. Studies conducted in a mouse model indicate that Imatinib treatment also leads to a selective inhibition of memory CTLs without affecting primary T or B cells responses. Recent reports describing the development of severe viral infections such as varicella-zoster and fatal hepatitis B virus reactivation in patients with CML treated with Imatinib along with *in vitro* data suggest that the potential for immune suppression and viral reactivation in patients treated with abl and src kinase inhibitors should be considered. We describe the development of a herpes 6 virus associated with treatment with Dasatinib (BMS-354825) in a patient with diagnosis of CML. The patient had an acute infection of parvovirus B19 however, in concordance with the published data, her immune system was able to control the infection, probably because Dasatinib like Imatinib, selectively inhibits the expansion of antigen-experienced memory CTLs without affecting primary T or B cell responses. Although the rash resolved within 10 days, the patient developed asthenia and daily low grade fever (38ºC), at this moment a >15 fold rise in the titers of IgG against HHV6 were detected indicating reactivation of latent infection. Moreover, presence of effector $CD8^+CD7^-CD57^+$ T cells subset also indicate an acute immune response to viral infection and chronic antigenic stimulation maybe due to inability to suppress HHV6 reactivation. These findings could indicate impaired function and expansion of $CD7^+CD8^+$ memory T cells during Dasatinib treatment. Interestingly, when treatment with Dasatinib was stopped, the immunosupression reverted, and the immune system was able to clear the virus without need for specific treatment (Foscarnet or Ganciclovir). Dasatinib was reinitiated at a lower dose 1 month after discontinuation of the medication. Repeated analysis demonstrated a decrease in the titers of IgG antibodies against herpes 6 virus. *Conclusions*. these clinical features reflect the need of memory T cells surveillance to control chronic latent infections. Patients treated with Dasatinib may develop reactivation of viral infections and require greater surveillance to detect infectious complications. Tyrosine kinase inhibitors dose modification could be sufficient to prevent immunosupression in some patients.

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CORD BLOOD LYMPHOCYTE SUBTYPES IN TERM INFANTS

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Background. Standards of public cord blood banking include volume reduction of samples/ Investigation of cord blood specific features is necessary for quality control and establishing new methods. *Aim*. Study of immunological parameters of cord blood lymphocytes and influence of cord blood volume reduction on number of lymphocyte subtypes. *Materials and methods*. We evaluated cord blood samples from 30 term newborns (37-42 weeks of gestation). Samples were collected from cord vein using closed system, containing 35 ml of CPDA, 5-15 minute after delivery. Lymphocyte subtypes were analyzed using flow cytometry before and after volume reduction according to the New York cord blood bank protocol. We found no differences in number of $CD3^+$ lymphocytes before and after volume reduction, and it was 17,9±1,44% and 19,2±1,4% from total nucleated cells, respectively. Number and ratio of $CD3^+CD4^+$ cells and $CD3^+CD8^+$ cells after volume reduction has also not changed and was: before volume reduction CD3+CD4+ cells-12,62±1,12%, $CD3^+CD8^+$ cells-5,62±0,53%; after volume reduction $CD3^+CD4^+$ cells-13,23±1,12%, $CD3^+CD8^+$ cells-5,52±0,48% from total nucleated cells. Number of natural killer cells $(NK)^-CD16^+CD56^+$ cells-in native cord blood nucleated cells was 5,97±0,67% and significantly raised after volume reduction-8,54±0,91% (p = 0,027). Number of $CD33^+CD13^+$ cells was 12,5±0,76% and it significantly increased 15,82±0,81% (p =0,005) after extraction. This is may be related to greater granulocyte shedding (lost?) during volume reduction. Number of active lymphocytes has not changed and was 0,40±0,08% before and 0,38±0,09% after volume reduction. Number of $CD14^+$ cells has not raised and was 8,65±1,58% before and 9,11±1,76% after volume reduction. We found a tendency to decreased $CD15^+$ cells number after volume reduction. This may be result of greater lost of this type of cells in comparison with other leukocyte subtypes during volume reduction n. Its number was 39,32±3,45% before and 35,23±3,26% after volume reduction. *Conclusion*. We evaluated and characterized cord blood lymphocyte subtypes and found no influence of cord blood concentration method on $CD3^+$ lymphocytes count. Number of NK-cells and $CD33^+CD13^+$ cells has significantly increased after procedure of volume reduction.

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MOBILIZATION OF PERIPHERAL BLOOD PROGENITORS IN MULTIPLE MYELOMA. HOW TO DEAL WITH HARD TO MOBILIZE MYELOMA PATIENTS-SIX YEARS CENTER EXPERIENCE

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Peripheral blood progenitor cells (PBPCs) mobilized with high-dose chemotherapy and hematopoietic growth factors are still used to support myeloablative therapy of multiple myeloma during the autologous setting. Variables having an impact on the ability to collect PBPC include age, month's prior previous chemotherapy, mobilization regimen and platelet count at the time of mobilization. Myeloma patients with low mobilizing capacity indicate the need of evaluating alternative mobilizing regimens. We analyzed 25 patients with MM that underwent PBPC mobilization at Department of hematology, University Clinical Center Skopje. Pts received Cyclophosphamide (3gr/m^2) followed by daily G-CSF (10 mcg/kg). In 15 pts we experienced a significant WBC nadir on median day +6, and began pheresis in recovered WBC up to 5.0×10^9/L on day +8 (median). Good mobilizers reached at least 2×10^6/kg CD34$^+$ cells with median 3 (ranges 1-6) apheresis procedures. In 9 MM patients we registered low mobilizing capacity. Remobilizing procedure was preformed with single G-CSF in a dose of 20 mcg/kg in a 5 days regimen. All remobilized patients reached sufficient CD34+/kg count with median 2 (ranges 1-4) aphaeresis procedures. In statistical data in both groups of good and hard to mobilize MM pts we followed several variables concerning the platelet count on day 1 of aphaeresis which correlated with the ability to collect over 5×10^6 CD34+cells/kg (p<0,001), age of patients < 60 yrs and >60yrs (p<0,001) and previously received chemotherapy cycles of 5 pts (27%) who started aphaeresis on median day +14 (p<0,001) in the Cy/G-CSF group. We can conclude that the 5 day regimen of single G-CSF in increased daily dose showed effective with efficient yields results for median 2 day leukopheresis procedure, well tolerated with possibility for mobilization in outpatient basis. This approach, if confirmed on larger series of myeloma patients could open new opportunities in stem cell mobilization for poor or non-mobilizers.

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INFLAMMATION IN HYPERTENSIVE PATIENTS WITH METABOLIC SYNDROME

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Background. Inflammation has been implicated in metabolic syndrome pathogenesis as it plays a key role in the development of atherosclerosis. *Aim of the study*. To evaluate inflammation index in hypertensive patients with metabolic syndrome. *Materials and methods*. Three groups of patients participated in the study. Group A: 175 (58 men- 117 women) non diabetic patients with metabolic syndrome. All patients were hypertensive under no medications. Group B: 103 hypertensive patients with no metabolic syndrome. Group C: 98 healthy volunteers. The values of CRP and omocysteine were measured in groups A, B and C. *Conclusion*. All inflammatory parameters are elevated in hypertensive patients with or without metabolic syndrome. This fact is correlated with the high prevalence of atherosclerosis in these patients.

Table 1.

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A METHOD FOR EVI-1 QUANTIFICATION BY RT-PCR IN HEMATOLOGICAL MALIGNANCIES

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A method for evi-1 quantification by rt-pcr in hematological malignancies

Introduction. Previous studies have demonstrated that EVI-1 overexpression is involved in the development of several leukemias. In these leukemias a transcriptional activation has been observed which does not allow them from reaching terminal differentiation. EVI-1 overexpression has a high prevalence on certain types of leukemias such as: Acute Myeloblastic Leukemia (AML), Myelodisplastic Syndromes (MDS), blastic crisis (BC) in chronic Myeloid Leukemia (CML), and according to other authors it appears with low prevalence on Acute Lymphoblastic Leukemias (ALL). *Aim*. The optimization of a procedure by Quantitative RT-PCR (QRT-PCR) for the detection of EVI-1 overexpression as well as to determine different expression levels depending on the type of hematological malignancy.

Table 1.

Cut-off EVI-1	SMPC Phi- n=14	LMC n= 29	LLA n=15	LMA n=31
>0.0074	1 (7.14%)	15 (51.74%)	4 (26,66%)	9 (29%)

Figure 1.

Material and methods. Samples: 120 samples were studied, 17 belonging to healthy donors, and 103 to patients with varied diseases (14 MDS, 31 AML, 14 MPCD Ph-, 15 ALL and 29 CML). Relative Quantification: EVI-1 expression was analyzed in bone marrow and/or peripheral blood samples by QRT-PCR, adapting the technique developed by M. Russell *et al.* (Blood 1994) to the LightCycler 480 system, using as a fluorescent tracer SybrGreen I. In order to confirm the specificity of the obtained product, a melting curve was performed. GADPH was used as a reference gene. Results obtained through quantification are expressed as the ratio between the patients' samples and the reference gene. This result was then compared to the ratio between EVI-1 concentration in cell line k562, which was used as a calibrator, and which was amplified in each PCR. All samples were analyzed twice. All samples with a value 2 standard deviations above the healthy population's mean were considered statistically positive. *Results*. We concluded that the Cut-off considered as positive is a EVI-1 value >0.0074. 2- Table 1 Figure 1 *Conclusions*. 1. RT-PCR is a quick, easy and sensible method to study EVI-1 expression. 2. Results have allowed us to establish a cut-off above which patients can be considered positive, not only in AML and MDS, but also on other hematological malignancies. 3.The highest EVI-1 expression levels belonged to AML patients

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RESVERATROL INDUCED P53 MEDIATED APOPTOSIS BY SIGNAL TRANSDUCTION PATHWAYS VIA RUNX3 AND PIK3CA GENE EXPRESSIONS IN LEUKEMIA CELL LINES

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Background. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a phytoalexin present in grapes and other food products, has been suggested as a potential cancer chemo-preventive agent based on its striking inhibitory effects on diverse cellular events associated with tumor initiation, promotion, and progression. *Aims*. The aim of the study is to determine the activation profiles of p53 (tumor antigen p53), RUNX3 (Runt-related transcription factor 3), which are tumor suppressor genes and EGFR (Epidermal growth factor receptor), PIK3CA (phosphoinositide-3-kinase, catalytic, α polypeptide), which are oncogenes in leukemia. To investigate the role of gene expressions PIK3CA, Runx3, p53 and EGFR in resveratrol-induced apoptosis of leukemia cells, we performed cytotoxicity, apoptosis and expression analysis, respectively. *Methods*. We determined IC50 values of resveratrol in these cell lines. ARH-77(human multiple myeloma), CCRF-CEM (Acute T-lymphoblastic leukemia), HL-60 (Human premyelocytic) and K-562 (Human eritroleukemia) cell lines grown in RPMI-1640 medium. Cytotoxic assays and determination of IC50 dose of resveratrol in leukemia cells were performed by using Trypan blue dye exclusion and XTT assay as indicated in manufacturers' instructions. Acridine orange/Ethidium bromide dye technique was used to evaluate apoptosis. Gene expressions were examined in leukemia cells by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). *Results*. Cytotoxic effects of resveratrol in ARH-77, CCRF-CEM, HL-60 and K-562 were detected in dose and time dependent manner with the IC50 doses of 10 μM, 0.1 μM, 5 μM and 100 μM respectively. Acridine Orange / Ethidium bromide method have shown remarkable apoptosis at 72nd hour in resveratrol treated cells. Although HL-60 is known as an EGFR negative cell line, we also found no EGFR expression in other leukemia cell lines. P53 expression was found to be induced in ARH-77 and HL-60 and reduced in CCRF-CEM and none in K-562 cell lines. Except ARH-77, MDM2 expression showed antagonist expression when compared to p53 expression in others. All cell lines exhibited down-regulation of PIK3CA expression in IC50 doses of resveratrol. RUNX3 expression was up-regulated in K-562 while the others were presenting reduced expression. *Conclusion*. Our data indicate that resveratrol induced apoptosis by p53-dependent signal transduction pathways in leukemia cell lines positive for p53 expression, and by using downstream pathways interacting each other and effecting some apoptotic pathways in p53 negative cells.

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NO INTRINSIC DEFECT OF BONE MARROW ERYTHROID CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. The pathogenesis of anemia in chronic lymphocytic leukemia (CLL) at diagnosis is multifactorial and it may be due to autoimmune hemolysis, deficiency of erythropoietic co-factors, blood loss, pure red blood cell aplasia or hypersplenism. If none of the above causes exist (anemia of unknown origin), bone marrow (BM) infiltration by malignant cells is often considered to be responsible for anemia. However, it is reported that BM infiltration does not correlate with low hemoglobin levels in CLL, as patients with extensive BM involvement are not always anemic, whereas others without extended BM disease present with anemia. *Aims*. We investigated whether intrinsic defects of BM erythroid cells may be implicated in the development of anemia of unknown origin in CLL. *Methods*. BM samples from 15 newly diagnosed CLL patients with anemia of unknown origin (Hb<12 g/dL for females and <13 g/dL for males), 15 newly diagnosed non-anemic CLL patients, and 12 healthy volunteers were studied. Extent of BM infiltration was estimated in trephine biopsies stained immunohistochemically and serum EPO levels were measured by ELISA. *In vivo* reserves of BM hematopoietic cells were assessed by flow cytometry. The potential of BM immunomagnetically separated $CD34^+$ cells to proliferate and differentiate into the erythroid lineage was evaluated by a clonogenic methylcellulose assay and in a liquid culture system, in which immature erythroid precursor cells [$glycophorin^+$ (GPA^+)] were generated in the presence of EPO, SCF and IL-3. The response of $CD34^+$derived GPA^+ cells to EPO was assessed by detecting the activation status of key intracellular signaling proteins upon EPO receptor stimulation with EPO using Western Blot and EMSA. *Results*. BM infiltration did not correlate with anemia in CLL patients studied. Serum EPO levels were appropriate for hemoglobin levels. Flow cytometric analysis of BM revealed significantly lower percentage of $CD\ 34^+$ cells in CLL patients compared to normal controls, whereas $CD34^+/CD71^+$(erythroid progenitor) and $CD36^-/GPA^+$(mature erythroid precursor) cells were significantly fewer only in anemic CLL patients compared to donors. BFU-E clonogenic capacity in methylcellulose medium of $CD34^+$ cells from anemic samples was increased compared to other groups, even though it was not statistically significant. $CD34^+$ cells from anemic CLL patients led to similar generation of erythroid precursor cells in the liquid culture as those from other groups. Furthermore, $CD34^+$derived GPA^+ cells from all groups revealed no defect in their EPO receptor signaling, as they exhibited normal phosphorylation of the downstream effector molecules STAT5, ERK1/2, AKT as well as normal STAT5 DNA binding activity upon stimulation with EPO. *Conclusions*. Our *in vivo* results show that BM of CLL patients with anemia of unknown origin has lower percentage of $CD34^+$ and erythroid cells. Nevertheless, the bone marrow $CD34^+$ cells of CLL patients maintain their potential to generate functional erythroid cells *in vitro*. We conclude that impaired erythropoiesis in CLL cannot be attributed to intrinsic defects of BM erythroid cells. Other factors such as BM microenvironment may play a role in the development of anemia of unknown origin in CLL.

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HEMOLYTIC SYNDROME IN COBALAMIN DEFICIENCY ANEMIA: DOES IT RESULT FROM A BYSTANDER PHENOMENON OR ANOTHER CAUSE?

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Background. Hemolytic syndrome in cobalamin deficiency anemia (CDA) is usually related to shortened life span of megalocytes in blood and increased destruction of megaloblasts in bone marrow. However, a marked hemolysis may challenge hematologist not to miss other causes. *Aims*. To assess causes of hemolytic syndrome associated with CDA. *Methods*. Complete blood count, peripheral blood smear, corrected reticulocyte count (cRets) adjusted to patient's hematocrit, serum lactate dehydrogenase (LDH), total and indirect bilirubin, plasma antithrombin III (AT-III), direct antiglobulin test (DAT), serum total homocysteine by FPIA, and plasma D-dimer by latex-enhanced immunoturbidimetry were measured. LDH and D-dimer have been given in times of the upper limit of normal (ULN, 618 U/L) or cutoff value (0.5 mg/L), respectively. Serum cobalamin (B12) and folate were measured by RIA or ECLIA and a percentage of marrow megaloblasts (Mbs) counted. As minimal diagnostic criteria a macrocytic anemia with decreased serum B12 (<170 pg/mL) and normal folate (>2.0 ng/mL) for CDA, a positive DAT for autoimmune hemolytic anemia (AIHA), and increased D-dimer, decreased AT-III, and predisposing condition for disseminated intravascular coagulation (DIC) were required, respectively. Microangiopathic hemolytic anemia (MAHA) was diagnosed if schistocytes (FRC, fragmented red cells) in peripheral blood smear, increased serum LDH and indirect (Ind.) bilirubin, and negative DAT were present. The data were given as medians and ranges and t-test has been applied. *Results*. Of patients diagnosed with CDA in the last 10 years, we found on the grounds of an increased serum LDH 13 ones with hemolytic syndrome. In 6 patients (46%) we revealed, besides B12 deficiency, another cause of hemolysis: AIHA in 2, DIC in 3, and MAHA in 2 cases (Table 1). There were 4 women and 2 men in a median age of 60.5 years (51-97), which had hemoglobin concentration (Hb) 5.4 g/dL (3.1-6.7), MCV 117 fL (103-119), serum B12 < 106 pg/mL (<20-<150), and marrow megaloblasts 18.9% (16.6-33.6). The others (n = 7), 2 women and 5 men, had comparable median age 62 years (45-78), Hb 6.7 g/dL (3.3-11.8), MCV 114 fL (110-124), serum B12 <100 pg/mL (10-127), and marrow megaloblasts 14.4% (8.8-25.0). The former had higher median serum LDH of 20.3 × ULN (3.6-29.1) (p<0.05) and lower median serum folate of 5.75 ng/mL (3.34-10.90) (p<0.02) than the latter, 6.6 × ULN (1.1-11.9) and 13.20 ng/mL (7.29-17.20), respectively, but cRets 0.8% (0.3-1.6) and 0.9% (0.2-4.4), and serum total bilirubin 1.9 mg/dL (1.5-5.5) and 1.9 mg/dL (0.8-3.6) did not differ. Prednisolone and low molecular weight heparin had

to be added to B12 therapy in AIHA and DIC, respectively, for patients to respond. In woman with MAHA and very high serum homocysteine, 112 µmol/L, B12 dose had to be doubled. After red cell transfusion, there were a doubling of LDH in AIHA and an eightfold increase of D-dimer in DIC. *Conclusions*. In CDA with greatly elevated serum LDH and low normal folate further search for other causes of hemolysis is warranted. AIHA, DIC and MAHA must be taken into account and when confirmed a therapy modified.

Table 1. CDA with hemolysis related to other causes than B12 deficiency alone.

Case	Causes of hemolysis	Age (yrs)/ sex	B12 (pg/mL)/ folate (ng/mL)/ Mbs (%)	MCV (fL)/ Hb (g/L)	FRC/ DAT	cRets (%)/ Ind. bilirubin (mg/dL)	LDH (x ULN)	D-dimer (x cutoff)
1	MAHA	63/M	<20/5.0/ 33.6	113/ 5.7	+/–	1/3.6	20.5	NA
2	DIC	58/M	<20/6.5/ 18.6	119/ 5.0	–/–	0.5/0.9	22.6	2.4
3	AIHA&DIC	97/F	112/3.34/ NA	119/ 3.1	–/+	0.4/3.3	3.6	2.1
4	DIC	90/F	<100/4,29/ 16.6	119/ 3.8	+/–	0.3/1.7	29.1	4.7
5	MAHA	55/F	<100/10.9/ 18.9	103/ 6.7	+/–	1.6/1.7	6.0	0.5
6	AIHA	51/F	<150/9.56/ 30.8	115/ 5.9	–/+	1.6/1.6	20.1	0.6

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CLINICAL AND ECONOMIC INEFFICIENCY OF ROUTINELY HARVEST AUTOLOGOUS STEM CELL AT FIRST COMPLETE REMISSION IN PATIENTS OF INTERMEDIATE AND HIGH RISK AML

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Background. For patients of intermediate and high risk adult AML, the relapse rate after standard induction and consolidation chemotherapy remains high. Once relapse of AML developed and HLA-matched allogeneic donor is not available, the treatment is limited and the outcome is dismal. Autologous stem cell can be an alternative in this situation. However, it is difficult to harvest autologous stem cell of good quality when leukemia had already relapsed. *Aims*. To evaluate the feasibility of routinely harvest and cryopreserved autologous peripheral blood stem cell (APBSC) at first complete remission (CR) in patients of intermediate and high risk AML and having no HLA-matched allogeneic donors. Once relapse of AML developed, the patients may then receive autologous transplantation. *Methods*. Patients of intermediate or high risk AML and had no HLA-matched donors received conventional induction chemotherapy (*I3A7* regimen) followed by consecutive 2 courses of high dose Ara-C (18g/sq.m in each course)/Mitoxantrone consolidation chemotherapy (in-vivo purging). APBSCs were routinely collected immediately after recovery from nadir phase of the second course consolidation chemotherapy. At relapse, the patients were treated with high dose chemotherapy or chemoradiotherapy followed by *in vivo-purged* APBSCT. For patient with first CR longer than 1 year, another course of salvage chemotherapy with high dose Ara-C will be given to induce second CR. *Results*. Of the consecutive 26 patients enrolled, sufficient APBSC (2 ? 10^6/kg BW) was successfully harvested in 17 (65.4%) and the result was not associated with age, gender, disease nature (intermediate or high risk), lapse time between diagnosis and stem cell harvest, and lapse time between second course consolidation chemotherapy and stem cell harvest. However, a lower nadir white blood cell count (<0.1 ? 10^9/L) was correlated with a trend for successful stem cell collection (p=0.078, Fisher's exact test). Of these 26 patients, 19 relapses were identified. 12 of them had sufficient APBSC and 9 patients proceeded to myeloablative conditioning followed by APBSCT. Although there was no transplant-related mortality and 6 achieved second CR, the duration of second remission was generally less than 1 year and there was only 1 long-term survivor (received transplantation at second CR). On the other hand, almost two third (65/105) of all the cryopreserved APBSC units were still maintained in liquid nitrogen without further clinical usage. *Conclusions*. Our data suggest that APBSCs were not easy to mobilize after consecutive 2 courses of high dose Ara-C chemotherapy. Besides, using this strategy to treat relapsed AML is clinically and economically inefficient. Nevertheless, this approach is safe and a significant portion (66%) of patients who received autologous transplantation achieved second CR. This strategy would become viable if the efficacy of APBSC mobilization can be improved (using novel agents) and some kind of consolidation therapy (such as HLA-mismatched cord blood transplantation, haploidentical transplantation, or target therapy) can be incorporated to decrease the relapse of AML following APBSCT.

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PROTHROMBIN MUTATION G20210A SEEMS NOT TO BE A RISK FACTOR FOR RECURRENT FETAL LOSS

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Background. In the recent years thrombophilia as a risk factor for pregnancy complications and fetal loss gained much attention in the scientific community. However, data on this topic in the literature are conflicting. *Aim*. the aim of this study is to estimate the risk factor of the presence of inherited thrombophilia genetic defects in women with recurrent fetal loss. *Materials and methods*. 115 women with a history of recurrent fetal loss were referred to our laboratory for thrombophilia genetic testing (detection of FV Leiden, prothrombin G20210A, MTHFR 677T) by PCR methodology during one year (2006). *Results*. 36 (31%) women had a positive genetic testing: 16 (14%) were heterozygous for factor FV Leiden, one (1%) had prothrombin G20210A, 17 (14.7%) were homozygous for the MTHFR mutation, one was heterozygous for FV Leiden and homozygous for MTHFR, and one had a double heterozygosity for both FV Leiden and prothrombin mutation. *Conclusions*. Our findings, as compared to the prevalence of these defects in the Greek population (6%for FV Leiden, 4% for prothrombin mutation G20210, 15% homozygous for MTHFR 677) showed that only FV Leiden is a risk factor for recurrent fetal loss (odd. Ratio: 2.3) and the prevalence of the prothrombin mutation and MTHFR is not different from those in the general population.

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UNMANIPULATED BONE MARROW TRANPLANTATION FROM HAPLOIDENTICAL RELATED DONOR FOR HEMATOLOGICAL MALIGNANCIES IN HIGH-RISK PATIENTS:UNICENTRIC EXPERIENCE

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In this pilot study we investigated the feasibility of an intensive regimen for GVHD prophylaxis , associated with an intensive conditioning, in unmanipulated bone marrow transplantation (UBMT) from HLA-haploidentical related donor for patients (pts) considered *high- risk* because of hematological disease, previous treatments and infectious events. Between August and December 2006, 4 pts (2 males, 2 females; median age 41 years, range 32-60) were transplanted for acute myeloid leukemia (AML) in first remission (n=2), plasmacellular leukemia (PL) in relapse(n=1) and Chronic Myeloid Leukemia (CML) in second blastic crisis . One of pts with AML was FLT 3 positive and she did not complete the induction treatment for toxicity; the other one was transplanted after autologugus bone marrow graft failure. Patients with CML and PL were heavy treated. All pts had previous infectious events. All donors were HLA identical at 1 haplotype and they were primed with Filgastrim at 4 ug/Kg/day for 7 consecutive days; bone marrow cells were harvested on the eigth day with a target volume collection of 20 ml/kg recipient bw and were infused fresh and unmanipulated. In 3/ 4 pts the conditioning regimen was: Cytarabine at a dose of 3g/sqm/d on days -7,-6,-5; Busulfan i.v. at a dose of 3,2 mg/kg/d on days -4,-3,-2 and Cyclophosphamide 45 mg/kg/d on days -5 and -4. The pt experienced graft autologous failure was prepared with Fludarabine alone at dose of 40 mg/bw/day from -4 to -2. All pts received Anti-Thymocyte globulin at dose of 5mg/kg/d from d- 4 to d -1. As graft-versus -host-disease (GVHD) prophyl axis a combination of 4 drugs was used: cyclosporine at 1.5 mg/kg/d i.v. from d-7 to d -1, 3 mg/kg/d i.v. from d 0 until bowel function recovered and the switched to oral route until day +365; Methotrexate 15 mg/bw on day +1 and 10 mg/bw on days +3, +6 and +11; Mycophenolate mofetil 1 g/day from d +7 to d +100; Basiliximab 20 mg on day 0 and + 4. The median dose of nucleated cells, $CD34^+$ and $CD3^+$ cells was 12×10^8/kg (8-15), 3.4×10^6 /kg (1.54-6.2), 53×10^6 /kg (33-66) respectively. All pts achieved full donor cell engraftment with a

median time of 21 days to reach >0.5×10^9/L PMNs and 40 days to reach >25×10^9/L platelets. Two pts showed hemorragic cystitis, which resolved with medical treatment. The patient transplanted for autologous graft failure had a second graft faiulure at day +90 and she underwent to a second infusion of T-depleted cells from the same donor: she achieved full donor cell engraftment. All pts are alive with no evidence of acute GVHD. All pts are in hematological remission after a median follow up of 135 days (range 60-190). The small number of pts does not permit any final conclusion. Nevertheless, the regimen of GVHD prophylaxis seems to be very effective and the conditioning regimen is well tollerated; these results are encouraging and it needs further study with more patients and longer follow-up.

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ORIGIN OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND BLOOD VESSEL ENDOTHELIUM IN PATIENTS WITH IDIOPATHIC MYELOFIBROSIS (IM)

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Background. IM is characterized by an icreased number of circulating CD34$^+$ cells. Within the CD34$^+$ cell population the fraction of CD34$^+$/CD133$^+$/VEGF-R2$^+$ cells is also increased, suggesting an expansion/mobilization of endothelial progenitor cells which may contribute to neoangiogenesis in the bone marrow and at sites of myeloid metaplasia, such as the spleen. *Aims*. These observations prompted us to examine the extent of constitutive mobilization and origin of endothelial progenitor cells in IM and their participation in the generation of new vessels. *Methods*. Two populations of putative endothelial progenitor cells were evaluated: the *colony forming unit-endothelial cells* (CFU-EC), that according to some Authors are derived from the hematopoietic system and do not form perfused vessels *in vivo*, and the endothelial colony forming cells (ECFC) which have a higher proliferative potential and form vessels *in vivo*. Clonality analysis based on evaluation of the X-chromosome inactivation pattern was performed on the colonies from 9 female patients, whereas the JAK2 mutation status was determined in the CFU-EC from 5 patients carrying the JAK2 V617F mutation and in mature endothelial cells microdissected from the spleens of 5 more patients with the JAK2 V617F mutation that were splenectomized because of worsening splenomegaly. *Results*. Both ECFC and CFU-EC were increased in patients with IM compared with controls and with patients with polycythemia vera or essential thrombocythemia (in these myeloproliferative diseases only CFU-EC were evaluated). Clonality analysis and the JAK2 V617F mutation assay showed that in IM CFU-EC are clonal and derive from the transformed hematopoietic stem cell. Clonality of ECFC is more difficult to evaluate since these cells are extremely rare in the circulation (median: 0, range: 0-1 in controls; median: 3, range 0-20 in IM), but the JAK2 V617F mutation was present in endothelial cells from most of the microdissected spleen vessels. *Conclusions*. Our results suggest that circulating CFU-EC in patients with IM are clonal. Mature endothelial cells in the spleens from IM patients are a mixture of normal polyclonal cells and of clonal cells derived from the transformed stem cells (hemangioblast?). The molecular characterization of ECFC in patients with the JAK2 or the MPL mutation is ongoing in our laboratory and hopefully will shed more light on the cellular origin of IM.

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ASSOCIATION BETWEEN CHROMOSOMAL CHANGES AND STANDARD PROGNOSTIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Cytogenetic abnormalities in multiple myeloma (MM) are one of the most important independent prognostic factors. Based on cytogenetic findings MM patients (pts.) could be divided into prognostic groups. *Aims*. To determine the correlation between the aberration of the chromosome 13, rearrangement of IGH gene, translocations t(11;14) and t(4;14), the deletion of 17p13 and the prognostic factors in the patients with newly diagnosed MM who underwent autologous transplantation (AT) according protocol of CMG 2002 trial. *Methods*. Fluorescence *in situ* hybridization and cytoplasm immunoglobulin staining (cIg-FISH) were used to detect monotypic plasma cells and aberrations mentioned above. Cytogenetic abnormalities were found in 70 newly diagnosed patients with MM, median of follow-up 17,5 months, median age 57 years (39-67), DS stadium: stage I 7,1%, II 24,3%, III 68,6%; A/B - 84.3%/15.7%. *Results*. Overall response (OR) rate after AT was 78,5% including 13% CR, 27% VGPR and 38,5% PR. Median of time to progression (TTP) was 17,5 (0,4-41,4) months. Median of overall survival (OS) was 22,9 (0,4-48,5) months. The aberration of the chromosome 13 was found in 58% (40/69), IGH rearrangements in 57% (37/65), t(11;14) in 36% (13/37), t(4;14) in 33% (12/37) and deletion of 17p13 in 50% (23/46) pts. We have correlated standard prognostic factors (MIG, LD, B2M, Hb, CRP, Ca, albumin), TTP, progression free survival (PFS), duration of response (DOR) and OS with the occurrence of the mentioned aberrations. Higher CRP, lower albumin and lower Ca concentrations were detected in patients with aberration of chromosome 13. Higher Ca concentration (p=0,042) was found in patient with t(11;14). We have observed trend for shorter TTP in patients with deletion of 17p13 and then in pts without this deletion (18,5 vs.21,9 months; p=0,287). As for this aberration we have found no significant difference when compared incidence of each chromosomal aberration for TTP, DOR, PFS and OS. *Summary/Conclusion*. We have analysed data of the homogenous group of patients undergoing autologous transplantation in the CMG trial of Czech Myeloma Group. Based on the results we conclude that patients with deletion of 13q14 have higher CRP, lower albumin and lower Ca concentration. This analysis will be extended for all centres of CMG 2002.

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JAK2 V617F MUTATION IN THE PROVISIONAL ENTITY REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH MARKED THROMBOCYTOSIS

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Background. JAK2 V617F mutation was recently identified as a pathogenic factor in the classic Philadelphia negative Chronic Myeloproliferative Disorders (CMPD)-Polycythemia Vera (PV), Essential Thrombocytemia (ET) and Chronic Idiopathic Myelofibrosis (CIMF). In other myeloid disorders such as Myelodysplastic Syndromes (MDS) this mutation has sporadically been found. In general it is not difficult to differentiate CMPD from MDS, however, a few entities have been described with several overlapping features and 2001 WHO classification designated it as MDS/MPD. Among these disorders MDS/MPD-Unclassifiable (MDS/MPD-U) is particularly poorly characterized and includes the provisional entity *Refractory Anemia with Ringed Sideroblasts associated with marked Thrombocytosis* (RARS-T). This disorder has recently been found to have a high prevalence of the JAK2 mutation (V617F). *Aims*. The main purpose of this study was to determine the prevalence of JAK2 V617F mutation among CMPD, MDS and MDS/MPD patients attending our Hematology Centre. *Methods*. MDS and MDS/MPD diagnosis were established according to WHO criteria; in CMPD classification ASH 2005 adjustments were considered. Patients DNA was extracted from total peripheral blood samples; screening for the JAK2 V617F mutation was done by PCR amplification and digestion with the restriction enzyme BsaXI and allele-specific amplification according to methodology described elsewhere. *Results*. Median age: 74 years; M/F=1/1. See Table 1.

Table 1. JAK2 V617F mutation screening on myeloid disorders.

JAK2 V617F allele was detected in about 70% (62/90) of CMPD: PV 85%, ET 65%, IMF 3 out of 5 and in the 2 MPD-U. One patient with PV and 1 with IMF were homozygous. JAK2 V617F was also found in heterozygous state in 5 out of 37 (13%) low risk MDS patients: 3 RA and 2 RCMD. The 5 patients with RARS were JAK2 V617F negative. In MDS/MPD group one of the CMML patients and 2 out of 3 RARS-T were heterozygous. The two patients with secondary AML were homozygous for JAK2 V617F. *Conclusions*. These data on JAK2 V617F allele prevalence among myeloid disorders are similar to the others in the literature. Concerning RARS-T, the 3 patients have mild anemia (Hb 10-11 gr/dL) and megakaryocyte features resembling ET; 2 are heterozygous for JAK2 V617F; one of these had serious thrombotic events needing citorreduction therapy for thrombocytosis. The presence of the JAK2 V617F mutation in RARS-T suggests a pathogenesis more closely related to CMPD than to MDS but still no correlation has been found between RARS features and ET. Further investigations will provide insight to its pathophysiology and determine the role of JAK2 V617F inhibitors in the management of this disorder.

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DONOR CELL-DERIVED ACUTE MYELOID LEUKEMIA POST BONE MARROW TRANSPLANTATION FOR PRIMARY GRANULOCYTIC SARCOMA: CASE REPORT

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Disease relapse is the commonest cause of mortality following stem cell transplantation. Usually it emerges from residual recipient cells that evaded conditioning and graft-versus-tumor effect. Rarely, leukemia can arise *de novo* in cells of donor origin. We describe a rare case of donor cell leukemia (DCL) following transplantation for granulocytic sarcoma. *Case Report*. A 34-year-old lady presented with small bowel obstruction and underwent laporotomy. Histology of the excised segment revealed granulocytic sarcoma. Bone marrow studies and cytogenetics were normal. Following 2 courses of chemotherapy she underwent allogeneic bone marrow transplantation from a matched volunteer male donor. Conditioning comprised cyclophosphamide, alemtuzumab and total body irradiation. Standard graft-versus-host disease prophylaxis of methotrexate and cyclosporin was employed. Neutrophil engraftment occurred on D+25, but platelet engraftment was not achieved. She developed CMV retinitis requiring prolonged courses of ganciclovir, leading to secondary graft failure. Continued remission was confirmed on serial bone marrow analysis and CT imaging. However, she remained transfusion-dependent and required long-term G-CSF and erythropoeitin support. Twenty-seven months post-transplant she received recombinant human SCF. Over 24 days WCC increased to 63.2×10^9/L. Blood film revealed circulating blasts and bone marrow studies confirmed acute myeloid leukemia. Conventional cytogenetics and FISH demonstrated monosomy 7 and male (XY) karyotype in all cells examined. Chimaerism analysis by short tandem repeat (STR) DNA amplification (at 5 loci) persistently confirmed 100% donor type, strongly suggesting donor cell-derivation of the leukemic clone. Re-induction chemotherapy was complicated by severe neutropenic sepsis and she died shortly afterwards. *Discussions*. Since first described in 1971, at least 34 cases of DCL have been reported. Early cases ascribed donor origin based solely on demonstration of sex-mismatch by conventional cytogenetics. However, in isolation this method has limited validity, given its restricted ability to detect cells in active mitosis, and the proven ability of leukemic clones to gain or lose sex chromosomes. More recently, STR sequence analysis has provided a robust method for confirming origin of leukemic relapse post-transplantation. DCL is of interest since it reveals useful insights into leukemogenesis. One hypothesis suggests that stem cells with premalignant potential, previously under immune surveillance in the donor, undergo clonal transformation when transplanted into a stromal microenvironment that is more favourable (either inherently or following intensive pre-treatment). Expansion may then be facilitated by immunosuppression. SCF is an endogenous growth factor that binds c-kit, stimulating proliferation in a wide range of normal and malignant hematopoietic cells. We hypothesise that in this case administration of exogenous SCF may have supported expansion of the leukemic clone *in vivo*.

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DARBEPOETIN ALFA 500 G EVERY 3 WEEKS (Q3W) IS AN APPROPRIATE TREATMENT OF ANEMIA IN PATIENTS RECEIVING CHEMOTHERAPY FOR CHRONIC OR ACUTE LYMPHOID MALIGNANCIES

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Background. erythropoietic agents have demonstrated efficacy in correcting chemotherapy-related anemia in cancer patients, reducing needs for transfusion, and improving quality of life when Hb is ♀ 110 g/l. Different agents and routes of administration have been proposed. *Aims*. aiming at improving patients comfort, we have tested the scheme of darbepoetin alfa 500°g Q3W in the particular context of lymphoid malignancies treated by chemotherapy. *Patients and Methods*. the observations of 47 patients treated in a single centre have been collected between november 2004 and march 2006. Chemotherapy and darbepoetin alfa therapy could start concomittantly or not, according to the degree of anemia and to medical decision. Evaluation was performed before treatment at day 0 (D0), after 9 weeks of treatment (W9), 24 weeks (W24) or at the end of therapy. Response to therapy was defined as achievement of a level of Hb ≥110 g/L during or at the end of treatment. Median age of the cohort of patients was 58 years (28-78). 32% of the patients were overweighed (BMI > 27 kg/sqm). 34% of the patients suffered from lymphoma or Hodgkin's disease, 23% from myeloma, 13% from various chronic lymphoid malignancies, 30% from acute lymphoblastic leukaemia or Burkitt's lymphoma. The median time between diagnosis and therapy was 4 months.18% of the patients had previously undergone autologous bone marrow transplantation, 9% irradiation. 15% of the patients had previously received erythropoietic agents (including darbepoetin in 2 patients) and 34% had received transfusions during the month before onset of therapy. *Results*. dose and frequency of administration were correct in 96% of the cases. Median Hb level increased during therapy, from 95 g/L at D0 to112 g/L at W9 and 121 g/L at W24 : this evolution was significant ($p<0.001$). The most important gain in Hb was observed for patients with pronounced anemia < 80 g/L (D0 72 g/L, W9 96 g/L, W24 120 g/L). The percentage of patients with Hb ≥ 110 g/L increased from 11% at D0 to 61% at W9 and 67% at W24 ($p<0.001$). Of note, the group of patients receiving intensive chemotherapeutic regimens for ALL and Burkitt's lymphoma responded similarly to chronic malignancies. At W9, 72% of the patients were still on therapy, 56% at W24. Among the 29 patients who did not complete 24 weeks of therapy, 14 (48%) interrupted treatment because of favourable response with Hb ≥110 g/L, 7 (24%) because of increase in Hb level above 130 g/L, 6 (21%) because of death due to underlying disease or chemotherapy adverse events, 3 (10%) by medical decision. . 54% of the patients received transfusions during therapy, most of them between D0 and W9. One patient presented a thrombophlebitis while on therapy but with low Hb level < 80 g/L. *Conclusions*. tolerance and efficacy of darbepoetin alfa 500 °g Q3W compare favourably with weekly administration of erythropoietic agents

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COMBINED INTENSIVE CHEMOTHERAPY, SURGERY AND RADIOTHERAPY FOR PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

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Background. Primary mediastinal large B-cell lymphoma (PMLBCL) has been recognized as an aggressive disease with a poor prognosis when patients were treated with CHOP regiments. *Purpose*. The evaluation of the most effective combined treatment strategy for patients with PMLBCL, including intensive chemotherapy (C–D, ESHAP or dexa-BEAM), surgery, and radiotherapy. *Methods*. We review the medical records of 44 adult patients observed between September 1999 and January 2007 at the Hematological Research Center, Russian Academy of Medical Sciences, Moscow, with the PMLBCL. The 28 patients were females, and 16 were males. The median age at diagnosis was 39 years (range 18 to 55).The treatment program consisted of: 1) 2- courses 14-days C–D following 2-3 courses of ESHAP (41 patients) or dexa-BEAM regiment (3 patients); 2) surgical excision of a residual tumour mediastinal mass (9 patients); 3) all patients after chemotherapy and surgery underwent irradiation. *Results*. Complete remission was obtained in 39 patients (89%) and no relapse was observed in this group, in 5 cases (11%) the treatment was ineffective. The 5-year overall survival (OS) rate and event'free survival (EFS) were 88±8% and 85±7% respective-

ly. *Conclusions*. 1. Combined intensive chemotherapy (C–D, ESHAP or dexa-BEAM) for PMLBCL allow to achieve complete remission in most cases. 2. Surgical treatment is effective in local residual diseases eradicating. 3. The radiotherapy should be conducted in purpose of consolidation of complete remission.

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VALUE OF GLYCOSYLATED HEMOGLOBIN (HBA1C) DETECTION ANALYSIS AS SCREENING METHOD FOR HEMOGLOBINOPATHIES

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Backround. HbA1c determination is a usual way to control diabetic patients, because it provides a way to check therapeutic adherence in the last three months. This analysis is performed by high resolution liquid chromatography (HPLC), which in some cases shows peaks belonging to pathologic hemoglobins. *Aim*. The aim of this study was to review graphics obtained through HPLC to determine Hb1Ac in diabetic patients, in order to detect abnormal peaks which would be then cross-checked by a HPLC hemoglobinopathies specific program, and see if they belong to known pathologic hemoglobins. *Materials and Methods*. Chromatograms belonging to 1500 diabetic patients were checked. HA-8160 (A. Menarini Diagnostics) analyzer was used for HbA1c detection. This analyzer uses reversed-phase cation exchange chromatography column, and dual-wavelength colorimetry (415-500 nm) to carry out its measurements. Together with HbA1c, this analyzer can also provide the value for HbA0, HbF, and other abnormal peaks belonging to other fractions. The software used is not able to correctly separate HbA0 from HbA2. All samples, extracted in EDTA tubes, that showed abnormal crests or >2% HbF values, were analyzed by HPLC with specific software for the detection of β-thalassemias as well as other hemoglobinopathies (Variant. Bio-Rad. Beta Short Program). *Results*. From the 1500 analyzed chromatograms, 12 (0.8%) showed abnormal peaks, being then cross-checked by VARIANT detecting; 1 HbD, high HbF in 4 patients, 2 HbS, and 1 HbC. None of these patients showed mycrocytosis or were previously known by the hematology department at our hospital. *Conclusions*. According to our results, it appears interesting to check chromatograms belonging to diabetic patients, because without any added cost, structural hemoglobinopathies without mycrocytosis could be detected. Although the diabetic populations is not the most adequate because of its median age for the screening of hemoglobinopathies, it could be very interesting to check pathologic chromatograms from these patients in order to detect possible previously unknown hemoglobinopathies in order to perform family studies and genetic counseling when necessary.

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FAILURE OF PUBERTY IN EGYPTIAN β THALASSEMIC PATIENTS: EXPERIENCE IN NORTH EAST REGION - DAKAHLIA PROVINCE

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Background. Endocrine complications in thalassaemia major (TM) are classically considered to be the result of iron deposition in the endocrine glands. Hypogonatotrophic hypogonadism, which still remains the commonest endocrinopathy in patients with TM, has been proven to be the result of hemosiderosis of the gonadotroph cells of the pituitary gland. *The aim of the study*. to evaluate the prevalence of delayed puberty and hypogonadotropic hypogonadism in transfusion-dependent patients with β-thalassemia major. *Patient and Methods*. Growth and sexual development of 40 patients with thalassemia major (20 males, 20 females) aged 12-22 years were evaluated. The following parameters were measured in every patient: age, sex, height, weight, Body Mass Index (BMI) and Tanner's pubertal staging. For all patients, the following investigations were done: opthalomogical evaluation, audiograms, skeletal survey, echocardiography, serum ferritin, liver function tests, hepatitis profile, serum calcium, phosphorus and blood sugar. Thyroid, parathyroid hormones, serum follicule stimulating hormone (FSH), luteinising hormone (LH), testosterone (T), and estradiol (E2) hormone were also measured. *Results*. Failure of puberty was present in 80% of boys and 75% of girls aged 12-22 years. Gonadotropin insufficiency was found in most of the patients with lack of puberty. Arrested puberty was noted in five boys (25%) and six girls (30%). Ten girls (50%) did not menstruate, two (10%) had regular menstrual cycles, one (5%) had irregular menstrual cycles, and two (10%) developed secondary amenorrhea. Using univariate analyses and stepwise logistic regression analysis after adjustment for confounding factors, serum ferritin at the time of the study was identified as an independent risk factor for hypogonadotropic hypogonadism, with an odds ratio of 28.40 (95% confidence interval 3.25-245.15), p= 0.003 with a B value of 3.24 (standard error, 1.12). *Conclusions*. We conclude that failure of puberty are very common in our thalassemic patients which necessitates newer protocols of treatment, correct blood transfusion and chelation therapy.

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THE EFFECTS OF LOSARTAN ON PLATELET AGGREGATION AND HEMATOLOGICAL PARAMETERS IN PATIENTS WITH NEWLY DIAGNOSED HYPERTENSION: (A CLINICAL STUDY)

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Background. Angiotensin II receptor blockers have antiproliferative, antihypertensive and preventive effects from atherosclerosis. There are controversial *in vitro* effects of angiotensin II receptor blockers on platelet aggregation in several experimental studies. In only one study, losartan decreased *in vivo* platelet aggregation with ADP, and ristocetin after 3 weeks. *Aim*. To evaluate *in vivo* effects of losartan on platelet aggregation with ADP, collagen, epinephrine, ristocetin and other hematological parameters. *Methods*. Nineteen (13 patients female and 6 male, mean aged of 55±7 years) with newly diagnosed hypertension were enrolled to this study. They treated with 100mg/day losartan. Before the treatment and after 2 months, we analyzed complete blood cells parameters (Beckman Coulter) and platelet aggregation with collagen, epinephrine, ristocetin, and ADP (Chrono-log, 570 blood aggregation systems, 2 West Park Road, Haverton,PA, 19083-4691, USA). The values were analyzed using two-paired t test. *Results*. Before the treatment platelet aggregation with ADP, collagen, epinefrin, ristocetin, were 82±20%, 69±24%, 93±15%, and 80±18.4%, respectively. After 2 months, these rates were 79.8±13%, 62.5±20%, 92±14%, and 63±25%. Although all parameters decreased, decrease in ristocetin was significant. (p=0.042). The levels of hemoglobin (before 14±1.6 g/dL, after 13.4±1.3 g/dL) and hematocrit (before 40.2±5%, after 38.5±3.7%, p<0.001 for both) decreased with losartan. There were no changes on platelet counts, platelet-crit, and mean platelet volume (p>0.05). *Conclusions* Losartan treatment decreased platelet aggregation with ristocetin and the levels of hemoglobin and hematocrit. Losartan with these hematological effects may be useful in the atherosclerosis, inhibiting platelet aggregation and decreasing viscosity, in addition to antihypertensive effect.

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SREENING OF HB S IN THE REGIONAL HOSPITAL OF MALABO (EQUATORIAL GUINEA)

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Background. The sickle cell anemia is one of the genetic diseases more important in the world, it appears mainly in the black race constituting in a disease of high prevalence in some countries and originating serious problems of public health. The greater prevalence of the sickle trait is in Equatorial Africa in where until 40% of the population are carrying, being in direct relation with the zones in which falciparum malaria has been rampant; the disease reaches a prevalence of to 2-3%. *Aims*. To determine the incidence of Hb S in the population of Malabo (Equatorial Guinea). *Methods*. A total of 328 blood samples of umbilical cord and placenta were compiled in the regional hospital of Malabo during the months of February and June of 2005. The conserved samples were gathered on paper of filter and in methanol to 70% to 4° C. In the Service of Hematology of the Hospital Clinico San Carlos of Madrid (Spain), the samples were analyzed in the months of October of 2005 and February of 2006, being used for the analysis a VARIANT (Bio-Rad) with the program Sickle Cell Short Program. *Results*. Of the 328 compiled samples only 273 could be analyzed of which 208 (76.2%) were normal; 14,2% (39) displayed the sickle trait (Hb S/Hb A); 8 (2.9%) only have Hb S (Hb S/Hb S); 18 (6.6%) were carrying of some other hemoglobinopatia non S (Hb X/Hb A) and 55 samples (16.8%) were degraded not being able to be studied. *Summary/Conclusions*. Equatorial Guinea is a country located in the coast the west of Central Africa in the denominated zone of high malaria prevalence. It is divided in a continental part on the gulf of Guinea and another insular one. In the total population it is of 504,000 inhabitants. The capital is Malabo in the island of Bioko. Considering

that the total population of Malabo is of 90,000 inhabitants hope that 1 of each 7 is carrying of HbS (~13.000 carrying), as soon as the HbS/HbS would be 2,610 individuals (1/34) and approximately 6,000 with some variant of structural hemoglobinopathie (1/15). In spite of the means shortage and rudimentary of the collection of the samples only 16.8% of the total they could not be analyzed. For that reason and by the high incidence of the HbS it would be precise that policies of cooperation for the diagnosis, treatment and prevention of the homocigous forms settled down, as well as to collaborate in the formation of the sanitary personnel to give the suitable genetic advice.

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SERUM HEPCIDIN LEVELS IN CHILDREN WITH SOLID TUMORS, INFLAMATORY BOWEL DISEASE AND IRON DEFICIENCY ANEMIA

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Background. Hepcidin is an hepatic peptide that regulates iron homeostasis by inhibiting iron absorption in enterocytes, the efflux of iron from macrophages and the release of iron from hepatocytes. Its synthesis is induced by inflammatory stimuli and iron overload, and inhibited by anemia and hypoxia. It has been suggested that hepcidin may be responsible for anemia in inflamatory diseases. The aims of this study were to determine the hepcidin level in anemia of chronic diseases and iron deficiency anemia in children, and to evaluate its correlations with inflammatory markers and iron parameters. *Methods.* The levels of serum hepcidin, high-sensitivity CRP, ferritin, serum iron, transferrin saturation and soluble transferrin receptor were measured by commercially available kits, and complete blood count and erythrocyte sedimentation rate by conventional methods in children with solid tumors (ST, n:16), inflammatory bowel disease (IBD, n:15), iron deficiency anemia (IDA, n: 15) and age matched healthy controls (n:15). The study was approved by our IRB. *Results*. The mean value of serum hepcidin was significantly higher ($p< 0.01$) in children with ST (661.5±217.2 ng/dL) (± SD) and IBD (775.7±395.8 ng/dL) than in age matched healthy controls (426.1±208.9 ng/dL) but not significantly different between ST and IBD. The mean value of hepcidin in children with IDA (303.5±81.5 ng/dL) was not significantly different from that in controls. Serum hepcidin showed positive correlation with serum ferritin in ST (r:0.77, p:0.01) and IBD (r: 0.58, p:0.02), and with transferrin saturation in IDA (r:0.59, $p<0.01$). There were no significant correlations between serum hepcidin and hemoglobin concentration or reticulocyte count. *Conclusions*. Elevated hepcidin level in children with ST and IBD may be due to inflammation as indicated by positive correlation with serum ferritin. In IDA, serum hepcidin level appears to be related with transferrin saturation rather than serum ferritin and soluble transferin receptor. However, regulation of serum hepcidin level in children with these disorders should be further evaluated.

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THE RELATION BETWEEN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA AND HELICOBACTER PYLORI INFECTION AND EFFECT OF ERADICATION TREATMENT ON COURSE OF PLATELET COUNTS

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Background. Recent papers reports a relationship between chronic idiopathic thrombocytopenic purpura (CITP) and helicobacter pylori infection (HP). But, most of cases are adult patients and results have discrepancies. Moreover HP incidence and HP strains are deferent in each countries. *Aims*. In this study we were determined the incidence of HP in our CTIP patient and tested the effect of eradication therapy for HP on CTIP course. *Methods*. The HP incidence was determined in 21 patients of CTIP and 59 healthy children. Ages of Patients and controls are 2 To 24 ages. HP positive patients with CTIP were treated with antibiotics for eradication of agents. The success of eradication was controlled by Urea-breath test after two months of treatment. Positive patients received a second treatment. The platelet count of CTIP patient was fallowed by 8 month after eradication. *Results*. HP incidence was equal and 52% in both groups. Eleven cases of CTIP patient were HP positive. HP infection was eradicated 8 of 11 patients. After first course of antibiotic treatment, HP was eradicated in two of three patients with persistent positive urea-breath tests. Remaining one patient was persistent after second treatment. No elevation of platelet counts occurs either in treated or untreated patients. *Conclusions*. HP incidence was not different CTIP patients from controls. HP positivity and eradication of HP has no effect on the course of CTIP. Our study suggests that HP test and HP treatment was not useful for CTIP patients.

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PREVALENCE OF HELICOBACTER PYLORI INFECTION IN CHRONIC AUTOIMMUNE THROMBOCYTOPENIC PURPURA PATIENTS

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Background. Helicobacter pylori (Hp) infection has been implicated in the pathogenesis of chronic autoimmune thrombocytopenic purpura (CITP). However there has been regional variation in the association between these two conditions. *Aims*. We determined the prevalence of Hp infection in CITP north Indian patients and studied various clinical & laboratory features of Hp infection with CITP patients. *Methods*. Fifty patients of CITP patients and 50 healthy age and sex matched controls were studied for prevalence of Hp infection. The diagnosis of ITP was made by demonstration of thrombocytopenia on peripheral blood smear with normal or increased megakaryocytes on bone marrow examination. CITP was defined as thrombocytopenia lasting for more than 6 months. The prevalence of Hp infection was studied by serum IgG antibodies (Accubind ELISA Microwells Anti-H pylori IgG, Monobind Inc, Lake Forest, CA, USA). All patients underwent a detailed history regarding the duration of disease, nature of bleeding, co-morbid illness, drug history and history of any auto-immune disorder. Investigations included antinuclear antibodies, antiphospholipid antibodies, Hepatitis B, C and HIV serology. Patients with secondary causes of thrombocytopenia were excluded. The controls were selected from hospital visitors during the study period whose platelet counts were normal and Hp status was unknown. The study was carried out in a blinded fashion and results of Hp serology were known at the end of the study. These results of Hp serology were then correlated with clinical and lab characteristics of CITP patients. *Results*. The mean age of the patients (68% females) and controls (62% females) were comparable (34.1 vs 29.7 years, $p>0.05$) respectively. The prevalence of Hp infection was 38% in CITP patients and 46% in the control group ($p>0.05$). The risk factors for acquiring Hp infection in CITP patients (positive vs negative) were male sex (62% vs 26.5%; $p<0.05$) and low socioeconomic status (52.6% vs 6.5%; $p<0.05$) whereas steroid intake & response to steroid did not have any bearing on acquiring Hp infection. The mean duration of thrombocytopenia from diagnosis till the time of testing for Hp infection was 28 months (range 6-84) in Hp infection positive patients while it was 55 months (range 6-480) in Hp infection negative patients ($p=0.083$). Ten patients (20%) had undergone splenectomy for thrombocytopenia and they had lower prevalence of Hp infection than nonsplenectomised patients (29% vs 71%, $p<0.05$). *Conclusions*. Though the role of Hp infection in patients with CITP cannot be excluded, this study did not find increased prevalence of Hp infection in CITP patients in this part of the world.

1395

A NEW POINT MUTATION AT CD7 G→T OF THE β GLOBIN GENE CAUSES β^0-THALASSAEMIA

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Background. β Thalassaemia is an autosomal recessive disorder characterized by reduced (β^+) or absent (β^0) β globin chain synthesis. β-Thalassaemia constitutes the most frequent hereditary disease in Greece. Heterogeneity at the molecular level is reflected in the presence of a panel of 15 single point mutations and small deletions/ insertions bearing frequencies >1% and another 15 rarely or sporadically seen. Since 1990, Greece became a country of acceptance of economic immigrants, 58% of which are of Albanian origin. The overall frequency of β thalassaemia and haemoglobin variants is 7.1% and 4.4% respectively. A panel of 11 mutations has been described at the Albanian population. Most of them are similar with those described in the Greek Population but some are detected for the first time in Greece. *Aims*. In this study, we present ♀ new substitution of the β globin gene, CD7 (G→T), identified in a couple of Albanian origin, at risk for β-Thalassaemia. *Methods*. Carrier identification was carried out by standard methods. Molecular study includes Polymerase Chain Reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), Allele Specific Oligonucleotide (ASO) hybridisation and DNA sequencing. *Results*. Enzymatic amplification of the β globin gene followed by DGGE analysis has revealed a pattern typical

to the presence of IVS1-6 mutation in the mother but an unknown pattern at the 1st exon in the father. DNA sequencing showed a point mutation G→T at CD7 of the β globin gene, confirmed also by ASO hybridisation, which transforms codon 7 GAG (Glu) to a termination codon TAG. This new allele causes β^0 Thalassaemia. Prenatal diagnosis has been performed by Fetal Blood Sampling in the 20th week of gestation as the couple presented late in pregnancy. The fetal sample has been analysed both with DNA techniques and globin chain biosynthesis. The fetus has inherited the CD7 mutation with biosynthetic ratio β/α=0,030 characteristic of heterozygous β^0 -thalassaemia. *Conclusions*. The identification of new or rare mutations is indicative of the molecular heterogeneity of β- thalassaemia in Albania. Moreover, it gives the possibility for genetic counselling and prenatal diagnosis at 10- 12 weeks of gestation.

1396

THROMBOCYTOPENIA AND PSEUDOTHROMBOCYTOPENIA AFTER TREATMENT WITH ABCIXIMAB. A PROSPECTIVE STUDY

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Background. Thrombocytopenia is a well-recognized adverse effect of abciximab therapy (1-2% of patients). Abciximab is a glycoprotein IIb/IIIa inhibitor widely used following coronary angioplasty to reduce the incidence of thrombotic complications after revascularization. Abciximab is a chimeric (human/mouse) monoclonal antibody that binds to the fibrinogen binding site of the GP IIb/IIIa complex, inhibiting platelet interactions and thrombus formation. Acute thrombocytopenia is a frequent side effect of abciximab affecting 1% of patients after the first exposure and 4% in subsequent exposures. This thrombocytopenia can produce excessive bleeding increasing the risk of hemorrhagic complications in coronary angioplasty and may require platelet transfusions and discontinuation the abciximab infusion. Pseudothrombocytopenia has also been described associated with abciximab treatment but much more infrequently, and with no need of treatment modifications. *Aims*. Our objective was to study whether the administration of abciximab had any impact on the platelet count immediately after the administration of the drug, and to detect any other platelet abnormalities. *Methods and results*. From January 5th to February 5th 2007, we performed a prospective study in 44 consecutive patients, 18 male and 9 female with a median age of 69 (range 45-79), with acute ischemic myocardiopathy treated with abciximab after coronary angioplasty. The patients received abciximab at a dose of of 0.25 mg/kg i.v. bolus, followed by a 24 hours infusion of 0,0625 mgr/kg/min. In addition the patients were treated with sodium heparin (5000 IU, i.v. bolus), clopidogrel (75 mgr/day) and oral acetylsalicylic acid (100 mg/day). Platelet counts were obtained prior to and in the following 24hr following abciximab in 27 cases. Statistical comparisons were made with the student-T test for paired data. The median platelet count prior to abciximab was 189×10^9/L (range 94-380). We found a small but statistically significant decrease in the platelet count post treatment with abciximab $177,5\times10^9$/L (range 0-362), (p=0,005). In addition we found one case of severe thrombocytopenia that required platelet transfusion and one case of pseudothrombocytopenia (Figure 1), in this series (4,5%).

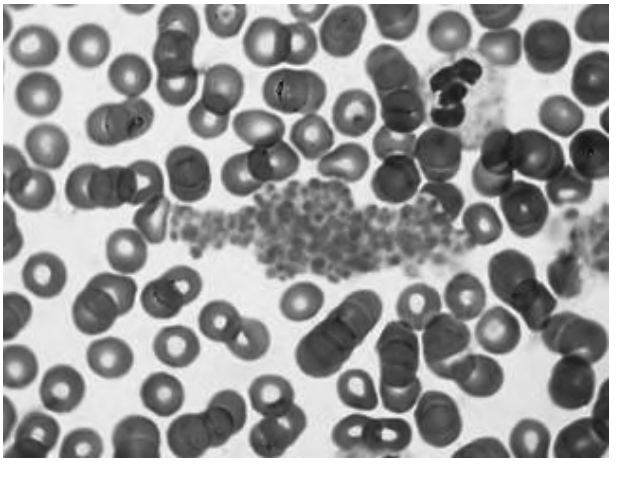

Figure 1.

In the first case we excluded the existence of anti-PF4/heparin complex antibodies (ID-PaGIA heparin/PF4 antibody test, DiaMed) prior to the platelet transfusion. The patient with pseudothrombocytopenia did not have any bleeding complications and did not required discontinuation of abciximab treatment or intervention with platelet transfusions. It has been proposed that clumping is due to antiplatelet antibodies directed against the GP IIb/IIIa complex but the mechanism of abciximab-associated thrombocytopenia is not clear. Evaluation of the peripheral smear, to get the automated platelet count in citrate anticoagulated blood samples, and the exclusion of PF4/heparin antibodies is essential to make the differential diagnosis of these complications with such a different management. *Conclusions*. Thrombocytopenia is not uncommon after abciximab therapy and the medical community should be aware of this complication. We have found a slight decrease in the platelet number probably related with abciximab infusion, but there was very mild and clinically irrelevant. The distinction of true severe thrombocytopenia from pseudothrombocytopenia and from heparin-induced thrombocytopenia in patients with acute coronary syndromes treated with abciximab is highly suggested.

1397

DIVERGENT PATTERNS OF SERUM VASCLAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PATIENTS WITH ACUTE LEUKEMIA AND LYMPHOMA

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VEGF has been implicated as the main endothelial pathway requied for tumor neovasclariztion . Although most of the initial studies in angigenesis were done on solid tumors, there were data suggesting the importance of angiogenesis in hematological malignancies such as non-Hodgkin's lymphoma and acute leukemia .we detemined serum VEGF by ELISA technique in patients with NHL(20 cases), acute lymphoblastic leukemia (13 cases), and acute myeloblastic leukemia (14 cases) in addition to 13 cases as a reference control group. Twenty five cases were followed up afrter therapy (10 with NHL, 8 with ALL and 7with AML).Signifcant high levels were only reported among patients with AML (M±SD=345.7±227.3) and NHL (357±214.4) when compared to controls control (189.2±70.5)(p< 0.05). On the contrary, a highly significant reduction of serum VEGF was elicited in patients with ALL (132.3±44.6) compared to controls (p< 0.01). Serum VEGF was significantly reduced nearly to control level after therapy in NHL (M±SD =142±47.9)and AML (210±69.5) as compared to the before therapy , while in ALL patients, serum VEGF was noticeably increased (168 ±49.2) nearly to control level after therapy as compared to to that before thrapy .We conclude that VEGF level in the serum may be used be as a valuable angiogenic marker for identifying the clinical outcome of patients with acute leukemias and NHL. Anti-angiogenesis is a promising target for therapeutic interventions in lymphomas and acute leukemias especially AML

1398

ACUTE MYELOID LEUKEMIA ASSOCIATED PLATELETS BEHAVIOR - FLOW CYTOMETRY ANALYSIS IN CLINICAL CONTEXT

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Background. Extensive bleeding is one of the most frequent causes of death in acute myeloid leukemia (AML). Previous studies on platelet behavior associated with this disease identified impaired activation and aggregation processes. *Aims*. The aim of this study was to examine platelet function in correlation with other haemorrhage risk factors (fever, sepsis, recent bleeding, uremia, leucocytosys , hemathocrit value, treatment). *Methods*. Whole blood flow cytometry analysis for platelets surface proteins (Glycoprotein Ib-IX [CD42b, CD42a], Glycoprotein IIb-IIIa [CD41, CD61], P-selectin [CD62P], granulophysin [CD63]) was fulfilled in patients with acute myeloid leukemia in different stages of diagnosis and therapy (n=22) in comparison with healthy human controls (n=10). *Results*. Our results show a significant decrease of fluorescence level associated with platelet activation markers (CD63 [14.11% vs. 40.78% p<0.05]; CD62P [15.26% vs. 28.23% p<0.05]); adhesion markers (CD42b [69.08% vs. 84.41% p<0.05]) and aggregation markers (CD61 [83.79% vs. 98.62% p<0.001]) in patients

compared to controls (Figure1). The levels of CD41 [80.62% vs. 86.31%, p=0.290] and CD42a [77.98% vs. 94.15%, p=0.99] demonstrate no significant differences in the two groups. Granulophysin (CD63) expression was negatively correlate with hemathocrit level (Ro=-0.447, p=0.037). Interactions between renal function determinants (BUN, uric acid, creatinine), coagulation indices (APTT, INR), clinical determinants (fever, cutaneous haemorrhage), treatment options (chemotherapy, antibiotic therapy) and platelets functionality were analysed without significant results. Due to relative reduced number of cases no correlation could be established with AML subtype or evolving stage of the disease. *Conclusions*. The AML patients present changes in adhesion receptors, i.e. decreasing of CD42b (von Willebrand factor) and activation markers, CD63 (granulophysin) and CD62P (P-selectin), suggesting a functional defect or a denatured intracellular signalling. The exposed data indicates that flow cytometry can effectively identify multiple platelet impairments regarding adhesion, aggregation, and secretion associated with in AML pathogenesis, and express the need for furthermore extensive investigations in order to integrate these platelet behaviour features in AML clinical context.

Expression of platelet surface markers in AML patients and controls. Results are presented in box plots indicating the median as the horizontal line, 25th-75th percentiles of the group distributions as boxes and 2.5% and 97.5% cumulative frequencies as whiskers. Outliners [identified by the 1.5 X inter-quartile range (IQR) criterion] are plotted as empty squares.

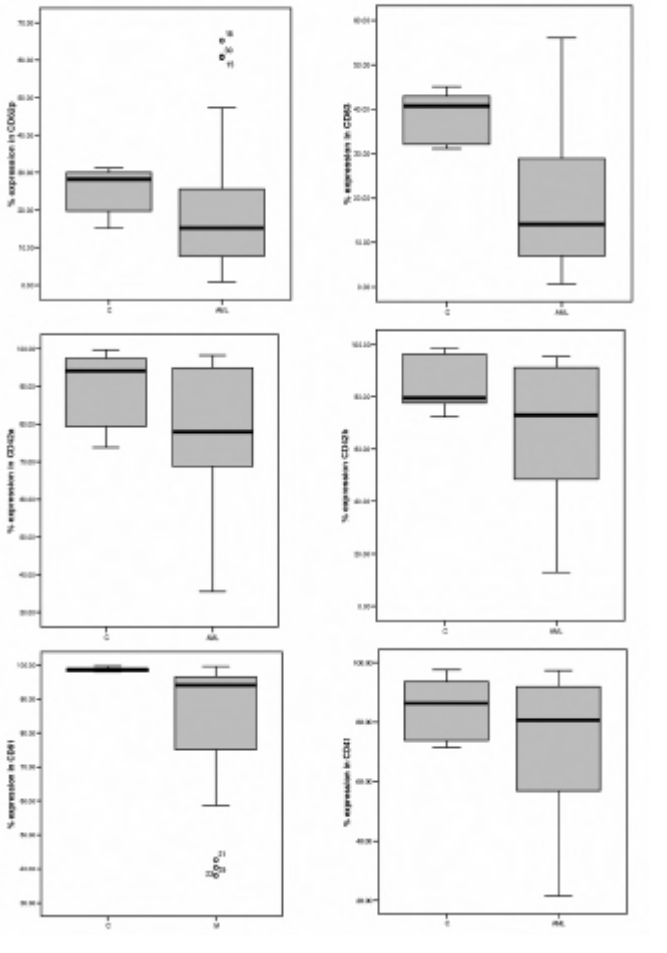

Figure 1. Expression of platelet surface markers.

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IMMUNE THROMBOCYTOPENIC PURPURA: A 10 YEAR EXPERIENCE FROM A SINGLE CENTER

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Background. Immune thrombocytopenic purpura (ITP) is a common acquired autoimmune disorder. The natural course of ITP is unknown and no standard therapeutic approach exists for the one third of patients who develop refractory disease. Aim of this study was to examine the clinical features, the laboratory findings and the effects of different treatment modalities in patients with ITP and to draw any useful conclusions. *Methods*. 59 patients (37 female, 22 male, age range 33-94 years, mean 61.7 years) who were diagnosed at our center between 1996 and 2006 were evaluated retrospectively. The diagnosis of ITP was based on history, physical examination and decreased platelet count with an otherwise unremarkable peripheral smear. All other causes of thrombocytopenia were excluded. Treatment was initiated when the platelet count was less than 30×10^9/L Relapse was defined as a blood platelet count less than 30×10^9/L and chronic refractory ITP was defined as the failure of any modality to keep the platelet count above 20×10^9/L for an appreciable time without unacceptable toxicity. *Results*. 33 patients (56%) of patients were asymptomatic at diagnosis and thrombocytopenia was found on a routine visit to a physician. Thirteen patients (22%) presented with easy bruising and/or petechiae, 12 (20%) patients with minor bleeding, such as gingival bleeding, epistaxis, macroscopic hematuria and one patient with severe intracerebral hemorrhage. Thirty-one patients (17%) had a positive autoimmune profile with main findings being positive antinuclear antibodies, antiplatelet antibodies and low C¬¬3. None of the patients developed an autoimmune disorder. With regard to therapy 3 patients received no treatment; as first line treatment 13 patients received steroids only and 43 patients received combination of prednisolone (1mg/kg) and IVIg (400 mg/kg) for 4-5 days. In 24 patients (40%) no relapse was noted. Out of the 45 patients who relapsed 12 patients (20%) experienced one relapse, 11 patients (18.6%) 2 relapses and 12 patients (20%) had more than 2 relapses. In patients with symptomatic relapse or severe thrombocytopenia steroids and IVIg was reintroduced and 12 of them achieved remission. Third line treatment consisted of danazol (10 patients), splenectomy (6 patients), iv anti-CD20 (8 patients), azathioprine (2 patients) and vincristine (4 patients). In 4 patients who relapsed post splenectomy iv anti-CD20 was given at a dose of 375 mg/m^2 weekly for 4-6 consecutive weeks. All 12 patients who received anti-CD-20 responded to treatment achieving a platelet count >50×10^9/L, however 3/12 patients relapsed 7. 9 and 24 months post infusion. Adverse effects of therapy were allergic reaction to IVIg (2 patients), iatrogenic Cushing and myopathy secondary to steroids (4 patients) and pneumonococcal pneumonia in a splenectomized patient. *Results*. ITP is an autoimmune disorder with variable clinical presentation and course. Patients with chronic refractory ITP are difficult to manage. AntiCD-20 therapy appears as a promising immunomodulatory agent for the management of these patients.

1400

BRAIN MRI FINDINGS IN LEBANESE SICKLE CELL DISEASE PATIENTS

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Background. Sickle cell disease(SCD) is one of the most prevalent hemoglobinopathies in Lebanon. Acute stroke defined as an acute neurological syndrome secondary to arterial occlusion or hemorrhage with resultant neurological symptoms and signs lasting for more than 24 hours is the most catastrophic brain injury seen in this disorder and has a prevalence of around 10% by 50 years of age. Silent cerebral infarcts (SCI), defined as an abnormal magnetic resonance image (MRI) of the brain and no history of neurologic deficit, are seen in 20-44% of children with sickle cell anemia (SCA) and represents a major risk factor for overt stroke. Identifying asymptomatic patients at high risk of developing sickle-related brain injury helps physicians implement preventive and effective therapies. *Aims*. 1. Determine prevalence of brain imaging abnormalities in a group of Lebanese patients with SCD and 2. Identi-

fy predictive factors for these abnormalities. *Methods*. A review of magnetic resonance imaging of the brain for 50 SCD patients followed in 2 centers specialized in inherited hemoglobin disorders was undergone. Of those 50 patients, 39 asymptomatic patients have had surveillance brain MRI. All patients underwent MR imaging at 1.0 T using 5 mms thick sections and all images were read by a single radiologist with a 10 years experience in neuroradiology . Fisher's Exact test (Wilcoxon two sample rank-sum test) was employed to study the association of binary data (continuous data)with the 2 groups. The SAS v8.2 (Cary, N.C.) was used to analyze the data. A p-value less than 0.05 was considered significant. *Results*. Overall prevalence of stroke in this group was 30% (15 out of 50). 11 patients (22%), median age 14 years and 6 months, had overt strokes. When patients with overt stroke were excluded, 4 of the remaining 39 patients (10.3%), median age 13 years and 5 months, had abnormal brain MR imaging compatible with SCI. Of those with overt strokes, 27% were females and 73% were males. 91% had SCA and 9% had sickle β thalassemia (ST). Bivariate analysis showed an association between the occurrence of overt stroke and premature death (p=0.006), regular blood transfusions (p=0.006), osteomyelitis (p=0.009) and acute chest syndrome (p=0.085). As for those with SCI, all 4 patients were males and were in the SCA group. Bivariate analysis showed an association between the occurrence of SCI and presentation of disease before 2 years of age (p=0.053, borderline) and acute chest syndrome (p=0.035). The small number of patients in this group did not allow for multivariate analysis. *Conclusions*. Stroke prevalence rate in Lebanese patients with SCD is lower than what was previously reported. This may be attributed to the small study size as well as to the imaging technology utilized which is less sensitive than newer MRI technology at detecting very small lesions.

1401

NEONATAL THROMBOCYTOPENIA AND PLATELET TRANSFUSION: OUR EXPERIENCE

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Background. Premature infants and at term newborns have an higher circulating blood volume per Kilogram than the adults (100 mL/Kg in premature; 80 mL/Kg in at term), for this reason, in case of neonatal thrombocytopenia, a specific hemocomponent, with a very high Platelet concentration, needs for transfusion therapy. The laboratory criteria for Platelet transfusion are the following: A) a PLT count < 150×10^9 cells/L if bleeding is observed; B) a PLT count < 20×10^9 cells/L without bleeding; C) a PLT count < 50×10^9 cells/L in newborns showing critical clinical conditions. *Aims*. In this study, we have reported the PLT transfusion therapy in Neonatal Intensive Care Unit (NICU) of Caserta's Hospital in the last four years. *Methods*. Effects of PLT transfusions have been followed in 37 children (31 premature infants and 6 at term newborns). The weight of premature infants ranged from 600-1800 g and at term newborn from 1800-4000 g. Gestation age of premature infants ranged from 26-36 weeks and of at term ones, of course, from 37-42 weeks. For every Platelet transfusion in these newborns, the volume of Platelet Concentrate has been of 10-20 mL/Kg, with a PLT count ≤ 700×10^9 cells/L. *Results*. In the study period, 77 PLT transfusions have been performed: 23 children have been only transfused one time, while multiple PLT transfusions (ranged 2-10) needed for 14 children according to clinical conditions. The observed clinical indications for transfusions have been the sepsis with haemorrhagic syndrome (26 cases), haemorrhagic syndrome without sepsis (7 cases) and neonatal alloimmune thrombocytopenia without haemorrhagic syndrome (4 cases). After 24 hours from transfusion therapy, the absolute PLT count and the Correct Count Increment have increased in all 37 little patients. The highest increase in PLT count was 45×10^9 cells/L, while the lowest 5×10^9 cells/L. No difference in the efficacy of therapy has been detected between premature group and at term group. 94% of children has been discharged from hospital in good general conditions without complications in following controls. *Conclusions*. In conclusion, we can affirm that PLT transfusion in premature infants and in at term newborns is an efficient and safe treatment of severe haemorrhagic conditions. However a collaboration between NICU, Paediatric Haematologists and Transfusion Centre is necessary to choice the adequate Platelet Concentrate's volume for transfusion and the best PLT donor for the newborn.

1402

HEREDITARY HEMOCHROMATOSIS GENE MUTATIONS IN THE SOUTH EASTERN REGION OF TURKEY

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Background and Aims. Hereditary hemochromatosis (HH) is a common disorder of iron metabolism with autosomal recessive inheritance and is characterized by tissue injury resulting from an abnormal accumulation of iron in various organs. The hemochromatosis protein (HFE) gene was identified on chromosome 6 by Feder *et al*. in 1996. Most affected patients are homozygous for the missense mutation which results in the substition of tyrosine for cysteine at amino acid 282(C282Y). A more common mutation is the substition of aspartat for histidine at amino acid 63 (H63D); this mutation may contribute to minor increases in iron levels but rarely iron overload in the absence of C282Y. S65C missense substitution accounted for nearly 8% of hemochromatosis chromosomes. Additional rarer mutations in the HFE gene have since been described, such as the V53M, V59M, H63H, Q127H, Q283P, P168X, E168Q, E168X, and W169X mutations. Diagnosis of HH is usually based on a combination of various genetic or phenotypic criteria. Genetically, it can be based on direct DNA testing for HFE gene mutations that associated with hereditary hemochromatosis. Percentage of transferrin saturation and serum ferritin level have been used to confirm the diagnosis of HH. We conducted a study to describe the HH mutations in Southeastern region of Turkey. *Patients and Methods*. We analyzed the HH mutations of the patients with high transferrine saturation, and with clinical suspicion or findings that attributed to HH. Prevalence of HH genes; C282Y, Q283P, H63D, and S65C, V53M, V59M, Q127H, P160delC, E168Q, E168X, W169X, TFR2; E60X, M172K, Y250X, AVAQ594-597del, FPN1; N144H, V162 del variants were analyzed and results were evaluated retrospectively. *Results*. Mean ferritine level was 285 ng/mL(196-1936). Transferrine saturation of 70 of 97 patients were between 38-45% and remaining 27 patients have transferrine saturation of 45-50%. 19 of 97 patients (19,5%) have hetezygote H63D mutations and no other mutation was detected. These result is similar to the results of other population based studies that were carried out in Turkey. Liver biopsy was performed in our 2 patients and no sign of iron overload was examined. *Conclusion*. In our medical center all of the hemochromatosis cases are associated with secondary iron overload and no patient with HH was detected. Although H63D heterozygosity is frequent in our population, heterozygosity of this mutation is not related with iron overload. It would be more reliable to determine the transferrine saturation cutoff value as higher than 50% , or even 60% for establishing the contributing role of these mutations in the diagnosis of HH. The results of other population based studies in our country supporting our data however further population based studies are required to confirm this data in our region. We know that there may be major racial variation in gene polymorphisms. Therefore, population based prevalence studies must be performed to understand its regional and racial frequency before using HH mutations as a diagnostic procedure.

1403

RAPID IDENTIFICATION OF HETEROZYGOUS OR HOMOZYGOUS JAK2V617F MUTATION IN MYELOPROLIFERATIVE DISORDERS USING MELTING CURVE ANALYSIS

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Background. An activating JAK2 mutation recently has been associated with a wide spectrum of myeloproliferative disorders (MPD) which included polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis with myeloid metaplasia (MMM). This newly identified somatic point mutation is a G-C to T-A transversion, resulting in the substitution of valine by phenylalanine at codon 617 (JAK2V617F). Mutational frequencies were the highest for PV (65-97%) followed by ET (32-57%) and MMM (43-50%). However, 25% of patients with PV displayed homozygosity for the mutant allele which was infrequent in other myeloid disorders. *Aims*. We report the advance and our experience in the diagnosis of JAK2 mutation in MPD. *Methods*. In the current study, mutation analysis for JAK2V617F was performed prospectively either bone marrow or peripheral blood cells using allele-specific polymerase chain reaction (AS-PCR) and fluorescence resonance energy transfer (FRET) probes with melting curve analysis. From January to December of 2006, we prospectively enrolled 78 patients with PV (N=21), ET (N=32), MMM (N=5), secondary PV (N=4), secondary thrombocytosis (N=2), acute myelocytic leukemia (AML) (N=4), chronic myelocytic leukemia (CML) (N=8),

and myelodysplastic syndrome (MDS) (N=2). *Results*. The detection rate was 76.2% in PV (homozygous in 14.3%), 46.9% in ET, 80% in MMM (homozygous in 20%) and none in other entities. In PV, the homozygous JAK2V617F patients displayed a significant longer duration of disease than heterozygous patients. In ET, the clinical parameters did not reveal any significant association between mutant and wild type of JAK2V617F patients. *Summary/conclusions*. We demonstrated that heterozygous and homozygous JAK2V617F mutation can be identified by a rapid and reliable assay based on FRET probes and melting curve analysis. Detection of this mutation, although not specific to particular disease entities, should assist in the diagnostic evaluation of patients with suspected BCR/ABL-negative MPD.

1404

EFFICACY OF RECOMBINANT FVIIA TREATMENT IN INHIBITOR POSITIVE HEMOPHILIC PATIENTS AND IN GLANZMANN THROMBASTHENIA: SINGLE CENTER EXPERIENCE

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Background and Aims. Management of bleeding in hemophiliacs with a history of inhibitor remains problematic. Recombinant factor VIIa (rFVIIa) was initially developed for the treatment of hemorrhagic episodes in hemophilic patients with inhibitors to factors VIII and IX. rFVIIa is approved as a *bypassing* agent to promote hemostasis in congenital or acquired hemophilia with inhibitors. Factor VIIa has also been used in thrombocytopenia, platelet function defects, and liver transplantation and after trauma or surgery. It has also been used to enhance hemostasis in nonhemophilic patients who experience bleeding episodes not responsive to conventional therapy. Evidence so far indicates that the use of factor VIIa in hemophilic patients with inhibitors is both safe and effective. Several questions pertaining to the use of factor VIIa require further investigation, including the mechanism of action; the optimal dose; definitive indications; ultimate safety; and laboratory tests for monitoring therapy. In this report we describe our experince of using FVIIa treatment in inhibitor positive hemophilic hemorrhages and in Glanzmann thrombasthenia refractory to conventional therapy between 2002 and 2006. *Patients and Methods*. We used the rFVIIa 5 times in intraarticular, one time in retroperitoneal intramuscular, and two times in scapular intramuscular bleeding in our patient with haemophilia A with high inhibitor status. Patient admitted to our hospital with severe bleeding and schok in two of these musculer hemorhage. We administered rFVIIa for intrarticular bleeding; initiated with doses 90µg/kg, and the same dose was repeated every 3 hours for a total three times. rFVIIa was administered for severe bleeding and shock; initiated with doses 90 µg/kg, and the same dose was repeated every 3 hours for a total three times, followed by tappering doses several days according to clinical response. We also used rFVIIa replacement therapy to perform the surgery because of hip fracture, and surgical procedure achieved succesfully in our hospital for the same patient; initiatal rFVIIA dose was; 90 µg/kg just before surgery, then every 3 hours for 10-30 hours, then gradually prolonging the administration up to 6-hourly intervals untill the sutures removed. We also used the rFVIIa for bleeding control in a pregnant patient after delivery and in another patient with vaginal bleeding refractory to convantionel therapies with Glanzmann thrombasthenia. *Results*. Our patients treated successfully with rFVIIa in all bleeding episodes and in surgical procedure in a patient with inhibitor positive hemophilia A. We did not see increased bleeding during or after surgery when compared to our experience with non-inhibitor hemophilic patients. rFVIIa was also effective treatment after delivery period with Glanzman thrombastenia. *Conclusions*. Our experiences illustrates the usage of rFVIIa as an effective treatment modality in a haemophilia A patient with previous history of inhibitor in surgery and bleeding episodes and also in Glanzmann thrombasthenia refractory to conventional therapy.

1405

CHARACTERIZATION OF β THALASSEMIA MUTATIONS IN THE NORTHEASTERN PROVINCES OF EGYPT USING REVERSE DOT BLOT HYBRIDIZATION

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Background. Thalassemias represent a heterogeneous group of heritable hypochromic anemias of varying degrees of severity. Underlying genetic defects include total or partial deletions of globin chain genes and nucleotide substitutions, deletions or insertions. A gene frequency ranging between 0.013 and 0.02 was reported. This, coupled with the relatively high inbreeding in the Egyptian population, leads to an appreciable incidence of this disease in the Egyptian families. This is confirmed by the finding of 1st cousin consanguineous marriage in about 38% of Egyptian thalassemia families with an inbreeding coefficient double that of the general population. These factors have made thalassemia in Egypt not only a medical problem but also a socioeconomic one. *Aims*. We attempted to find the distribution of main thalassemia mutations among our locality (East and North part of the Nile Delta). *Methods*. Forty six children with β-thalassemia (26 male and 20 female) with mean age 7.8±4.6 years were included in this study. For these cases DNA extraction was done with further PCR amplification for the ,-globin gene and characterization of mutation by specific restriction enzyme analysis. Simple technique of hybridization of patient DNA samples with fixed probes on nylon membrane for detection of 8 common mutations was used. Informed consent was obtained from the parents. *Results*. Most genotypes of β-thalassemia of our cases were presenting with hepatic enlargement and mild to moderate splenomegaly especially with genotypes (IVS1:110/IVS1:110, IVS1:110/IVS1:6). The most frequent mutations were in order IVS1:110 (35.9%), CD39 (17.4%), IVS1:6 (13.04%) and IVS1:1 (11.96%). We have observed higher frequency of the mutation types IVS1:110, IVS1:1 and IVS1:6 and CD39 in the Eastern provinces while mutation types of IVS1:110 and CD39 were more frequent in the Northern provinces. It was found that mutation type IVS1:110 was the commonest mutation in Mansoura district (10.9%). As regard Damietta we found four mutations that IVS1:110, IVS2:745, IVS1:6 and IVS1:1 (1.1% of each). In Gharbia we found three common mutations that IVS1:110 (4.3%) and IVS1:6 and CD39 (2.2% of each). In Damnhour two mutations were found IVS1:110 and CD39 (1.1% of each). *Conclusions*. We recommend screening of other parts of Egypt especially Upper Egypt for detection of the distribution of β-thalassemia mutations to help establishing good prenatal diagnosis program.

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1406

DISTRIBUTION OF ABO BLOOD GROUPS IN CHILDHOOD ACUTE LEUKEMIA

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Background. ABO blood groups are associated with altered risk of a number of malignancies and diseases, e.g. gastric carcinoma (increased incidence in A blood group) and duodenal ulcer (increased incidence in O blood group). If the risk of hematological malignancies was also known for different ABO blood groups, it could serve as an epidemiological marker or a primary screening aid to identify high-risk populations. *Aims*. We opted to determine the distribution of ABO blood groups among a large number of children with ALL and AML in a multi-centric study and compare it with that in normal subjects. *Methods*. The blood group data were collected from the records of children with acute leukemia who were admitted to the hospitals and whose data were available in the hospitals registry. The data of ABO blood groups in the registration profile of 1000 Iranian municipal employees were considered as controls.The overall distribution of blood groups in ALL, AML and control groups were compared using the chi-square test, the 95% confidence intervals (CI) were calculated using binomial distribution probability, and Bonferroni's correction method was applied for subset analysis when the p values reached the cut off value of 0.05.There were four subsets, corresponding to the four ABO blood groups. Therefore, p values were multiplied by 4 when a statistically significant difference was observed in the overall ABO distribution in the initial analysis. *Results*.There were 682 (377 males and 305 females) ALL and 224 (133 males and 91 females) AML patients up to 12 years. The overall distribution of blood groups was significantly different in both ALL and AML groups from the control group ($p<0.001$). In the ALL group there were 56.5% (95% CI: 45.8-67.1) more patients with O blood group, 35.8% (95% CI: 27.0-44.5) less patients with A blood group, and 26.9% (95% CI: 12.7-39.2) less patients with B blood group. Distribution of blood groups was not significantly different between male and female ALL patients.In the AML group, there were 28.8% (95% CI: 10.8-46.9) more patients with A blood group.Again, males and females were not significantly different regarding ABO distribution. *Conclusion*.This study demonstrates that there is an increased proportion of O blood group and decreased proportions of A and B blood groups in ALL. It also shows that there is an increased proportion of A blood group in AML.The present study is the first one carried out in Iran and the one with the largest sample size of more than 900 patients with acute leukemia.Our results demonstrate an association between ABO blood groups and childhood

acute leukemia. However, we cannot say based on these results whether this association corresponds to a pre-leukemic event (i.e. certain blood groups as risk factors) or a consequence of malignancy *per se*. Using newborn cord blood for ABO blood group determination with re-determination of blood groups at intervals in a prospective cohort study is an idea for future research to evaluate the role of blood groups as risk factors of leukemia.

1407

ANTITHYMOCYTE GLOBULIN FOR GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS AFTER MISMATCHED UNRELATED PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKEMIA

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Background. Anti-thymocyte globulin (ATG) has been introduced in preventing acute graft-versus-host disease (AGvHD) in several studies. Many of them suggest that ATG reduces the risk of severe AGvHD but increases the risk of infections. *Aims*. We tried to investigate the role of ATG in HLA-mismatched unrelated hematopoietic stem cell transplantations (uHSCT), specifically in patients who received G-CSF mobilized peripheral blood stem cells (PBSCs) or from allele(s)/antigen mismatched unrelated donors. *Methods*. Thirty five patients with intermediate to unfavourable risk AML who received HLA-mismatched uHSCT from the available Asian as well as Caucasian donors were retrospectively reviewed. We compared 2 different groups according to the use of ATG (group 1) or not (group 2). The addition of ATG (thymoglobulin, Sangstat), at a dose of 1.25 mg per kilogram of body weight per day for 2 consecutive days, for recipients who received PBSCs from mismatched unrelated donors (group 1, N=10); this was added to prevent the development of AGvHD together with our standard regimen which consisted of methotrexate (10mg/m^2 intravenously bolus on day +1; and methotrexate 5 mg/m^2 intravenously bolus, on days +3, +6, +11) and tacrolimus starting at day -1. G-CSF was administered in all patients at a dose of 5 ug/kg subcutaneously per day from D+7 after transplantation until neutrophil recovery. The median age was 36 (range, 16-53) and the median follow-up duration was 21 months (range, 5-60). The majority of patients had intermediate or unfavourable cytogenetic features. The main conditioning regimen consisted in cyclophosphamide plus total body irradiation. *Results*. All transplanted patients were successfully engrafted. The overall incidence of AGvHD and chronic GvHD was 34% and 40%; 30% and 33%, 36% and 44% for patients with group 1 and group2, respectively. Four (11%) patients were relapsed so far. The comparison of estimated probability of disease-free survival rate at 2-year for each group was 100% vs 74%, respectively. When we considered the incidence of transplant-related mortality (TRM) between two groups, there was a significant difference of event-free survival rate, i.e. 86% vs 47%, respectively. The overall 2-year non-relapse TRM was 26%. *Conclusions*. These results demonstrate that the uHSCT performed with an appropriate dose of ATG, specifically either from mismatched multinational donors allografted with PBSCs, are associated with a favourable outcome.

1408

COAGULATION DISORDERS AND INHIBITORS OF COAGULATION IN CHILDREN: CASES FROM MANSOURA, EGYPT

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Background. Disorders of coagulation in children provide an interesting challenge to the medical care team. *Aim of the Study*. to assess spectrum and prevalence of coagulation disorders among children attending Mansoura University Children Hospital (MUCH), Mansoura, Egypt. *Subjects and Methods*. A total of 105 pediatrtc patients were referred to MUCH. They were divided into 2 groups: congenital coagulation disorders (75 cases, age 45.36±48.59 mo), and acquired coagulation disorders (30 cases, age 56.13±61.61 mo). All patients were subjected to thorough history taking including the nature of bleeding, family, past history, mode of inheritance, and detailed physical findings. Hemostatic tests include: Platelet count, Bleeding time(BT), Prothrombin time (PT), Activated partial thromboplastin time (APTT), Thrombin time (TT). Specific tests in the congenital group include assay of coagulation factors according to each disorder, Von Willebrand factor assay, Ristocetin aggregation test, APTT mixing study for detection of inhibitors in complicated hemophilia cases, F VIII C to VWAg ratio with cut off 0.7 for detection of carriers in some hemophilia A families. *Results*. Congenital disorders constituted 71.4% of the studied cases versus 28.6% for acquired disorders. Hemophilia A (42.85%), hemophilia B (14.28%) and liver diseases (14.28%) represented the majority of the studied cases. Mild and moderate cases of hemophilia A and B are more frequent than severe cases in both types. Male sex is more frequent than female in congenital group (94.7% v 5.3% $p<0.001$). Direct correlation existed between factor level assay and severity of hemophilia (r=0.73, $p=0.006$).Three mothers and one sister were identified as carrier out of 4 families. Anti- clotting factors inhibitors was detected in 18.2% of patients with hemophilia A and in 9.1% with hemophilia B. *Conclusions*. Hemophilias are the most prevalent congenital coagulation disorders among children. Attention must be given for detection of hemophilia carriers and inhibitors of clotting factors.

1409

THROMBOSIS COULD OCCUR AT ANY PHASE OF ESSENTIAL THROMBOCYTHEMIA WITH JAK2V617F MUTATION

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Background and aim. The JAK2V617F mutation is an activating kinase that is constitutively expressed in the vast majority of patients with polycythemia vera and in approximately half of those with essential thrombocythemia (ET). The association between JAK2V617F mutation in ET and thrombosis is still controversial. *Methods*. We used the database of JAK2 mutational status in 49 patients with ET in our institute. The JAK2V617F mutation was determined using by the semiquantitative sequence- specific primer-single molecule fluorescence detection (SSP-SMPD) assay. *Results*. ET patients with JAK2V617F showed a significantly higher incidence of thrombotic episodes (10/31 versus 1/18: $p=0.0308$), high leukocyte count ($p=0.0092$) and hemoglobin level ($p=0.0044$), when compared to those with wild-type JAK2. ET patients with thrombosis had higher hemoglobin (14.5+1.5 g/dL versus 13.3+1.5 g/dL: $p=0.0283$) at diagnosis, while no significant differences were noted in leukocyte count ($p=0.140$), hematocrit level ($p=0.0801$), or platelet count ($p=0.0877$). Of the ET patients with JAK2V617F mutation (n=31), no significant differences were noted in hematologic parameters regardless of whether they developed thrombosis or not. Among ET patients followed for at least 1 year, we found 11 patients who suffered thrombosis: 6 ET were found by thrombosis, and the remaining 5 had events during the stable phase of the disease. Hematologic parameters at the time of ET diagnosis of these two groups did not significantly differ, including leukocyte count ($p=0.2703$), hemoglobin concentration ($p=0.1306$), hematocrit level ($p=0.4472$), or platelet count ($p=0.9662$). *Conclusions*. Some ET patients will be initially detected due to thrombotic events. However, the remaining ET patients with JAK2V617F may develop thrombosis during hematologically stable condition, indicating that there is a risk of thrombosis in ET patients, especially those with JAK2V617F, at any phase of the disease.

1410

PROGNOSTIC VALUE OF IMMUNOGLOBULIN VARIABLE HEAVY CHAIN GENE MUTATION STATUS AND ITS RELEVANCE FOR RISK-ADAPTED THERAPEUTIC STRATEGIES: A LONG TERM FOLLOW-UP STUDIES

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Background. B-cell chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease, with many patients surviving for decades with minimal or no treatment, whereas others succumb rapidly to their disease despite therapy. Classical staging systems and laboratory features help predict survival in CLL, but they do not distinguish patients who will progress from those whose disease will remain indolent. Also, many years ago it was established that the prompt treatment of early stage of the disease has no benefit. This fact was based on series of CLL patients treated ineffectually with Chlorambucil, with out stratification according to the prognostic markers. *Aims*. In recent years, new molecular prognostic factors have emerged that have significantly improved prediction of the risk for disease progression. The mutational status of the immunoglobulin variable heavy chain genes (VH) is one of the major molecular prognostic factors and the aim of our study was to test its relevance as a prognostic marker for indicating treatment in early stage CLL patients. *Methods*. In our study we evaluated the association between the immunoglobulin VH gene mutation status and the clinical characteristics and outcome in 108 CLL patients that had been followed for a considerably long period at our institution. *Results*. At diagnosis,

patients with unmutated VH genes had higher median lymphocyte counts (p=0.001), higher total tumor mass score (p=0.001) and more often presented at an advance clinical stage (p=0.005) compared to patients utilizing mutated VH genes. Treatment was initiated for symptomatic or progressive disease CLL cases. Patients with early stage and stable disease were not treated. First line therapy consisted of Chlorambucil in 73 patients (67.59) and Fludarabine in 16 patients (14.81%). Early stage unmutaed cases had very short median time to progression (11 months). Also, unmutated cases had worse response to treatment (p=0.005) compared with mutated CLL patients, regardless of the treatment. Moreover, the median survival of patients with unmutated VH genes was considerably shorter (VH unmutated, 56 months, VH mutated, 125 months; p< 0.001). *Summary/Conclusions*. Our data confirmed the prognostic value of immunoglobulin VH genes mutational status in CLL, which divides the disease in two prognostic subsets in terms of overall survival and clinical characteristics of the disease. Results from the treatment of unmutated cases indicate that they could have increased treatment benefit if they are treated earlier with more intensive first line therapy. We consider that analysis of the mutational status of the immunoglobulin VH genes may allow for an individualized approach to CLL treatment in the near future and we suggest clinical trials of more effective treatment stratified by this single prognostic marker to be designed.

1411

CLINICAL ASSESSMENT OF THE SENSITIVITY OF LEUKEMIC CELLS TO CHEMOTHERAPY IN PROGNOSIS OF 7 YEARS DFS SURVIVAL IN CHILDHOOD ALL

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Background. The determining *in vitro* drug resistance may reveal clinically relevant information for prognosis the efficacy of chemotherapy in childhood leukemia. *Aims*. of this study was to evaluate the correlation between blast cells sensitivity profile to chemotherapy *ex vivo* (according to range scale) and the probabilities of 7-year disease-free survival. *Methods*. The blast cells sensitivity to chemotherapy (10 drugs) was examined in MTT-assay. The material of the study-bone marrow from 169 children with newly diagnosed ALL. *Results*. For each drug (Prednisolone, Dexamethasone, Vincristine, L-Asparaginase - PDVA) LC50 was determined. The degree of cytotoxity was estimated in ranks scale: each step of a drug dilution (from greatest to lowest) was corresponded to rank (from 1 up to 7). The high sensitivity to a drug - LC50 value from 1 to 4 ranks; low sensitivity - LC50 value from 5 to 7 ranks. The present data on analyze correlations between these potential prognostic factors and well-known favourable and unfavourable markers of prognostic in ALL. The probability of 7-year disease-free survival was significantly higher in patients with high sensitivity (1-4 ranks) to Prednisolone (p=0.036), Dexamethasone (p=0.043), Vincristine (p=0.041), L-Asparaginase (p=0.042), in comparison with the patients with low sensitivity (5-7 ranks). The probability of 7-year disease-free survival in patients with high simultaneous sensitivity to 3 of 4 drugs (PDVA) was 0.88 versus 0.62 in patients with low simultaneous sensitivity to three of these drugs (p=0.037). *Conclusions*. the initial sensitivity of leukemic cells to Prednisolone, Dexamethasone, Vincristine, and L-Asparaginase is very significant in prognosis of 7 - years disease-free survival in pediatric ALL. High simultaneous sensitivity to at least 3 of 4 above mentioned drugs appears to be good prognostic factor.

1412

DONOR TRANSMITTED LYMPHOMA MALIGNUM IN A KIDNEY-TRANSPLANT RECIPIENT

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Introduction. Transmission of cancer from organ donors is considered as a fatal risk following solid organ transplantation. The Danish Cancer Registry (2002) quantified the risk in a population-risk register of 1.3% for having a donor with an undetected malignancy and 0.2% risk of getting a transmitted cancer. In the most recent commentary from the UNOS/OPTN a cancer transmission rate of 0.018% was identified utilizing a cadaveric donor source (2005). To the best of our knowledge, no example exists of a acute lymphoblastic leukemia (ALL) T-cell accidentally transplanted to recipient of renal from donor with the malignant lymphoma, lymphoblastic T-cell. *Case report*. A 19-year old donor suffered from deep cerebral anoxia due to a choked with tablet, without any history of malignant. His family consented for multiorgan transplantation. On June 2005, at the time of organ retrieval no abnormalities were detected. After transplantation autopsy was performed, this demonstrated the limited infiltration of the mediastinal tumor arising in thymus. The diagnosis of lymphoma type lymphoblastic of T cell origin was made. A recipient of first kidney, 59 year old woman with a history of end stage renal disease secondary to glomerulonephritis, having been informed of the donor autopsy findings, decided to retain her allograft. Immunosupresion consisted of tacrolimus, mycophenolate mofetil and prednisolone. At the first, third and 5-month assessments she was asymptomatic and renal function remained good. Ultrasound scans of the graft were reported as normal. On december 2005, 6 month after transplantation she developed general malaise. It was noted a mild leukopenia and thrombocytopenia. A bone marrow specimen revealed massive infiltration of lymphoblasts. The immunophenotyping analysis showed the same antigenic profile and immunoglobulin monotypic restriction of the abnormal T cells, both in the thymus of the donor and the bone marrow of the recipient. FISH studies for sex chromosomes were performed to evaluate the origin of the blastic cells. The gene rearrangement studies confirmed the clonal origin of the blasts in the recipient. These findings lead us to speculate that the clonal origin of the tumor was the same in the both donor and recipient. At nearly the same time after transplantation, the identical type of tumor were observed in the other centers, in the second renal and liver recipients with grafts from same cadaveric donor (data unpublished). It is probable that lesions in the grafts were metastases that developed in the recipients over several months. This malignant clone was more aggressive and became at a disseminated stage, partly due to the effects of immunosupression. In conclusion, organs transplant recipiens shouls be carefully followed up for malignancy, despite the absolute risk of cancer transmission with solid organ transplantation is low but real.

1413

ACTIVATION OF AKT PREDICTS POOR OUTCOME IN NEUROBLASTOMA

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While aberrant activation of the PI3K/Akt pathway, a key survival cascade, has previously been linked to poor prognosis in several human malignancies, its prognostic impact in neuroblastoma has not yet been explored. We therefore investigated the phosphorylation status of Akt, S6 ribosomal protein as target of mTOR and ERK in 116 primary neuroblastoma samples by tissue microarray and its correlation with established prognostic markers and survival outcome. Here, we provide for the first time evidence that phosphorylation of Akt at serine 473 (S473) and/or threonine 308 (T308), S6 ribosomal protein and ERK frequently occurs in primary neuroblastoma. Importantly, we identified Akt activation as a novel prognostic indicator of decreased event-free or overall survival in neuroblastoma, whereas phosphorylation of S6 ribosomal protein or ERK had no prognostic impact. Also, Akt activation correlated with parameters of aggressive disease, including MYCN amplification, 1p36 aberrations, advanced disease stage, age at diagnosis and unfavorable histology. Monitoring Akt at T308 or both phosphorylation sites improved the prognostic significance of Akt activation in neuroblastoma specimens compared to S473 phosphorylation. Parallel experiments in neuroblastoma cell lines revealed that activation of Akt by IGF-1 significantly inhibited TRAIL- or chemotherapy-induced apoptosis in a PI3K-dependent manner, since the PI3K inhibitor LY294002 completely reversed the IGF-1-mediated protection of neuroblastoma cells from apoptosis. By demonstrating that activation of Akt correlates with poor prognosis in primary neuroblastoma *in vivo* and with apoptosis resistance *in vitro*, our findings indicate that Akt presents a clinically relevant target in neuroblastoma that warrants further investigation.

1414

SPONTANEOUS SPINAL EPIDURAL HEMATOMA IN POLYCYTHEMIA VERA. REPORT OF A CASE

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Background. Bleeding manifestations in polycythemia vera (PV) are commonly observed during therapy of anticoagulants or antiplatelet

agents. They involve primarily the skin and mucous membranes. Gastrointestinal haemorrhage is observed less frequently. Although reported, intraarticular, retroperitoneal, central nervous system and deep intramuscular hematomas are unusual. We report the case of a patient diagnosed of PV with spontaneous spinal epidural hematoma. *Case*. A 73 years old woman diagnosed three years ago of PV was admitted to the hospital due to back pain for the last 24 hours and weakness and plegia of her left leg. The magnetic resonance (MR) revealed the presence of a spinal epidural hematoma that compressed spinal cord from T11-L1 on its left side. She underwent descompressive surgical treatment (laminectomy) without complications. The patient didn´t refer aspirin, anticoagulants or similar agents intake and/or prior trauma; the last phlebotomy was performed 3 months ago. Tests: Hg 14.8 g/dL, VCM 66.3 fL, Leucocytes 23.3×10^3/µL, Platelets 138×10^3/µL, APTT 30.7 sec (30-41), Prothrombin time 13.04 sec (10-14), Fibrinogen 3.0 g/L (1.4-4.0), PFA-100®: col/ADP 121 sec (71-118), col/Epi > 300 sec (85-165), FvWAg 183%, FvWRCo 150.1%, FVIII:C 160.6%. Platelet optical aggregometry: decreased primary and secondary aggregation to ADP and epinephrine, decreased response to collagen. Flow cytometry: Normal expression of GpIb; p-selectin expression (without ADP): 64,67% (normal controls: 4-18.4%), p-selectin expression (with ADP) 82.2% (normal controls: 11.3-57.4%) showing platelet hyperreactivity. 36 hours after surgery she presented acute paraplegia due to hematoma in the surgical area from skin to spinal cord, surgical evacuation was required. Platelet transfusion every 2 days was administered for a week until no evidence of bleeding complications was observed. Our patient is now under spinal cord injury rehabilitation program. *Conclusions*. 1) Even without anticoagulants or antiplatelet agents intake and with normal platelet counts, patients with chronic myeloproliferative disease (CMPD) may develop severe bleeding. 2) Co-existence of platelet hyperreactivity and changes in platelet aggregation patterns has been described in CMPD in the same patient, as observed in this case. 3) Neverthless there is no specific assay to predict hemorrhagic diathesis in CMPD patients.

1415

NEW RESTRICTIVE PROPHYLACTIC PLATELETS TRANSFUSION THRESHOLD IN CANCER PATIENTS WITH STEM CELLS TRANSPLANTATION

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Background. Prophylactic platelets transfusion are usually administered to patients receiving stem cell transplantation (SCT) when their platelets count falls below 10×10^9/L. Some observations suggest that lower platelets count can be used in patients with stable condition but the safety of lower threshold is uncertain. *Aims*. To compare the risk of bleeding complications and the number of platelet and red cell transfusion administered using two different prophylactic platelets transfusion protocols. *Methods*. We evaluated 30 patients with hematological malignancies and solid tumors who received autologous and allogenic stem cells transplantation. 12 patients have received prophylactic platelets transfusion when their platelets count decreased below 10×10^9 µ/L and 18 patients received at threshold below 5×10^9/L. *Results*. Decreasing the threshold of prophylactic platelet transfusion from 10×10^9 /L to 5×10^9µ/L resulted in reduction of platelet transfusion units from (10.4±6.1 unit/patient) to (1.3±4.1 unit/patient) during the period of bone marrow aplasia (95 percent confidence interval for mean was 6.5to14.3 and 0.9 to 2.7respectively) and decreased the number of patients received platelets transfusion from 100% to 11.1%(p=0.001). The incidence of bleeding was statistically non significant in comparison of patients with threshold 10×10^9/L(10%) with patients with threshold 5×10^9 (11.1%) (p=0.125). Logistic regression analysis of complications in both groups with other variables (age above and below 40 years, sex, duration from diagnosis till bone marrow transplantation, platelet refractoriness, days of thrombocytopenia, platelets count in nadir, and number of platelets units transfused) showed only significant regression with both, platelet refractoriness (p= 0.0117) and odds ratio (97.8)and with the number of transfused platelet units (p= 0.022) and odds ratio (1.3) *Summary/Conclusion*. Prophylactic platelets transfusion threshold can be decreased safely from 10×10^9/L to 5×10^9/L in patients undergoing SCT with stable condition, normal organs functions and intact haemostatic system, which makes further clinical studies are required to answer the question of whether there is even a need for prophylactic platelet transfusion or whether platelet transfusion should be administered only when active bleeding is documented.

1416

BLOODSTREAM INFECTIONS IN ADULT PATIENTS WITH MALIGNANT BLOOD DISORDERS AND NEUTROPENIA: MICROBIAL SPECTRUM AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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Background. Bloodstream infections are a major cause of morbility and mortality among patients with haematological disorder. *Aim*. The aim of the present study was the bacterial spectrum and antimicrobial susceptibility pattern of organisms causing bloodstream infections among patients hospitalized in a hematology center. *Methods*. A total of 146 episodes of bacteraemia and fungaemia in 109 patients were identified. All patients were treated in the haematology ward during a 7-year period (2000-2006) and had an underlying haematological disorder (lymphoma, leukaemia and myeloma). There were 73 male and 36 female patients, aged 17-80 years old. *Results*. A total of 164 microbial strains were isolated in 146 episodes. Forty-seven (47.6%) isolates were Gram-negative bacteria, 48.1% Gram-positive bacteria and 4.3% yeasts. Polymicrobial bacteraemia was observed in 17 from 146 (11.6%) episodes. The dominated pathogens were coagulase-negative Staphylococci (CNS) 26.2%, Pseudomonas aeruginosa 18.3%, E. coli 14%, Enterococcus spp. 7.9% and S. aureus 5.5%. The mortality rate was 19.3% (21 patients) being higher in patients with bacteraemia caused by P. aeruginosa, CNS and Klebsiella spp. Oxacillin resistance was detected in 3/9 (33.3%) of S. aureus isolates and in 29/43 (67.5%) of CNS. Vancomycin resistance was detected only in two strains Enterococcus spp. (15.4%). Four strains E. coli produced ESBL (17.4%). Among P. aeruginosa isolates, 16 were resistant to colistin (53.3%) and 22 to carbapenems, fluorquinolones and ceftazidime (73.3%). *Conclusions*. The identification of the microbiology profile of bloodstream infections and antimicrobial susceptibility pattern of isolated strains may help in managing these infections in haematology patients, and reviewing infection control and antibiotic policies.

1417

PRIMARY LYMPHOMA OF THE LIVER: CLINICAL FEATURES AND OUTCOME OF 10 PATIENTS

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Background. Primary liver lymphoma (PLL) is a rare lympho-proliferative disorder, presenting in less than 1% of all extranodal lymphomas. The etiology of PLL is unknown and the prognosis is dismal. Early stage, localized PLL generally does better than that with advanced stage. Lymph node and bone marrow involvement are considered as poor prognostic factors. *Aims*. A retrospective study to review our departmental experience with PLL. *Methods*. From 1985-2001, 10 patients who fulfilled the diagnostic criteria for PLL were treated at our hospital. All patients underwent a thorough work-up and were staged accordingly. *Results*. There were six males and four females [age range, 45-81 years; mean 63 years]. Main presenting symptoms were abdominal pain (localized to the right upper quadrant with/without radiating character), weakness, loss of appetite and weight. No patient presented with jaundice. Only three patients exhibited clear *B* symptoms. Three patients presented with hepatomegaly. Liver functions tests were elevated in all patients and LDH in two patients. Imaging (CT, US, Gallium scan) exhibited typical, unspecific findings, such as solitary or multiple lesions, filling defects, signs of hepatocellular damage and pathological uptake. Five patients had stage I/II and five had stage IV disease. A total of 80% exhibited diffuse, large, B-cell lymphoma. The rest had immunoblastic, mixed or anaplastic (high grade) lymphomas (all B-cell type). Eight patients were treated with conventional CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) and two were treated with a high-dose CHOP regimen; all achieved good partial remission or complete remission for a mean of six months. Relapses occurred at multiple sites. Relapsing patients were treated with DVIP (cisplatin, etoposide (VP-16), ifosfamide, dexamethasone) with a good partial remission for about three months. One patient died of a massive stroke after eight months of complete remission with no evidence of disease. Another patient developed massive recurrent disease following 35 months of sustained complete remission. *Conclusions*. PLL should be considered in the differential diagnosis of any patient with non-specific solitary or multiple liv-

er lesions. PLL is fatal but relatively chemotherapy-sensitive. The introduction of more intensive chemotherapy plus/minus localized radiation might achieve long-term complete remission with better survival rates. The role of consolidation radiation therapy following local control should be evaluated.

1418

ANGIOGENESIS MARKERS AND JAK2 MUTATION IN PATIENTS WITH POLYCYTHAEMIA VERA AND ESSENTIAL THROMBOCYTHAEMIA

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The role of the acquired mutation of the tyrosine kinase JAK2 (V617F) in human myeloproliferative disorders is under investigation. There is evidence that the mutation arises as a secondary genetic event and we now know that has got a key role in signal transduction from multiple haemopoietic growth factor receptors. The mutation is noted in more than half of patients with myeloproliferative disorders (MPDs), and in 95% approximately of patients suffering from Polycythaemia Vera (PV). Recent studies have determined that the JAK/STAT pathway is involved in mediating processes related to angiogenesis. VEGF whose receptor is a receptor tyrosine kinase, has surprisingly been shown to activate specific STATs. We tried to investigate the role of the existence of the mutation JAK 2 (V617F) mutation in MPDs and its relation with angiogenesis markers in 17 patients with MPDs (5 with Polycythemia Vera and 12 with Essential Thrombocythaemia, ET). The angiogenesis process in all patients have been examined by the microvascular density (MVD) count per 400x high power field (HPF)using light microscopy of bone marrow trephines immunostained with anti-CD34 monoclonal antibody and with the estimation of angiogenesis markers in serum by enzyme immunoassays. The angiogenic factors estimated were VEGF (vascular endothelial growth factor), Angiogenin and MMP-9 (matrix metalloproteinase 9). The patients enrolled were divided in two groups according to the presence or not of the mutation JAK2 (V617F). In our group of patients the mutation was found in three patients with PV and in six patients with ET while it was not found in two patients with PV and in six patients with ET. We found no differences in MVD, VEGF, Angiogenin and MMP-9 levels among carriers of the mutation and carriers of the wild type gene. More specifically, MVD count was found 9,1 ±7 vs 7,9±4,2 vessels per HPF, among V617F carriers and the wild type carriers respectively (p=0,74). VEGF serum levels were found in the subgroup of V617F carriers 289,9±515,5 vs 278,7±254 pg/mL (p=0,28). Angiogenin levels were found as 400,3±83,1 vs 326,2±102,2 pg/mL (p=0,56) among (V617F) carriers and wild type carriers respectively. Finally MMP-9 levels were found 420,4±364,9 vs 601,2±244,6 pg/mL, (p=0,28) among (V617F) carriers and wild type carriers respectively. Our data although preliminary give evidences that the presence of the presence of the JAK2 (V617F) mutation is not probably involved in angiogenesis procedure in MPDs. Of course the role and the aetiology of increased angiogenesis in MPDs is under investigation as well as the novel molecular marker, the JAK 2 (V617F).

1419

MDM2 PROTEIN EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN RELATION TO INITIAL WHITE BLOOD COUNT

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Background. White blood count (WBC) at presentation is the one of most significant prognostic factor in children with acute lymphoblastic leukemia (ALL). Elevation of initial WBC over 50000/μL, seen in over 20% of children with ALL is uniformly associated with poor prognosis. High WBC may result from ineffective spontaneous apoptosis which does not counterbalance increased proliferation of leukemic cells. Both proliferation and apoptosis are controlled by P53 gene. MDM2 protein is a known endogenous inhibitor of p53 protein. This study was undertaken to assess in a clinical setting the hypothesis that overexpression of MDM2 protein in leukemic cells of children with ALL may be associated with high initial WBC. It was comprised of 35 children (15 girls, 20 boys, aged 6-192 months, median 95 months) with *de novo* ALL. All children were treated according to BFM ALL 90 protocol. The follow up time was 1-42 months (median 21 months). *Methods*. Peripheral blood mononuclear cells were collected prior to and after 6 and 12 hours from prednisone administration. Cytospin preparations of these cells were stained with mouse monoclonal anti-MDM2 antibodies (DakoCytomation) followed by goat anti-mouse antibodies conjugated with APC (Molecular Probes) and mouse monoclonal anti-p53 antibodies conjugated with FITC (DakoCytomation) respectively. Nuclear DNA was stained with propidium iodide (PI). MDM2 - associated long red fluorescence and p53-associated green fluorescence and were measured by laser scanning cytometer (LSC, CompuCyte, USA). In order to assess rates of apoptotic cells respective slides were stained with polyclonal rabbit anti-PARP p85 fragment followed by FITC conjugated swine anti-rabbit antibody. Cell expressing p89 fragment of PARP were considered apoptotic. Red fluorescence of DNA-bound PI was used as a contouring parameter. Values of long red integrated fluorescence and green integrated fluorescence were recorded as .FCS 3.0 files by WinCyte 3.4 software. *Results*. The mean pretreatment values of MDM2 expression were lower in the group of patients with initial WBC below 50000/μL. (p=0,056). After 12 hours from prednisone administration these patients had significantly higher mean p53 expression values (p=0,047) as well as significantly higher rates of apoptotic cells (p=0,03). They also did better with p-EFS significantly higher than for those with initial WBC over 50000/μL (0,961 vs 0,667; p=0,018). *Conclusions*. These data seem to indicate that preatreatment overexpression of MDM2 protein may contribute to high initial WBC in children with ALL. It may also inhibit apoptosis in response to prednisone treatment, thus influencing the outcome in these patients.

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COMBINED SYSTEMIC AND LOCAL TREATMENT OF DISSEMINATED BRAIN ASPERGILLOSIS IN A 14-YEAR-OLD GIRL WITH APLASTIC ANEMIA AFTER MUD HSCT

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In spite of advances in diagnosis and treatment, invasive aspergillosis in immunocompromised patients still remains a problem in clinical practice. The worst prognosis is associated with central nervous system aspergillosis with a mortality rate exciding 90%. This high rate of treatment failure results from limited penetration of most of the antifungal agents through the blood brain barrier. We report on a case of a 14-year-old girl with congenital aplastic anemia, who at the age of 12 underwent hematopoietic stem cell transplantation from a matched unrelated donor. She achieved hematologic reconstitution, however she developed chronic graft versus host disease treated with glucocorticoids and cyclosporin A. The post-transplant period was complicated by pulmonary aspergillosis which was succesfully treated with liposomal amphotericin B. Twelve months later she developed sudden loss of consciousness, seizures, diplopia and left-sided hemiparesis. A brain MRI revealed several disseminated lesions with the two biggest lesions located in the parietal and frontal lobes (Figure 1).

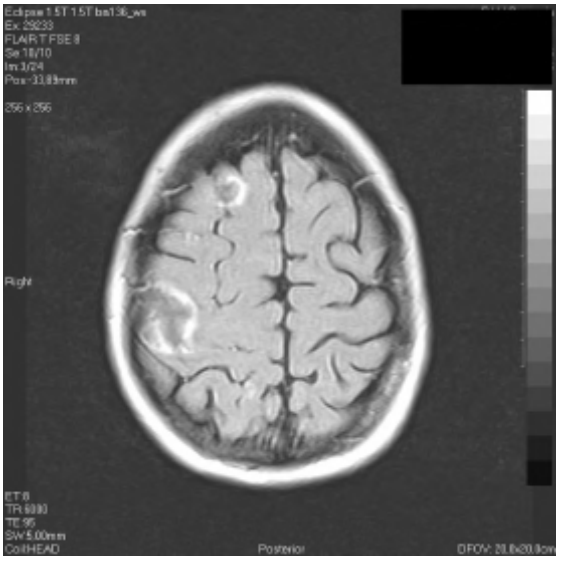

Figure 1.

A lung CT scan revealed the reactivation of pulmonary aspergillosis. A galactomannat test was positive. The patient was given intravenous

voriconazole for 2 months followed by oral administration of the drug. This resulted in the resolution of pulmonary and small brain lesions however those located in the right temporal lobe remained almost unchanged. Intrathecal amphotericin B was introduced however this method of the drug's administration was not tolerated. Two Rickham reservoirs were inserted in the right lateral ventricle and the biggest abscess in the right frontal lobe, thus allowing evacuation of serous masses from the lesions as well as direct administration of amfotericin B. A month later neurosurgical resection of the two largest abscesses was performed followed by voriconazole and amphotericin B systemic treatment. Ten months after diagnosis the girl is well with slight neurologic deficits and discrete brain lesions seen on MRI scan.

1421

NEW INSIGHT INTO THE PATHOGENESIS OF MULTIPLE MYELOMA: CHROMOSOME 11 ABERRATION, P18 DELETION AND CYCLIN D1 EXPRESSION AND THEIR CLINICAL RELEVANCE

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Background and hypothesis. Multiple myeloma is a currently incurable malignancy of terminally differentiated plasma cells with a median survival of two to three years. In recent years much has been learnt regarding the biology of the myeloma clone; especially on the chromosomal alterations that can be more frequently found and on the involved oncogenes, in particular those regulating the cell cycle. *Aims*. Therefore the frequency and the clinical and prognostic implications of chromosome 11 aberration, cyclin D1 expression and p18 deletion were examined. *Methods*. The study included 22 patients with *de novo* multiple myeloma. Fluorescence *in situ* hybridization was used to examine t(11;14)(q13;q32) and chromosome 11 polysomy. P18 deletion was tested using PCR and cyclin D1 expression was evaluated by immunoblotting. *Results*. Chromosome 11 aberrations were identified in 36.3% of patients (27.2% t(11;14) and 9.1% 11 polysomy). Cyclin D1 protein was overexpressed in 59.9% of cases. There was a significant link between t(11;14) and cyclin D1 expression. The two cases having 11 polysomy demonstrated cyclin D 1 overexpression. There was no significant correlation between any of the three aberrations and the selected clinical and laboratory data. The only significant association was observed between t(11;14) and serum β2-microglobulin. In Kaplan-meier analysis, t(11;14), 11 polysomy and cyclin D1 overexpression were significantly unfavorable parameters as regard to overall survival. The cell cycle inhibitor p18 demonstrated deletion of exon 1 in 22.7% of patients. No significant difference was observed regarding clinical, laboratory and overall survival between p18 deletion positive and negative patients, unless the deletion is associated with other aberration. *Conclusions*. Cyclin D1 overexpression is a common event in MM patients and is associated with t(11;14) and extra copies of chromosome 11. The three aberrations could be considered as unfavorable parameters for MM. The low frequency and lack of prognostic significance of p18 deletion in MM in this study indicate the limited role of p18 *per se* in the pathogenesis of the disease, however its deletion could facilitate the effect of cyclin D1 overexpression in making cells progress through the cell cycle rapidly. Further studies are required to determine the role of inhibiting cyclin D1 expression and restoration of p18 as interesting targets for new therapeutic strategies

1422

PLASMABLASTIC LYMPHOMAS OF THE ORAL CAVITY IN PATIENTS WITH THE HUMAN INMMUNODEFICIENCY VIRUS INFECTION

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Non Hodgkin Lymphomas associated with the Human Immunodeficiency virus infection (HIV) are heterogeneous in clinic and pathology characteristics. Plasmablastic lymphoma (PL) is a recently described entity from B cell lineage, with affinity for extranodal localization in oral cavity. Immunohistochemical studies show lack of more common lineage B antigens, expression of markers associated to plasma cells, like VS38c, CD138 and in a variable shape light chains of cytoplasmatic immunoglobulins and cytokeratine. The usual presentation is a tumor arising from the gingiva, palate or floor of the mouth, rapid growth, mobility or loss of teeth, irruption in nearby structures causing edema and facial deformity. A different presentation was descripted, with nodal and splenic involvement, HIV negative and association with multicentric Castleman disease and HHV8. We present a series of 3 cases attended in the Hematology and Stomatology Departaments, Oncology Hospital Marie Curie. *Discussions*. The knowledge of this entity will allow the differential diagnosis with other lymphomas, plasmocytomas and large cell tumors. Nevertheless HIV negative cases were described, the association with HIV infection is relevant, and lead to unknown diagnosis.

Table 1. Characteristics and immunochemistry.

CHARACTERISTICS												
Patient	sextage	risk group	diagnosis HIV	treatment HIV	primary tumor	stage at diagnosis	CD4	CD4/CD8				
VD	F29	no	6 years ago	yes	gingiva,upper jaw	IAE	180	0,25				
HF	M50	homosexual	5 years ago	irregular	gingiva, lower jaw	IAE	21	0,06				
ED	M31	no	unknown	no	gingiva and palate upper jaw	IAE						
IMMUNOHISTOCHEMISTRY												
	CD45	CD20	CD3	CD56	KERATINA	KAPPA	LAMBDA	EMA	CD138	S-100	HMB45	VIMENTINA
VD	positive	neg	neg	neg	neg	positive	neg					
HF	neg	neg	neg		neg			positive	positive	neg	neg	
ED	neg	neg	neg	focal +	neg	neg	positive	positive		neg		neg

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THE ASSOCIATION BETWEEN THE METABOLIC SYNDROME AND THE NON-HODGKIN LYMPHOMA

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Background. Some studies suggest that overweight and obesity are risk factors for several malignancies. *Aim*. Our study was examined to determine whether the Metabolic Syndrome (which includes obesity) is associated with non-Hodgkin's lymphoma development. *Methods*. We have studied a group of 216 patients which were hospitalized in the Medical Departments of the County Clinical Hospital from Sibiu, Romania, during 15th of September and 30th of October 2006. The 216 patients were divided into two groups: group A - 57 patients with non-Hodgkin's lymphoma and group B - 159 patients-control group. We have studied the next parameters: age, gender, BMI, the abdominal circumference, the seric cholesterol and triglycerides level, the levels of the liver enzymes, the hemoglobin and hematocrite level, the presence or the absence of the diabetes mellitus and essential arterial hypertension. We have establish the prevalence of the metabolic syndrome in each group, using the metabolic syndrome's definition which was adopted by the International Federation of Diabetes. The results were statistically analyzed using the relative risk and the t Student test. *Results*. The average age of the study population was 63.15 years. A medical diagnosis of diabetes was reported by 27.5% of the patients from group A and 26% of thoses from group B. A medical diagnosis of essential arterial hypertension was reported at 46.8% of the controls and at 50.8% of those with non-Hodgkin lymphoma. The laboratory results showed that 35% from the patients with non-Hodgkin lymphoma had high triglycerides level, and 34.7% from the control group had hypertriglyceridemia. From the total of 57 patients with non-Hodgkin lymphoma, 20 (35%) were categorized as normal weight (BMI 18-25 kg/m^2), 21 (37%) as overweight (BMI>25-30 kg/m^2), and 16 (28%) as obese (BMI>30 kg/m^2). The relative risk of developing non-Hodgkin lymphoma at the overweight patients was 1.353, at the hypertensive patients was 1.058, at the diabetic patients was 1.0015 and at the dyslipidemic patients was 1.066. 122 patients from the whole group presented metabolic syndrome. From all these, 42 had the non-Hodgkin lymphoma diagnosis. The relative risk of the appearance of these diseases at the patients with metabolic syndrome was 2.16. The relative risk of developing non-Hodgkin lymphoma at the patients with 4 components of the metabolic syndrome comparing with those without metabolic syndrome was 2.937. *Summary/Conclusions*. At the patients we have studied, not only obesity, but every component of the metabolic syndrome too, is associating with a higher risk of non-Hodgkin lym-

phoma. The patients with metabolic syndrome have a twice higher risk to develop non-Hodgkin lymphoma. The risk increases with the number of components of the metabolic syndrome they present.

1424

THE CURRENT OPINION OF THE PATIENTS FROM SOUTHERN TRANSYLVANIA REGARDING THE BLOOD TRANSFUSION

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Background. Together with Romania's attendance to the European Union, it is stipulated that blood transfusion should become honorific and this can have implications on the act of transfusion. *Aim*. We established this study in order to see the patients' attitude concerning the blood donation and the blood transfusion. *Methods*. We have performed a transversal study in which we have included all the 112 patients from the medical departments of the Clinical County Hospital from Sibiu during 19-23.02.2007, who have agreed to answer at a questionnaire with 10 questions regarding the blood transfusion. Each question had 3 variants of answer. We have analyzed the rates of the answers, their significations and we have drawn useful conclusions for medical practice. *Results*. Even though the majority of the questioned patients (96%) agree that a patient can be transfused if this act can save his life or ameliorate his evolution, 66% of them did not donate blood ever. 97% would donate blood for a family member, but 11% would not donate for an unknown person. 38% from them consider that the blood donation must be compensated with money. Most of them (92%) agree that all the patients who need transfusions to get them, not only those who do not have cancer. 39% of the questioned people do not know if the blood transfusion can prolong the life of a patient with acute leukemia. A part of them (16%) do not know if viruses or bacteria can be transmitted through a blood transfusion; 37% do not know and 24% are not sure which are the risks of a blood transfusion. 34% from the questioned patients declare that they would not accept the blood transfusion from an anonymous donator, knowing that it can be risky, 56% would accept it and 10% do not know. *Conclusions*. The majority of the questioned patients agree that all those who need blood to be transfused. Only a third of them donated blood at least once. More then a third would still like that the blood donation to be compensated with money. Many of them are insufficiently informed about the risks and benefits of the blood transfusion, this thing been well reflected in their attitude on receiving blood from unknown donators and donating blood to unknown people.

1425

AUTOTRANSPLANT AFTER PURGING *IN VIVO* IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASE A POOR PROGNOSYS

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Autologous stem cell transplantation is a efficacy therapy for limphoproliferative 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 December 2006 we have treated with Autologous stem cell transplantation, purged *in vivo* with monoclonal antibodies, 20 patients (5 F; M 15 median age: 57 years) with limphoproliferative diseases to poor prognosis (3 Burkitt lymphoma; 4 mantle cells; 3 CLL; 1 NHL- peripheral T cells; 2 follicular and 7 large cells) and we have evaluated the results and the feasibility. In all patients, the purged *in vivo*, has been effected administering a dose of monoclonal antibodies (anti CD20 in B-NHL and anti CD52 in CLL and T-NHL) before the harvest and after the infusion of the stem-cells. To the transplantation 6 patients were in CR (3 Burkitt lymphoma; 2 large cells and 1 mantle cells) 10 in PR (1 CLL; 3 mantle cells; 2 follicular and 4 large cells lymphoma) and 4 in resistant disease (2 CLL ; 1 large cells and 1 NHL peripheral T cells) All patients have harvest (median CD34:4 $\times 10^6$/Kg) and median minimal residual disease in the harvest has been < to 2%. All the patients have been conditioned with BEAM and the graft are documented in 18/20 patients (2 patient is dead to the day +4 and +10 for gastric haemorrhage and septic shock respectively) with neutrophils> 1000 in media to day + 14 (range 10-19 days). After transplantation 17/18 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 8 months after transplantation (range 2-47) 14/17 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 +13 months respectively. Two patients (1 large cells and 1 CLL) are died at months +3 and + 7 for CMV reactivation and interstitial pneumonia respectively. The DFS and EFS projected at 47 months are of the 75% and 70% respectively. 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 much 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 limphoproliferative disease.

1426

PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE SPLEEN

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Background. Two-third patients of primary diffuse large B-cell lymphomas of the spleen had unfavorable prognostic factors by International Prognostic Index (IPI). The optimal treatment remains controversial *Aims*. efficacy of induction chemotherapy standard CHOP regimens and intensified regimens by NHL-BFM-90 protocol in adult patients of primary Diffuse Large B-Cell Lymphomas of the spleen plus involved-field radiotherapy after spleenectomy are to be clearly defined. *Methods*. We have our own experience of spleenectomy, chemotherapy and plus involved-field radiotherapy of 14 patients (7 men and 7 women from 42 to 73 years; means 60 years) with primary diffuse large B-cell lymphomas of the spleen. All patients were treated between1996 and 2006 years in our center. There were 9 patients in I group (treated by spleenectomy, chemotherapy CHOP plus involved-field radiotherapy) and 5 patients in group II (treated by spleenectomy, intensified chemotherapy modified NHL- BFM-90 regimen and plus involved-field radiotherapy). 7 patients of I group were older than 60 years and had 3 unfavorable prognostic factors by IPI. All patients of II group were younger than 60 years and also had 3 unfavorable prognostic factors by IPI. *Results*. in group I there were 9 complete remissions at median follow-up 54 months (range 4-99 months); in group II there were 5 complete remissions at median follow-up 11 months (range 4-20 months) *Conclusions*. primary diffuse large B-cell lymphoma of the spleen is curable disease. It is still necessary to clear up the advantages of intensified induction chemotherapy

1427

MOLECULAR EPIDEMIOLOGY OF HEPATITIS C VIRUS AMONG HEMATOLOGICAL PATIENTS' GROUP IN UZBEKISTAN

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Background. Differences in the hepatitis C virus (HCV) genotype influence the severity of HCV related liver disease and response to interferon therapy. HCV infection is frequent in hematological patients who have been exposed repeatedly to multiple HCV genotypes through non HCV virally inactivated blood products. In Uzbekistan, all blood products are of domestic production and have never been imported from abroad. A nation-wide screening of blood donors for anti-HCV has begun since 1996.The distribution of the various HCV genotypes in hematological patients in Uzbekistan is unknown. *Aims*. This study aimed to estimate the overall HCV genotype distribution and to reconstruct the HCV genotype-specific incidence in multi-transfused hematological patients in Uzbekistan during the recent decades. It also focused at the identification of genotype 2k/1b recombinant subtype in Uzbekistan. *Methods*. A total of 72 chronically infected HCV patients with detectable serum HCV RNA by RT-PCR, belonging to different hematological diseases groups (56 hemophilia and 18 acute leukemia patients) were studied. Amplified products from the 5'-noncoding region were

typed using a commercially available assay based on the reverse hybridization principle. Genotyping for PCR-positive samples was carried out with HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a and 6a specific primer mixtures for the core region [Ohno *et al*., 1997]. To confirm the results of genotyping and to carry out phylogenetic analysis, the nucleotide-sequencing assay has been used. *Results*. HCV genotype 1b was the most prevalent in a acute leukemia patients 10(55.5%) followed by genotype 3a 7(38.7%) and 1a 1(5.6%). A high prevalence of genotype 3a 26(48.1%) and subtypes1b 21(38.9), 2a 6(11.1), 2k/1b 1(1.9%)recombinant subtype were fined in haemophilia patients group. Data on the temporal patterns of HCV genotype-specific incidence in Uzbekistan revealed a moderate increase for genotypes 3a in non IVDU groups from 2001 to 2006. 2k/1b recombinant subtype (described before in Russia) was fined first time in Uzbekistan in a haemophiliac patient who also had history of IVDU. There was no association between the HCV genotype and the severity of haemophilia, alanine transaminase levels, or the presence of portal hypertension. Two main mechanisms of HCV infection distribution were observed in this grous: HCV 1b genotype infection is widespread through blood products, and HCV 3a genotype infection is spreading through the growing number of intravenous drug users in hemophilia patients group. *Summary*. Genotype 1b and 3a are common in multi-transfused hematological patients in Uzbekistan. After a nation-wide screening of all donors for HCV a moderate increase for genotypes 3a in non IVDU groups were observed. Determination of the patient's virus load and of the infecting subtype of HCV may be helpful in planning interferon α therapy. The viral molecular epidemiology investigation is ongoing.

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PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA (A SINGLE CENTRE EXPERIENCE)

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Background. Peripheral blood stem cell transplantation (PBSCT) is the therapy of choice for the treatment of multiple myeloma (MM) patients. *Aim*. To evaluate the effectiveness of PBSCT in MM. *Methods*. Between 2001 and 2006, Autologous-PBSCT were performed to 14 patients with MM (11 men, 59±13 years of mean age) after conditioning with high-dose intravenous 140-200 mg/m^2 melphalan (293±77 mg of mean melphalan dose). Syngeneic-PBSCT was done to another patient. 13% of the patients were only light-chain disease. 47% and 67% of the patients were IgG type and stage-IIIA, respectively. Before PBSCT, 13 and 2 patients were treated with VAD and melphalan plus prednisone regimens, respectively. 8 and 7 patients were in complete and partial remissions, respectively. Stem cell mobilization was done with 10 µg/kg filgrastim plus 4 g/m^2 cyclophosphamide. When the counts of CD34 positive cells were more than 20 µL, stem cells were collected. 69±92×10^6/kg CD34 positive stem cells were infused. The counts of mononuclear cells were 1.7±1.1×10^8/kg. Neutrophil and platelet engraftments were detected in 14±4 days and 15±6 days, respectively. Febrile neutropenia was seen in 87% of the patients. Causative micro-organism was found only in 53% of the patients with febrile neutropenia. *Results*. Transplant-related mortality rate was 13%. The causes were multi-organ failure due to sepsis and pneumonia. At 100 day after PBSCT, 11 of 13 patients were in complete remission. Relapse was seen in 4 (27%) of the patients at followed-up. Relapses were extra- and intramedullary. These patients were treated with bortezomib, thalidomide, dexamethasone, and radiotherapy. 3 of 4 patients with relapse were died from progression. Interferon-α was performed to the patients in remission least for 24 months. The remission duration was 18 months. After Kaplan-Meier analysis, overall survival after diagnosis and transplantation were 54 and 42 months, respectively. Now 10 (67%) patients are alive. *Conclusion*. PBSCT with high-dose melphalan which is the therapy, prolongs survival.

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THERAPEUTIC APHERESIS: THE RESULTS OF A SINGLE CENTER IN TURKEY

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Background. Therapeutic apheresis (TA) is carried out a broad spectrum of diseases and syndromes. *Aims.* We retrospectively evaluated the results of therapeutic apheresis (TA) including plasma-exchange, therapeutic platelet-apheresis, and leukapheresis during 2000-2006. *Methods*. A total of 195 procedures were performed in 44 patients (25 male and 19 female, with mean age 52±15 years). These procedures consist of 165 plasma-exchange, 20 therapeutic platelet-apheresis, and 10 leukapheresis. The most common indications were hematological, neurological, and metabolic diseases. Eighty-three percent of plasma exchange, 100% of platelet-apheresis and leukapheresis belonged to indication Category I or II, according to the guidelines of the American Society for Apheresis. *Results*. While hemoglobin levels significantly increased (p<0.05), platelet counts decreased (p<0.005) after plasma-exchange. Hematological parameters did not statistically significantly changed with leukapheresis (p>0.05). Platelet counts significantly decreased with platelet-apheresis (p<0.001). Total complication was detected in 21% of the procedures. Adverse events (AE) were seen in 17% of procedures. None of the patients died (Grade-IV) from any complication. AE occurred in 14% (Grade-I), 1% (Grade-II), and 2% (Grade-III) of the procedures. The most common of AE were nausea/ vomiting, hypotension, and abdominal pain. *Conclusion*. TA, an important procedure in Transfusion Medicine, is safely carried out in our center in several hematological, neurological, and metabolic diseases which are similar to previous reports.

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COMPARATIVE STUDY OF THE EFFECT OF RECOMBINANT ERYTHROPOIETIN β ON ERYTHROPOIESIS INDICES IN CASE OF ANEMIA IN PATIENTS WITH HODGKINS DISEASE AND DIABETIC NEPHROPATHY

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Background and Aims. Preparations of human recombinant erythropoietin (rhEPO) were found to be effective in the treatment of anemias both in patients with lymphoproliferative diseases, including Hodgkin's disease (HD), and in those with diabetic nephropathy (DN). It has been shown that anemia in DN is mainly determined by insufficient endogenous EPO (eEPO) as a result of a decrease in its production by injured kidneys and, thus, exogenous EPO administration compensates this insufficiency. The mechanism of the favourable effect of rhEPO in HD is studied to a lesser degree. The purpose of the work was to find out whether normalization of EPO level in HD patients represents a main mechanism of the favourable effect of exogenous EPO. *Methods*. 33 patients with HD (II-IV stages of the disease) and 32 patients with DN and marked anemia (Hb<10 g/dL) in which blood serum eEPO level was initially determined using immunoenzymic method (ELISA), and then with a therapeutic purpose rhEPO (epoetin β, Hoffmann-La Roche) was prescribed subcutaneously at a dose of 2000 IU twice a week for 8 weeks. The control group comprised 30 healthy subjects of the same age and gender. Erythropoiesis indices: hemoglobin (Hb, g/dL), erythrocytes (RBC, 1012/L), hematocrit (Hct,%), mean content of Hb (MCH, pg), mean concentration Hb in RBC (MCHC, g/dL, mean volume RBC (MCV, fb) were determined using a hematological analyzer (Coulter). *Results*. Patients with DN were characterized by a marked normochromic anemia (Hb - 7.89+0.28 g/dL; RBC - 2.69+0.10×10^{12}/L); Hct -24.4+1.17%) and a low concentration of eEPO (3.82+0.94 versus 13.48+2.08 mU/mL) in healthy subjects. A good correlation was noted between Hb content and eEPO concentration in blood. In patients with HD hypochromic anemia was prevailing (Hb - 6.27+0.31 g/dL; RBC - 3.26+0.14×10^{12}/L; Hct - 25.6+1.15%), and a decreased eEPO level, though not so significant (9.92+3.84 mU/mL) as in case of DN, and in most cases there was no correlation between this index and Hb content. After 4 weeks of rhEPO use, in patients with DN there was evidence of a marked increase in Hb, RBC, and Hct (p<0.05) without any significant changes in MCH, MCHC, and MCV indices, and in case of HD an increase in Hb, RBC, and Hct indices (p<0.05) was associated with an increase in MCH and MCHC indices. In both groups of patients treated with rhEPO there was evidence of a decrease in the number of hemotransfusions and an improvement of QOL. *Conclusions*. The data obtained suggest that anemia in case of HD is determined not only by EPO insufificency but also by a specific inhibition of erythropoiesis, including cytostatics effect. The mechanism of development of anemia and its treatment with rhEPO in case of HD and DN are not quite the same.

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FORMATION OF LECTIN-INDUCED HAPTENIC-SUGAR-RESISTANT AGGREGATES OF PLATELETS FROM PATIENTS WITH UNSTABLE ANGINA

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Background. Platelets are postulated to participate in the early stages of atherogenesis, but data addressing this notion are contradictory. Pro-

posed by many authors method of measuring of platelet aggregation by various agonists don't give positive information about the progression of coronary atherosclerosis. *Aims*. In this study we examined platelet disaggregation to determine the formation of haptenic-sugar-resistant (HSR) intercellular contacts (as indicator of post-binding signaling) in platelets from patients with unstable angina (UA). *Methods*. We studied platelets isolated from control subjects (n=10) and patients with UA (n=16). The spectrophotometrical technique was used to assess extent of formation of HSR-contacts by determination gaptenic sugar-induced disaggregation of platelets stimulated by N-acetyl-D-glucosamine (GlcNAc)-specific lectin from Triticum vulgaris (WGA) and galactose-specific lectin from Viscum album (VAA). *Results*. The degree of platelet aggregation from patients with UA induced by WGA and VAA was statistically indistinguishable from that of control subjects. The degree of GlcNAc-induced dissaggregation of platelet of patients with UA in response to WGA was significantly increased in comparison with control subjects (p<0,05). The disaggregating response of VAA-activated platelets to lactose of patients with UA was statistically indistinguishable from that of control subjects. Thus WGA-stimulated platelets from patients with UA exhibited less formation of HSR-contacts that the cells from control subjects. *Conclusions*. Since PECAM-1 serves as the main receptor for WGA in platelets the obtained results suggest that disease-dependent disturbances of stability of lectin-induced aggregates of platelets from patients in acute coronary ischemia can be associated with alterations of PECAM-1-dependent processes.

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CORRELATION OF TELOMERASE ACTIVITY TO APOPTOSIS AND SURVIVAL IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA;AN EGYPTIAN SINGLE CENTER STUDY

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Background. Telomerase is activated in most tumors but suppressed in normal human somatic cells. Evidence indicates that reactivation is a critical step in carcinogenesis with a close relationship to apoptosis. The goal of this study was to investigate the levels and relationship of telomerase activity to apoptosis and its impact on survival of Egyptian adult acute lymphoblastic leukemia patients. *Patients and Methods*. Telomerase activity was quantified by polymerase chain reaction (PCR) and detected by Enzyme Linked Immunosorbant Assay (ELISA) while apoptosis was measured at single cell level by Fluorescence *In Situ* Detection using flow cytometry in 15 control subjects and 40 ALL patients at presentation. *Results*. Telomerase activity in ALL patients was negatively correlated to apoptosis (% and MFI)(p<0.001 for% and <0.001 for MFI) and to the 4 year survival rate (p<0.05) to which apoptosis (% & MFI) was consequently positively correlated (p<0.001 for% and p<0.05 for MFI).For telomerase the highest positive predictive value (PPV) for mortality (93.3%) was at a cut off value of 13 amol/mL while those for apoptosis(85% for percent of apoptotic cells and 90.9% for MFI)were at a cut off 8% and 0.19 MFI .This makes measurement of telomerase activity in ALL patients a potential tool to predict disease with unfavorable outcome and a candidate tumor marker.

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FACTOR XIII GENE V34L MUTATION IN THE LEBANESE POPULATION

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Background. Previous reports from the Lebanese population indicate important and unique genetic features namely Factor V Leiden, Factor V H1299R, ApoE E3 allele, and I/I frequency of ACE gene. This report studies the prevalence of an important gene, Factor XIII. *Aims*. To describe the Factor XIII Val / Leu gene polymorphism frequency. *Methods*. We randomly selected 205 samples from donors logged into our HLA registry and representing unrelated healthy Lebanese people originating from different areas and religious communities of the country. Their DNA was originally extracted using the PEL-FREEZ extraction kit (PEL-FREEZ, DYNAL, USA) and stored at -80°C for later use. To test for the various genotypic profiles of the FXIII gene, the CVD StripAssay (ViennaLab, Austria) was used and its protocol was followed as described by the manufacturer. This assay screens for several gene mutations including FXIII gene polymorphisms. Briefly, *in vitro*, the different gene sequences are simultaneously amplified and biotin-labeled in a single amplification reaction (Multiplexing). The thermocycler program consists of an initial step of 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 3 minutes. Finally, the amplification products are selectively hybridized to a test strip which contains allele-specific (corresponding to V or L) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. *Results*. Our data showed that the V34V Normal genotype was the most prevalent (74.2%) followed by the Heterozygous genotype V34L (22.4%) and the Homozygous genotype L34L (3.4%) with an allelic frequency of 0.14. The sampled Lebanese population showed that the prevalence of V34L carriers (25.8%) was lower than Caucasians in general (44.3%) and, interestingly, with a low allele frequency of 0.14 similar to that in Blacks and South Asians. *Conclusions*. The Leu allele frequency in the Lebanese population is 0.14 and thus, looks more fit into the range of South Asians and Africans although, ethnically and geographically, the differences among these populations are obvious. Since the V34L mutation of Factor XIII has been reported to be associated with protection against a variety of cardiovascular events, the low prevalence of this mutation in the Lebanese population leads to a major question: Are Lebanese individuals more prone for cardiovascular problems than other Caucasian individuals or ethnic groups in different communities?

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UP-FRONT AUTOLOGOUS HAEMATOPOIETIC CELL TRANSPLANTATION (AHCT) IS HIGHLY EFFECTIVE IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMA (PTCL)

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Introduction. Patients (pts) with peripheral T-cell lymphomas have a poor outcome after conventional chemotherapy. High dose chemotherapy with AHCT is currently recommended for patients with refractory and/or relapsed PTCL. The role of up-front AHCT is still unclear. We analysed first 11 patients who underwent AHCT as a consolidation after achieving complete (CR) or partial remission (PR). *Material and methods*. Between 1991-2006, we performed 964 autologous transplants for haematologic malignancies included only 11 pts (1%), (5 male / 6 female), median age of 41 years (yrs) with PTCL. In these pts, CHOP regimen was used as an induction treatment and 7 out of 11 pts (64%) achieved CR. The remaining 4 pts reached PR after second line therapy. The median number of chemotherapy lines needed to achieve CR/PR was 8 (range 3-20). As a conditioning, 10 pts received CBV regimen (cyclophosphamide, BCNU, etoposide), 1 pt was treated with BEAM (BCNU, etoposide, ara-C, melphalan). *Results*. The 100-day mortality rate was 0%. The median number of transplanted mononuclear cells equaled 3.5×10^8/kg (range 2.02-6.9), including $CD34^+$ 6.2×10^6/kg (range 1.1-17.2). The median time of recovery of granulocytes $>0.5\times10^9$/L and of platelets $>50\times10^9$/L equaled 14 days (range 12-18) and 16 days (range 10-30), respectively. Relapse and progression occurred in 4 out of 11 pts (36%, 1CR, 3PR at transplant) after a median of 7 months (range 4-9) after AHCT. The relapse in the single CR patient occurred after 4 months. 3 out of 4 pts died in progression, one pt achieved PR2 and underwent allogeneic stem cell transplantation. With a median follow-up of 20 months (range 6-52), six pts (55%) remained in CR. 3 year overall survival (OS) and progression-free survival (PFS) equaled 60 and 62%. The obtained results of up-front AHCT for PTCL are consistent with the data published elsewhere and they are significantly better if compare to results of AHCT for refractory or relapsed pts. *Conclusions*. These preliminary results are encouraging, but further studies are warranted to confirm benefit of this strategy.

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LONGITUDINAL FOLLOW UP OF ABL KD MUTATIONS' LEVEL USING A SENEITIVE AND QUANTITATIVE MALDI-TOF BASED ASSAY

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Background. Imatinib therapy have revolutionized the treatment of CML patients (pts), however a proportion of pts develop imatinib resistance. The most investigated mechanism for acquiring resistance to imatinib is the acquisition of point mutations in the ABL KD. In pts harboring ABL KD mutations, follow up of mutation level may be important in assessing response to new generation kinase inhibitors. *Aims*. the fol-

low up of ABL KD mutations' level in imatinib resistant pts. *Patients and Methods*. Pt 1 is a 73 years old lady with CML of 11 months duration treated with nilotinib for accelerated phase CML. Pt 2 is a 30 years old man with CML of 8 months duration treated with nilotinib for blast crisis and on further progression with MK-0457. ABL KD mutation level was determined on cDNA extracted from peripheral blood, with a sensitive high throughput MALDI-TOF based assay using the SEQUENOM MassARRAY® system and specific primers designed for the detection of each of 27 ABL KD mutations. The assay involves PCR amplification of the region containing the mutation of interest, SAP treatment to remove excess dNTPs, addition of DNA polymerase along with a mixture of dideoxy and deoxy NTPs that allows extension of the hME primer through the mutation site and generates allele-specific extension products, cleanup of the extension reaction to remove salt, spotting of the extension product into 384 SpectroCHIPs and the analysis of the spotted product using the MALDI-TOF mass spectrometry. We have previously published the sensitivity of our method which is 1.5-3% mutated clone in the sample analyzed (Leukemia 2007, in press). Results were confirmed by direct cDNA sequencing analysis. *Results*. Two ABL KD mutations were found in pt 1 before commencing therapy with nilotinib: M244V and F359V; the relative proportion of M244V mutation to wild type (WT) ABL was 45% and of F359V mutation to WT ABL 20%. Mutation levels were further analyzed at 2 months intervals during therapy with nilotinib and showed a decrease in the relative proportion of M244V mutation until disappearance and an increase in the relative proportion of F359V mutation that reached 40% in the last sample analyzed. Clinically, the pt did not achieve CHR after six months therapy with nilotinib, suggesting that F359V but not M244V is associated with clinical resistance to nilotinib. Pt 2 was treated with nilotinib for 3 months after which he developed a second blast crisis. At that time T315I mutation was found with a relative proportion of 50% to WT ABL. After 2 courses of MK-0457 there was a decrease in peripheral blast count with a disappearance of the T315I mutation, suggesting persistence of blasts with WT ABL. *Conclusion*. Follow up of ABL KD mutation level during therapy with new generation kinase inhibitors is informative and may influence future therapeutic decisions.

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CLINICAL CHARACTERISTICS AND OUTCOMES OF HEPATITIS C VIRUS POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Most of subtypes in previous studies about association between hepatitis C virus (HCV) infection and non-Hodgkin's lymphoma (NHL) are low grade NHL, while limited data are available to characterize HCV-related diffuse large B-cell lymphoma (DLBL). We conducted this retrospective study to investigate distinctive clinical characteristics and outcomes of HCV-positive DLBL. *Methods*. Total 32 cases of HCV-positive DLBL from 9 institutions in Korea were analyzed for evaluation of clinical characteristics and outcomes. We compared the clinical characteristics and outcomes of HCV-positive DLBL to those of 371 patients with HCV-negative DLBL. *Results*. The HCV-positive DLBL was associated with a higher portion of old age (≥60) at diagnosis (59.4% vs 36.1%, p=0.009) and less likely to have extra-nodal involvement (53.1% vs 71.1%, p=0.044) than HCV-negative DLBL. The nodal presentation was only independent factor favorably influencing the event free survival (EFS) in HCV-positive DLBL (HR=0.11, 95%CI; 0.01-0.95, p=0.012). In comparison to patients with HCV-negative DLBL, HCV-positive DLBL patients had a superior EFS, especially in cases of age≥60 and nodal presentation (p=0.047, p=0.023) *Conclusions*. HCV-positive DLBL is more common in old age and relatively less presented with extranodal involvement than HCV-negative DLBL in Korea. The good prognosis of HCV-positive DLBL seems to be correlated with transformed low-grade NHL.

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A PHASE I/II STUDY OF ALL-TRANS RETINOIC ACID COMBINED WITH CONVENTIONAL CHEMOTHERAPY IN INFANTS WITH MLL-REARRANGED LEUKEMIA

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Background. MLL-rearranged leukemia in infants is a unique leukemic subset with short latency, extremely aggressive clinical course and unacceptable poor survival. MLL-fusion genes are participating in early progenitors' differentiation block with following transformation to the leukemic clone. In order to improve treatment results in infants with MLL-leukemia we conducted Phase I/II clinical study of all-trans retinoic acid (ATRA) application in combination with conventional chemotherapy. ATRA-a natural derivate of provitamin A has been approved in treatment of acute promyelocytic leukemia and demonstrates differentiating, antiproliferative and proapoptotic activity. We are hypothesizing that ATRA is able to affect the differentiating arrest in the leukemic cells and induce maturation to the normal progenitors. *Aim*. To evaluate the tolerability and efficacy of the ATRA containing regimen in infants with primary diagnosed MLL-leukemia. *Patients and Methods*. Treatment design included alternating 1-2 weeks ATRA courses in daily dose 25mg/m^2 started on the day 36 of induction chemotherapy. Chemotherapy is referred to the ALL-MB 91 for the intermediate risk group and to the modified ALL-BFM 90 high risk (HR) branch with high dose methotrexate reduction. Maintenance therapy included 14 days ATRA courses together with reinduction pulses of vincristin, and dexamethasone. To avoid severe adverse effects cranial irradiation has been omitted and substituted by five additional intrathecal triplets. Patients with MLL/AF4 and nonresponders on the day 36/43 were determined as a HR group. All the rest patients were stratified to the intermediate risk schedule. Trial has been approved by the local Ethical Committee. Parents have signed the informed consent. From September 2003 till the October 2005 four patients aged 1-10 months with MLL-rearranged acute leukemia have been admitted to our clinic and are the subjects of this study. All of them have had bulky extramedullary disease and high white blood cells (WBC) count: 410×10^9/L; 242×10^9/L; 131×10^9/L and 70×10^9/L. Patient with the highest WBC level has been considered to be positive for CNS involvement due to the traumatic first lumbar puncture. All the patients demonstrated BI immunophenotype. Three of four patients have presented t(4;11)(q21;q23) and MLL/AF4. Translocation t(11;19)(q23;p13) and MLL/ENL was detected in patient with the lowest initial WBC. *Results*. All the patients responded well to the initial treatment and achieved complete remission on the day 36. But despite of good hematological response, fusion genes were detectable by nested RT-PCR in all cases. Three of four patients (one with MLL/ENL and two with MLL/AF4 chimeric transcripts) became PCR negative after the first ATRA course. In fourth case MLL/AF4 transcript gradually decreased and disappeared later - after the 7th course of ATRA. The follow up time is as follow: 42, 22, 17 and 16 months. At the present time all the patients are in molecular remission for: 38, 19, 16 and 9 months respectively. No severe adverse effects of ATRA have been registered. *Summary*. Our study demonstrated acceptable tolerability and satisfied efficacy of ATRA containing treatment in infants with MLL-leukemia. Referring to this encouraging experience we have developed a pilot MLL-Baby 2006 protocol intended for treatment of infants' leukemia.

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CHRONIC LYMPHOID LEUKEMIA (B-CLL) & LOW GRADE NON HODGKIN LYMPHOMA (LG-NHL) TREATMENT WITH FLUDARABINE (1995 2007)

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Background. fludarabine (F) has become the standard first line therpay for chronic lymphoid leukemia (CLL) in younger patients. *Aims*. To assess the efficacy, safety and quality of life of F in previously untreated LLC(B) and low grade Non Hodgkin Lymphoma (NHL-LG) in a Group of Medical Institutions in Uruguay. *Methods*. 213 patients in the period 1995-2007 were evaluated.165 of them received the intravenous formulation (1995-2007) and 48 the oral one (2002-2007). CLL 131 patients and NHL-LG 82. Age: 44-85 years old, media 67 years old. Gender: male 115: female 98. Inclusion criteria for CLL-B was Binet stages B, C and A progressive (Ap), 18 to 85 years old, non multiorganic failure, performance

status 0-2 (WHO), informed consent. Inclusion criteria for NHL-LG was: non previous treatment, Ann Arbor stage III or IV (nodal or extranodal), a measurable mass, age > 18 years old, WHO performance status 0-2 and informed consent. WHO performanse status: 0: 125 pts, 1: 75 pts. & 2 13 patients. Staging: CLL-B: Binet Ap 8/131, B 99/131 & C 24/131. NHL-LG: IIIA 16/82, IIIB 5/82, IVA 45/82 & IVB 16/82.Treatment: as first line therapy all the patients received (minimum): 6 cycles of Fludarabine (Fludara®, Schering) 25 mg/m²/daily (5 days) e/ 30 days or Oral Fludarabine, 40 mg/m²/daily (5 days), 6 cycles *Results*. on the CLL-B cohort the overall response rate (ORR) was 78% (CR+PR), 80% of them have immunophenotypic response. Safety: on the 744 cycles in 131 patients, the toxicity was 1 AHAI, 2 pancytopenia, 3 plaquetopenia,. Infection 1,3% ; degree 3 and 4. No alopecia was observed in any patient. Kaposi sarcoma (0,7%). Mortality rate: 1,5% (2/131 patients). On NHL-LG the overall response rate was 78% (PR+CR); complete response 44%, partial response 34%. Stable disease 2%. Progressive disease 23%. Time to progression 15 months. Overall survival at 60 months was 68%. LDH in serum was an adverse prognostic factor for time to progression an overall survival. Other adverse factors to overall survival were, age over 65 (p=0,0001) and hepatic impairment (p=0,0001). Toxicity: (WHO>2): granulocytopenia 28%, thrombocytopenia 8%, infection 2%, alopecia 0%. Causes of death: NHL 52%, sepsis 5%, associated disease 14%, second malignancy 9% and others 30%. Comparing oral with intravenous formulation in overall survival the results were: CLL 34% vs 36% and NHL-LG 82% vs 77% (p= NS). *Conclusions*. fludarabine monofosfate (Fludara®) is an effective and safe treatment for CLL-B and NHL-LG. The oral and intravenous formulations have a similar response rate in elderly and young patients. A longer follow up and a larger trial, could be necessary to confirm these results.

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ANALYSIS OF CHROMOSOME ABERRATIONS IN CHILDREN WITH HEMATOLOGICAL MALIGNANCES, BASHKORTOSTAN REPUBLIC, RUSSIA

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Background. Hematological malignances are the most common cancer in childhood. Chromosome rearrangements determine the prognosis of diseases and play an important role in choosing of treatment protocols, allow controlling minimal residual disease during and after treatment. *Aims*. The aim of this study was to detect the more common mutations in patients with hematological malignances in Bashkortostan Republic. *Materials and methods*. During the 2005-2006 years, 39 bone marrow samples of patients with leukemia tested to the presence of chromosomal aberrations by the method of reverse transcription-polymerase chain reaction. There were 28 patients with acute lymphoblastic leukemia (ALL), 6 children with acute myeloid leukemia (AML), two-with chronic myeloid leukemia (CML), three-with myelodysplastic syndromes (MDS). *Results*. We detected three patents with t(1;19) E2A/PBX among 28 ALL samples. This translocation define unfavorable prognosis. Patients with t(1;19) received high-risk treatment protocol, that permit to reach molecular remission and decrease risk of relapses. Among ALL patients - three were with t(12;21) TEL/AML1. This translocation define favorable prognosis. Patients with t(12;21) received standard-risk treatment protocol, that permit to reach molecular remission and decrease risk of complications. Between ALL patients-one was with t(9;22) BCR/ABL p210. This translocation define absolute unfavorable prognosis. This child has received high-risk treatment protocol in combination with STI571 (Glivec) 400 mg daily. Amid 6 AML patents, one was with AML-M3, translocation t(15;17), PML/RARA confirmed the diagnose. This patient has received AML-APL protocol in combination with ATRA (Tretionin). We detected t(9;22) BCR/ABL p210 translocation in two samples in patients with chronic phase of CML. These patients have received treatment with Glivec 400 mg daily. After 6 and 12 months of treatment molecular remission was achieved, t(9;22) has not detected. Among three patients with MDS, in one child we tested t(9;22) BCR/ABL p210, titer 10-2. This patient was moved to CML group and began to receive (Glivec) 400 mg daily. *Conclusion*. The most common mutations among ALL patients were t(1;19) E2A/PBX and t(12;21) TEL/AML1. In CML patients we detected t(9;22) BCR/ABL p210 translocation Molecular-genetic analyses play an important role in diagnostics, choice of treatment and prediction of relapse in hematological malignances.

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PHENOTYPIC ABNORMALITIES OF PERIPHERAL BLOOD NEUTROPHILS HAVE A RELATION WITH SURVIVAL OF MYELODYSPLASTIC SYNDROMES

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Background. the main prognostic factors in myelodysplastic syndromes are degree of peripheral blood (PB) cytopenias, number of bone marrow (BM) blasts as well as cytogenetic findings. Flow cytometric (FCM) analysis has been used in MDS for diagnostic purposes. However, number of phenotypic abnormalities per patient, present a correlation with IPSS and WPSS. *Aim*. We examined the relation between FCM abnormalities and survival of the patients. *Methods*. quantitative FCM analysis of BM erythroblastic, granulocytic and monocytic cell lines was performed in consecutive newly diagnosed patients. The relation of phenotypic abnormalities WPSS and overall survival (OS) was examined by the Cox model. *Results*. 31 patients entered the study. Median age: 60 years (18-93). WHO categories: 11 refractory anemias, 2 sideroblastic anemias, 10 refractory cytopenias with multilineage dysplasia, and 7 refractory anemias with excess of blasts. WPSS: 6 very low risk, 9 low risk, 9 intermediate and 6 a high risk. Median total number of FCM abnormalities per patient was 3 (1-8). In the univariate Cox regression had impact on OS: WPSS, PB platelets and number of $CD34^+$ cells (but not BM percentage of blasts) as well as the total number of FCM alterations. Among the FCM variables were significantly associated with survival: SSC of promyelocytes, SSC of granulocytic precursors, MFI of CD13 of myelocytes, metamyelocytes and mature neutrophils and CD45 of mature neutrophils, MFI of CD13 in monocytes and MFI of CD45 of mature neutrophils. The model chosen for multivariate analysis included PB platelet count, WPSS and expression of CD45 and CD13 as well as SSC of mature neutrophils. In this model only PB platelet count, SSC and expression of CD13 were independent prognostic factors. *Conclusions*. in the present analysis, we could construct a prognostic model for MDS based only of PB parameters, avoiding the use of BM aspiration.

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EVI-1 GENE EXPRESSION CHANGES ACCORDING TO IMATINIB RESPONSE AND PROGRESSION TO BLASTIC CRISIS IN CHRONIC MYELOID LEUKEMIA

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Background. Previous studies have shown high expression levels of EVI-1(a trancription factor codifying gene) in cell lines derived from Chronic myeloid leukemia (CML) as well as in samples from CML patients in blastic crisis (BC). Based on these results that relate EVI-1 expression to transformation into BC, and taking into account that its early detection is vital for an effective therapeutic intervention, EVI-1 expression has been studied both in cell lines derived from CML before and after treatment, and in CML patients at diagnosis and after treatment. *Materials and Methods*. Bone marrow (BM) and/or peripheral blood (PB) samples from 36 CML patients (19 M, 17 F) were analyzed. Of the total, 34 of them belonged to patients in chronic phase (CP) and 2 in BC (1 patient was studied in CF and BC). *Cell cultures*. cell line K562 was treated for 3 days with 1 μM Ara-C, 2000 U/mL IFN-α, 0.5 mM Hydroxyurea (HU), 0.5 mM Busulphan (BU) and 2.5 μM Imatinib. Qualitative RT-PCR was used to analyze EVI-1 expression in patients at diagnosis, while Quantitative RT-PCR (QRT-PCR) was used during follow up and on RNA from cultures through relative quantification. *Results*. 1. 22 patients were positive at diagnosis (20 in CP and 2 in BC). Opposite to other studies in which incidence has been found to be low, in this case an incidence of 61% was detected. Upon follow-up of these patients, 14 on CP were treated with Imatinib, observing a decrease in EVI-1 level expression similar to those found in healthy people. A normalization of EVI-1 expression was also detected on one patient with loss of molecular response who underwent an Allogeneic SCT. It is important to note that 2 out of the 9 patients with negative EVI-1 determination at diagnosis, a progressive increase in its expression was observed coinciding

with development of BC/AP. 2. EVI-1 expression in K562: expression was analyzed in this cell line after treatment with commonly used drugs for CML (BU, HU, Ara-C, IFN, Imatinib). A decrease in the gene expression was only observed after treatment with Imatinib, while its levels remained unchanged after the use of the other drugs, even though they have an antiproliferative effect on K562.

Figure 1.

Conclusions. a) A high percentage of CML show an increase in EVI-1 expression at diagnosis and during BC, disappearing after treatment with Imatinib. b) EVI-1 expression decreases alongside BCR-ABL expression. Loss of response to Imatinib is associated to recovery of EVI-1 expression. c) A repression of EVI-1 is detected on K562 treated with Imatinib. This is not found when treated with the other above mentioned drugs, suggesting that EVI-1 expression depends on the activity of BCR-ABL. d) EVI-1 expression could be a marker of the antileukemic effect of anti TK drugs such as Imatinib.

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CUTANEOUS LESIONS ASSOCIATED WITH HIGH RISK PRIMARY MYELODYSPLASIA

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Background/Aim. Most previews studies assessed the autoimmune inflammatory rheumatic disorders in patients with myelodysplastic syndrome (MDS). There is no study of skin manifestations in a cohort of MDS patients nor that correlated the cutaneous findings with immunologic parameters and prognostic features of MDS. The aim of the present study was to assess the cutaneous findings in a cohort of 84 MDS patients in relation to immunologic parameters or prognostic features of MDS in order to clarify their potential clinical significance. *Methods*. We studied a cohort of 84 newly diagnosed MDS patients in order to assess the cutaneous findings present at the time of diagnosis or during 1 to 3 years of follow-up. We described the clinical variety of cutaneous findings ascertained by histology. We also looked for any association between the group of MDS patients with skin manifestations and MDS subtype, immunologic and prognostic features highlighting transformation to acute leukaemia (high and low risk patients). The laboratory exams performed at the time MDS was diagnosed were marrow and trephine biopsy, peripheral blood count, erythrocyte sedimentation rate (ESR), serum protein electrophoresis (SPE), determination of anti-nuclear antibodies (ANA), rheumatoid factor (RF) and direct Coombs test. The blood specimens used in this study were collected prior to the initiation of any therapeutic approach such as chemotherapy and/or blood transfusion. Statistical analysis of the data was performed using SPSS version 10 statistical software package. *Results*. 21 MDS patients presented cutaneous manifestations: 1 patient developed leukemia cutis, 6 patients photosensitivity not associated with autoimmune disease, 3 prurigo nodularis, 2 Sweet's syndrome, 6 leukocytoclasitc vasculitis, 2 ecchymoses and purpura associated with preexisting relapsing polychondritis, 1 patient subcutaneous nodules associated with Wegener's granulomatosis (an exceptional finding) and 1 patient with malar rash and oral ulcers associated with preexisting SLE. Adjusted for age and gender, the presence of skin findings constitutes a significant predictor of the high risk MDS subgroup (OR: 3.59, 95% C.I: 1.18-10.92). Hypergammaglobulinemia was significant higher in the MDS subgroup with skin findings (p=0.03). *Conclusion*. In conclusion, most MDS patients with cutaneous manifestations belong to the high risk MDS subgroup. This investigation highlights the need to search meticulously for cutaneous manifestations by performing early skin biopsies in hematologic patients, as the skin could reveal prodromal signs of an underlying bone marrow disorder in transformation.

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LUPUS ANTICOAGULANT (LA) IN INFECTIOUS DISEASE . A REPORT OF THREE CASES

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Background. Transient and non related with bledding and/or thrombosis LA tend to appear after infections, and generally is not associated with any underlying disorder. This fact has been reported in children with viral infections in most cases in literature. The finding of LA is usually detected during routine laboratory investigation of a prolonged aPTT. *Aim*. We describe 3 patients with AL following infectious disease studied in our center for a period of five months. *Patients*. In all patients LA was detected after evaluation of prolonged aPTT in the course of infectious disease. Clinical and laboratory findings are reported in Table 1. Biologic assay of factors II, V, VII, VIII, IX, X, XI, XII and von Willebrand factor were at normal levels in patients 1 and 3. Patient 2 who had a prolonged PT presented a mild hypoprothrombinemia (FII 42.2%) without hematological symptoms. Patient 3 presented mild postraumatic subcutaneous bleeding that improved spontaneously within a week. Patient 1 and 2 were asymptomatic. Infectious disease improved in all patients.

Table 1.

	Case1	case 2	Case 3
Gender/Age (y)	Female/74	Female/84	Male/2
PT (8.5-1.3")	12.30	32.2	13.0
aPTT (26-39")	71.6	79.7	66.0
Mixing test	50.7	43	55.2
dRVVT ratio (<1.2)	1.86	1.37	1.73
Platelet count	$176x10^9$/L	$98x10^9$/L	$398X10^9$/L
Anticardiolipin IgM (<40 U/mL)	-	430 U/m	17.7 U/mL
Symptoms	Pyelonephritis	Urologic sepsis	Influenza
Microbiologic agent	Klebsiella pneumonia	Escherichia coli	
Time follow up (weeks)	7	21	8
Confirmatory AL test	-	positive	-

Confirmatory AL and anticardiolipin test according to the Sapporo reviewed criteria (Journal of Thrombosis and Haemostasis 4: 295-306; 2006) was performed only in patient 2 (follow up less than 12 weeks in patient 1 and 3); dRVVT ratio was 2.14 and IgM 155.73 U/mL and no clinical antiphospholipid syndrome criteria were present in any of the patients. FII levels improved in this patient at 12 weeks follow up. *Conclusions*. a) Even when this finding has been described mainly in children we have to take it into consideration when a prolonged aPTT appears during the course of an infectious disease; b) Altough viral infections are more common, bacteria can be present too without thrombosis and /or bleeding as we describe here, in contrast to the reviewed in literature; c) Low levels of FII may be present with prolonged PT in some of these patients, maybe due to a rapid clearance of antigen-antibody from circulation, as proposed in literature reviewed.

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PROGNOSTIC FACTORS IN SECOND-LINE THERAPY FOR AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background. Seventy percent of patients with advanced, aggressive non-Hodgkin's lymphoma [NHL] can achieve complete remission following 1st line treatment, although about half of these patients will relapse and will be treated with a 2nd line regimen. There are various studies concerning the importance of initial prognostic factors for the outcome of treatment and for the creation of an International Prognostic Index. *Aims*. The purpose of this study was to define prognostic factors for various outcome parameters (complete remission, time to tumor progression, survival) in patients with persistent/recurrent aggressive NHL undergoing 2nd line chemotherapy and their implementation in the therapeutic decision planning. *Methods*. This was a retrospective study of 70 patients (30 males, 40 females) ranging in age from 18-86 years (mean: 54 years) with recurrent (following complete remission) or persistent (primary refractory) aggressive NHL who were treated with CHOP (cyclophosphamide, adriamycin, vincristine, dexamethasone - conventional or high dose) as 1st line treatment. Second-line treatment included DVIP (cisplatin, VP-16 (etoposide), ifosfamide, dexamethasone), DAIP (cisplatin, VP-16, cytosine-arabinosid, dexamethasone) or DVIP-high dose methotrexate regimen. All patients had a follow-up of at least 1 year from the start of their salvage regimen. *Results*. The following parameters were analyzed for their influence on complete remission (CR) rate, time to tumor progression (TTP) and survival: performance status (PS),*B* symptoms, bulky disease, lactate dehydrogenase (LDH), hemoglobin and albumin levels, location/number of nodal and extranodal sites, and splenic involvement. CR was achieved in 46% and partial remission in 28% of patients. Median TTP and survival were 6 months and 10 months, respectively. The following parameters were associated with better TTP: high-dose chemotherapy (HD-CT), higher hemoglobin level at the onset of 2nd line treatment, and splenic involvement at initial diagnosis. Improved survival was associated with HD-CT, good PS before introduction of 2nd line chemotherapy and splenic involvement at initial diagnosis. Longer duration of CR following 1st line chemotherapy and higher initial hemoglobin level at initial diagnosis predicted higher CR rate with 2nd line chemotherapy. *Conclusion*. Assignment (or classification) of various risk factors in aggressive, recurrent or therapy-refractory NHL might have paramount importance in treatment decision making. Patients with poor predictive factors should be treated with a more intensive treatment schedule, such as HD-CT with bone marrow or peripheral blood stem cell support.

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PNEUMONITIS AND RENAL TOXICITY WITH SIROLIMUS BASED GVHD PROPHYLAXIS FOR RIC ALLOGENEIC HSCT

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Background and Aims. A challenge with reduced intensity conditioning (RIC) allografting is to adequately manage acute graft versus host disease (aGVHD) without negating a graft versus leukaemia/lymphoma (GVL) effect. Previous studies (Antin *et al.*) suggest reduced rates of aGVHD with sirolimus based GVHD regimens. The aim was to assess sirolimus based GVHD prophylaxis in RIC HSCT in a single transplant centre. *Methods*. The RIC included fludarabine 30 mg/m^2 x5 and melphalan 140 mg/m^2 x1. Intravenous Tacrolimus was administered at 0.02 mg/kg daily, oral sirolimus 1mg daily (for 28 days) and methotrexate 5 mg/m^2 (days 1,3,6 and 11). Sirolimus dose was reduced to account for our use of azole antifungal prophylaxis. After the first 15 patients, tacrolimus was substituted with intravenous cyclosporin (1 mg/kg daily) because of renal toxicity and difficulties with drug monitoring and dosing. *Results*. Twenty-five consecutive patients with median age of 54 (37-67) and high risk features were enrolled. 13 had matched unrelated donors and 12 sibling donors. 72% of the patients survived at a median follow up of 351 days (70-1056). Median relapse free survival was 351 days (49-1055) and overall survival 356 days (70-1057). Seven died (4 sepsis, 3 relapsed disease). 15/25 is alive in complete remission and 3/25 is alive with relapsed disease. Transplant related mortality at 100 days was 8%. Grade 2-4 GVHD was seen in 11 of 13 (85%) unrelated donor RIC HSCT and 2 of 12 (17%) sibling HSCT. Toxicity from this regimen included renal impairment (13), thrombotic thrombocytopaenic purpura (3) and pneumonitis (2). Renal impairment was a major management problem in 13 patients with two requiring temporary renal dialysis. The two patients who developed sirolimus related pneumonitis required a temporary period of mechanical ventilation. *Conclusions*. This pilot study found that sirolimus based GVHD prophylaxis in RIC HSCT led to low transplant related mortality at 100 days with acceptable relapse free and overall survival for this poor risk group of patients. Secondly, the introduction of sirolimus did not significantly reduce rates of aGVHD in unrelated RIC HSCT. Thirdly, the combination of sirolimus and a calcineurin inhibitor resulted in significant toxicity. Finally, to our knowledge, this is the first report of sirolimus associated pneumonitis in bone marrow transplant patients, which has only been reported in solid organ transplant literature.

Reference

1. Antin, J. etal. Sirolimus, tacrolimus, and low dose methotrexate for graft-versus-host disease prophylaxis in mismatched related donor or unrelated donor transplantation. Blood. 2003;102:1601-1605.

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SEVERE HYPOXIA ENHANCES EX VIVO HAEMATOPOIETIC EXPANSION UNDER *CLINICAL GRADE* CONDITIONS

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Background. Haematopoietic cells isolated from bone marrow or mobilized peripheral blood, to become suitable for therapeutical haematopoietic reconstitution, often require to be expanded *ex vivo*. Enhancement of stem cell self-renewal is the natural issue of such a procedure, as an increased number of stem cells is a key factor in the overall numerical expansion of the explanted cell population. In a number of studies carried out over the last 15 years, we demonstrated that very low oxygen tensions *in vitro* (severe hypoxia) help to preserve the stem cell potential within haematopoietic cell populations obtained *ex vivo* (Blood 82:2031-2037, 1993; Leukemia 14:735-739, 2000). Severe hypoxia was shown to influence the balance between stem cell differentiation commitment and self-renewal in favour of the latter and to restrain the effects of cytokines boosting commitment and clonal expansion (Exp. Hematol. 30:67-73, 2002). *Aims*. The target of this study was the development of protocols for the *in vitro* expansion of haematopoietic potential under good medical practice (GMP) conditions, suitable for clinical use. Serum-free incubation media were therefore used, supplemented with a combination of stem cell-active cytokines, to incubate explanted haematopoietic populations in order to compare their *in vitro* expansion under severe hypoxia with that in normoxia. *Methods*. Cells were obtained from mobilized peripheral blood of 3 donors. CD34$^+$ cells were isolated from buffy-coat by the Miltenyi Biotec *indirect* immunomagnetic technique, using for the first passage the *midi* column and for the second the *mini* column. Cells were then analysed by flow cytometry using anti-CD34,-CD133, -CD61, -CD3, -CD19 antibodies. The purity of the isolated CD34$^+$ cells was 35-75%. Cells were cultured in the HP01 medium (MacoPharma), in the presence of the FKT6 cytokine combination, including Flt3-ligand, Stem Cell Factor/Kit-ligand, ThromboPoietin and Interleukin-6. Incubation under severe hypoxia (0.1% O2) was carried out in a water-saturated Ruskinn Concept 400 anaerobic incubator, flushed with a preformed gas mixture (0.1% O2, 5% CO2, 95% N2); incubation in normoxia (21% O2) was carried out in a 5% CO2, 95% air, water-saturated atmosphere. Culture expansion was measured after 14 days of incubation, with respect to either the overall number of viable cells or the number of colony-forming cells (CFC), as determined following cell transfer to secondary FKT6-supplemented MethoCult (StemCell Technologies Inc.) semisolid cultures and a further 7-day incubation therein, in any case in normoxia. *Results*. Haematopoietic expansion in hypoxia was 2.8-fold higher than in normoxia as determined by counting the overall number of viable cells, and 3.5-fold higher as determined by counting the number of CFC. *Conclusions*. In the presence of the FKT6 cytokine combination, incubation in severe hypoxia resulted an efficient method to markedly improve the expansion of haematopoietic cell populations under *clinical grade* conditions.

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THE IMPACT OF DISPARITY IN SHORT TANDEM REPEATS (STRS) ANALYSIS ON THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (ALLO-HCT) FROM HLA-IDENTICAL SIBLING DONORS

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STRs were widely used for monitoring of chimerism after allo-HCT. The effect of STR disparity on allo- HCT has been analyzed in a few studies. We aimed to evaluate the impact of STR disparity on allo-HCT outcome retrospectively in our single center cohort. Between Sep 2000 and May 2005, total 88 patients (F/M:45/43) underwent allo-HCT from an HLA-identical sibling donor (PB/BM: 71/17) Median age was 34 ys (16-64). Multiplex PCR was performed to amplify 16 STR loci (D3S1358, HUMvWA, D16S539,D2S1338, Amelogenin,D8S1179, D21S11,D18S51, D19S433, THO1, FGA, D7S820, CSF1PO, D13S317, TPOX, D5S818) (ABI Prism 310). These loci were classified as full- and partial-matched, and full mismatched(mm) between donors and recipients. *Results*. D2S1338 locus (full matched, 21.6%-n=19; partial matched 50%-n=44 and full mm 28.4%-n=25) was the most informative one, but CSF1PO (43.2%, n=4; 52.2%, n=46 and 4.5%, n=38, respectively) was the least. The general incidences of acute(a) severe and chronic(c)GvHD were 29.5% and 61.8%. Full mm in TPOX locus (p=0.04) led to more acute severe GvHD than in partial matched or full matched loci. However, full mm in D2S1338 locus significantly decreased the incidence of aGvHD in patients with partial matched and full matched loci (5.3%, 29.5% and 48%, respectively p=0.009). However, more cGvHD was only seen in the patients with full matched D7S820 donors (p=0.04). In our cohort,2-year probability of disease free survival (DFS) and overall survival(OS) were 53.1±5.9% and 66.2±5.6%, respectively in mean 46.5±3.8 moths of follow-up. Although the full-mm in D8S1179 locus increased the relapse incidence (62.5% vs 33.3% vs 18.8%, p=0.04), there was a tendency to a decrease of DFS in patients with full-mm CSF1PO or partial matched D7S820 (p=0.06). Early transplant-related mortality increased in patients with D7S820 full-mm (p=0.02). This unfavorable effect was reflected on OS (p=0.002).Besides, patients with full-mm TPOX locus had a decreased probability of OS. TPOX and D7S820 were also less informative resembling CSF1PO.We were not able to show any correlation of stem cell source, conditioning regimen, donor or recipient age, and sex mm on early TRM, aGVHD, cGVHD, and OS rate. *Conclusions*. The presence of STR disparity could be associated with the development of complications and unfavorable survival probability after allo-HCT from HLA-identical siblings.This analysis approach should be repeated on partial matched and unrelated donor transplants.

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EVALUATION OF THE SURVIVAL OF INPATIENT WITH ALL AND INTERMEDIATE RISK TREATED ACCORDING TO PETHEMA-96 PROTOCOL

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Introduction. Acute lymphoblastic leukemia (ALL) is the most common cancer in chilhood and represents around the 25% of neoplasias diagnosed in younger children of 14 years old. They are treated according to defined risk groups based in clinical and laboratory features. For children who seem to have less cure likelihoods, more aggressive treatment generally is given. *Materials and Methods*. Descriptive study in patient with ALL of intermediate risk treated in Pediatric Oncohaematological Service of our centre according to the PETHEMA-96 protocol. Period: August 1998-January 2007. *Results*. 38 inpatients (52% girls) with mean age of 4,8 years (1 to 14 years), have been evaluated. The most frequent clinical presentation were adenopathy and the hepatomegaly (57,9%), with anaemia (Hb< 8 g/dL) in 36,8% (14) and leukocytosis (>50.000/mm^3) in 26,3% (10). L1 are the 73,7% (according to the FAB classification), B-II (EGIL classification) 65,8% and a 13,1% were immunophenotype T. Cytogenetic study was performed in the 68,4% (26) of the inpatients being normal the 34,4% and pathological the 65,6% remaining, emphasizing the presence of hypodiploidies (13,2%), hyperdiploidies (18,4%) and complex karyotype in the 15,8%. The molecular biology study (BCR/ ABL, E2A/ PBX1, MLL/AF4, TEL/AML) was realized of uneven way in the studied inpatients (between the 28,9% and the 65,8% of the cases) finding TEL/ AML reordering in 2 inpatient (5,3%). During the treatment the 7,9% (3) relapsed and a 13,2% (5) died, 2 in the induction treatment (both by infectious causes) and the 3 remaining during relapse. The maximum follow-up is 101 months (mean 52 months). The complete remission (CR) rate was 86,8% having completed treatment the 63,2% (24). *Conclusions*. Complete continuous remission in childhood with risk factors agreements with PETHEMA-96 protocol is similar to the obtained inpatients of standard risk that they have received another treatment protocols.

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BONE MARROW/PERIPHERAL BLOOD POSITIVITY OF BCL-2/IGH REARRANGEMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA BEFORE TREATMENT HAS NO PROGNOSTIC SIGNIFICANCE

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Background. Bcl-2/IgH rearrangement represents a typical cytogenetic aberration in patients with follicular lymphoma where it occurs in 40-90% of the cases at diagnosis. However, its clinical impact remains unclear. *Aims and methods*. The aim of this study was to evaluate Bcl-2/IgH rearrangement in 50 patients with follicular lymphoma before treatment by means of nested PCR technique and to correlate molecular genetic findings with clinical characteristics and results of treatment in subgroups-with vs. without Bcl-2/IgH rearraangement. 91 samples from peripheral blood and/or bone marrow from fifty patients (median age, 56.5 years; male/female ratio: 33/17) were analysed. Samples from both compartments were available in 41 cases. Bcl-2/IgH rearrangement was analysed by nested PCR technique for both major breakpoint region (MBR) and minor cluster region (mcr). *Results*. Twenty-six out of fifty patients (52%) were positive for Bcl-2/IgH rearrangements; 24 in MBR and 2 in mcr, remaining 24 patients were negative. Regarding the initial characteristics, patients under 65 years were more likely to be Bcl-2/IgH positive than negative (88% vs. 58%, p=0.02). Patients with initial bone marrow involvement were also more often Bcl-2/IgH positive than negative (88% vs. 58%, p=0.02). High correlation between Bcl-2/IgH rearrangement in peripheral blood and bone marrow was noted as well as correlation between PCR Bcl-2/IgH detection and immunohistochemistry bcl-2 protein detection in bone marrow. No differences were found according to initial Bcl-2/IgH status with respect to clinical course of disease in terms of complete remission achievement, improved overall survival or progression-free survival in comparison with Bcl-2/IgH negative group. *Summary*. Molecular positivity of Bcl-2/IgH rearrangement in patients with follicular lymphoma before treatment had no impact to prognosis of the disease although additional breakpoint regions may play important role. Further investigations in this field are needed to determine definitive role of this molecular finding to prognosis of patients with follicular lymphoma.

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HEMATO- AND IMMUNOPOIESIS REGENERATION OF LETHALLY IRRADIATED MICE BY CELLS ISOLATED FROM INTESTINAL EPITHELIAL LAYER

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Background. The study of stem cell plasticity has large significance in development of the cellular therapy. Recent studies shown that adult stem cells are able to differentiate into cellular types of different tissues. Many adult non-hematopoietic stem cells is known to have hematopoietic potential. As shown earlier, cells of the intestinal epithelial layer have ability to form splenic hematopoietic colonies (CFU-9). *Aim*. This study aims to investigate ability of intestinal epithelial layer cells (IELC) to hemo- and immunopoiesis regeneration of lethally irradiated mice. *Methods*. Recipient animals were (CBAxC57Bl/6)F1 mice that were lethally irradiated with 9 Gy given in one dose. IELC or bone marrow cells (BMC) were introduced intravenously. Control group was non-irradiated mice. At 1, 3 and 6 months after transplantation, delayed-type hypersensitivity reaction, IgM antibody formation, blood cells counts were investigat-

ed. The white blood cell count (WBC), haematocrit (HCT), platelet number (PLT) and red blood cell (RBC) count were determined in an automatic counter (Cell Dyn 900). *Results*. Analysis of blood cell composition. WBC count in mice reconstituted with IELC (or BMC) amounted to normal values after 3 months, platelet number and RBC count-only after 6 months. However HCT and RBC count in mice reconstituted with BMC were statistically significant reduced in comparison with mice reconstituted with IELC. The obtained data are likely to be explained by highest proliferation potential of intestinal cells. Functional activity of immune system cells in reconstituted mice was studied. The cell-mediated immunity was estimated according to the degree of manifestation of the delayed type hypersensitivity reaction (DTHR), namely, the degree of paw edema upon administration of the resolving dose of T-dependent antigen (sheep erythrocytes; SE) to sensitized animals. During 3 months after transplantation, the pronouncedness of DTHR to SE introduction to mice of both experimental groups was significant reduced in comparison with the control group. Reconstruction of the cell-mediated immunity in mice reconstituted with IELC (or BMC) occurred after 6 months. Statistically significant differences weren't found between experimental groups. The humoral immunity was estimated according to the level of primary humoral response (IgM) to T-dependent antigen (SE). The number of antibody-forming cells (AFC) in the spleen of experimental mice was assessed according to the number of local erythrolysis areas in a semi-liquid medium on day 4 upon immunization. During 3 months after transplantation, the number of AFC (IgM) in the spleen of experimental mice was reduced in comparison with the control group. However the number of AFC in the spleen of mice reconstituted with cells of intestinal epithelial layer were statistically increased in comparison with the other experimental group. Reconstruction of the humoral immunity in mice reconstituted with cells of intestinal epithelial layer (or bone marrow cells) occurred only after 6 months. *Conclusions*. Thus, transplantation of intestinal epithelial layer cells in lethally irradiated mice resulted in reconstitution of hemato- and immunopoiesis. The obtained data testify to high hematopoietic potential of intestinal cells.

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INFLUENCE OF IRON OVERLOAD ON THE DEATH REASONS AMONG CHILDREN WITH ACUTE LEUKEMIAS

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Background. At the majority of children during the initial period of acute leukemia (AL) normochromic normocytic anemias with elevated serum ferritin level (SF) are revealing. There is a problem of total body iron overload in these children at the stages of treatment. *Aims*. To define SF in dynamics of leukemia process and estimate the patient's death reasons with taking into account SF . *Methods*. At 185 children with AL (152 - ALL, 33 - AML) SF levels have been investigated according to the disease period and the patient's death reason. Children were surveyed in the Ist (n = 77), in the IInd (n - 35) and in the IIIrd acute periods (n = 5), in the Ist (n =54) and in the IInd remissions (n = 14). The control group included 30 healthy children. The SF were defined by radioimmune analysis. *Result*. In the Ist acute period the SF was 329,1±19,9 ng/L, during the IIIrd acute period - 1365,7±190,9 ng/L, in the Ist remission - 226,7±30,5 ng/L, in IInd remission - 724,2±76,6 ng/L. At 10/19 patients with AML and 23/58 children with ALL in Ist acute period SF were >500 ng/mL. At children of comparison group mean SF was 42,4±11,8 ng/L. From 72 patients, who have died, 37/72 patients had SF >500 ng/mL (mean 872,2±29,4 ng/mL); in 35/72 patients SF were <500 ng/mL (mean 148,8±13,5 ng/mL). Patients with high SF died from cardiovascular failure occurrence (CVF) at the progression of leukemia, there were more boys (26/37) and previous resistance to chemotherapy (RC) was at 27/37 patients . Mean age of these patients was more than in children with <500 ng/L SF (10,8±2,0 against 5,8±1,7 years). The death reasons of patients with SF <500 ng/mL were hemorrhages and brain edema and RC were in 12/35 children (p<0,05). Overall survival of patients with >500 ng/mL SF was two times shorter, than of patients with SF <500 ng/mL. Direct correlative connection between initial SF and number of the subsequent RBC transfusions has been revealed (r = +0,43). On myocardium autopsy of 5 patients the fibrous layers, tela conjunctiva bands and iron-positive fine granules (2-3 microns) in the cardiocytes, located focally, has been revealed. At the myocardium of 3 children (SF -732÷895 ng/mL and cumulative dose of anthracyclines >240 mg/m^2) these specified changes were more expressive. *Conclusions*. At half of children with AL the elevated level of SF in the initial period of diseases was observed. At the subsequent stages of treatment (anthracyclines, RBC transfusions) advance of primarily existing disturbances of iron metabolism occurs, the cardiotoxicity is develops and resistance to the treatment is observed; that negatively influences to overall survival of patients and enlarges the CVF death risk.

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THE INCIDENCE OF MULTIDRUG RESISTANCE 1 (MDR1) GENE OVEREXPRESSION DOES NOT DIFFER IN ADULT PATIENTS WITH DIVERSE HEMATOLOGICAL MALIGNANCIES

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Background. Data concerning the incidence of Multidrug Resistance 1 (MDR1) gene / P-glycoprotein (P-gp) overexpression in untreated patients with hematological malignancies are extremely heterogeneous and the actual prevalence of this molecular abnormality in a particular disease remains unknown. The reported discrepancies are generally related to differences in the applied approaches for MDR/P-gp status evaluation and in criteria used for overexpression threshold. *Aim*. To determine the incidence of MDR1 gene overexpression in different hematological neoplasms using a validated and standardized molecular approach. *Patients and Methods*. MDR1 gene expression was evaluated in a cohort of 525 untreated patients with haematological neoplasms, including: acute myeloid leukemia (AML) (n=189), acute lymphoblastic leukemia (ALL) (n=116; 72 adults and 44 children); chronic phase of chronic myeloid leukemia (CML) (n=148), essential thrombocytemia (ET) (n=60), and polycytemia vera (PV) (n=12). MDR1 expression was determined by a semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) for MDR1 mRNA with co-amplification of b2-microglobulin, incorporated as an internal control. *Results*. MDR1 gene overexpression was detected in overall 249 patients (47.0%). The incidence of overexpression did not differ among the different malignancies in adult patients: 47.1% in AML (89/189), 54.2% in ALL (39/72), 48.6% in CML (72/148), 50.0% in ET (30/60), and 41.7% in PV (5/12) [p>0.05]. In contrast, the frequency of MDR1 over-expression was found to be significantly lower in children with ALL-31.8% (14/44), and particularly in those <10-yrs. of age-26% (7/27) [p<0.02]. *Conclusions*. The overall incidence of primary MDR1 gene overexpression is similar in adult untreated patients with different hematological malignancies, which suggests that a common pathogenetic mechanism might be responsible for this abnormality, regardless the precise nature of the disease.

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THERAPY OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS: A FIFTHTEEN YEAR EXPERIENCE AT FLORENCE UNIVERSITY HOSPITAL

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Background. Primary central nervous system lymphoma (PCNSL) is a rare tumor, accounting for approximately 1% of all intracranial neoplasms and less than 2% of non-Hodgkin's lymphomas (NHL). Unlike malignant gliomas, in PCNSL an appropriate therapy can result in lasting complete responses (CR) and progression-free survival (PFS). Standard regimens for systemic NHL (such as CHOP) are ineffective in PCNSL, mostly because of the blood-brain barrier. High-dose methotrexate-based 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. *Aims*. We studied retrospectively all patients with a diagnosis of PCNSL and treated in our hospital. *Methods*. To confirm primary extranodal disease all patients with diagnosis of PCNSL were staged with CT-scan of neck, chest and abdomen, bone marrow biopsy, ophthalmologic examination including slit-lamp evaluation and CSF cytopathology if lumbar puncture could be perform safety. Patients were treated with WBRT alone or methotrexate-based chemotherapy alone or a combination of WBRT and methotrexate-based chemotherapy; these different approaches reflect the evolution of guideline in the therapy of PCNSL. *Results*. Since December 1991 to January 2005 25 patients with proven diagnosis of PCNSL were treated at Radiotherapy Unit and Haematology Unit of Careggi University Hospital. Thirteen patients were male (52%), median age at diagnosis was 62 years (range 20-73 years). Disease was localized in the cerebral lobes in 17 patients (two with multiple sites) and in deep structures in eight patients (4 in cerebellum, 2 in corpus callosum, 2 in basal ganglia). Raised intracranial pressure was present in eight patients, eight had cognitive deficits,

three had visual field defects, two had hypostenia, two had seizures. Twelve patients were treated with WBRT alone, seven with chemotherapy alone and six with a combined treatment. At the end of therapy 19 patients were in CR (76%) and 2 patients obtained a partial remission (8%), with an overall response rate of 84%. Four patients progressed during therapy. The CR rate was 66% (eight out 12) for those treated with WBRT alone, 86% (six out seven) in patients treated with chemotherapy alone and 83% (five out six) in those who underwent CMT. These differences was not statistically significant. After a median follow up of 33 months (range 2-105 months) 18 patients died (sixteen due to disease) and 7 are alive and disease-free. The actuarial overall survival was 42% at 3 years and 27% at 5 years. PFS and disease-specific survival (DSS) at 5 years were 22% and 31% respectively. Multivariate analysis for OS showed the significance of multiple lesions and the newly developed Memorial Sloan-Kettering Cancer Center prognostic model, based on age and performance status. *Conclusions*. We observed a higher (but not statistically significant) rate of CR in patients 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.

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NUCLEAR MORPHOMETRIC VARIABLES AS PROGNOSTIC FACTORS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Age, immunophenotype, peripheral leukocyte number at diagnosis, and specific cytogenetic abnormalities are well-known risk factors in ALL. Recently it has been shown that nuclear texture in Giemsa-stained smears reflects the DNA methylation pattern. Overall methylation frequency is higher in adults than in childhood ALL. *Aim*. to compare the prognostic value of variables of nuclear texture with the current risk factors in ALL. *Material and Methods*. in newly diagnosed patients, clinical and laboratory data, as well as those of quantitative flow cytometric analysis, DNA ploidy and proliferation rate were obtained. BCR/ABL, AF4/MLL, E2A/PBX1 and TEL/AML1 were also examined. Blasts from the diagnostic bone marrow aspirate were digitalized, segmented and morphometric variables of nuclei were examined. Their influence on overall survival was examined using the Cox proportional hazard model, stratifying patients according to T- and B-lineage ALL. *Results*. 49 cases entered the study: 18 with age <18 years and 31 >18 years. T-ALL: 11 cases. Median age: 19 years, median PB leukocyte count: 81.5×10^9/L. B-ALL: median age: 29 years; median PB leukocyte count: 32.7×10^9/L. Gene rearrangements were only found in B-ALL: BCR/ABL in 4 cases and AF4/MLL and E2A/PBX1 2 cases each. In the univariate analysis were related a higher risk: age (p=0.009), PB leukocyte count (p=0.09), presence of risk-associated gene rearrangements (p=0.06), nuclear area (p=0.09) and mean optical density (p=0.01). Mean gray level (p=0.01) and minimal gray level (p=0.003) were related to a lower risk. None of the flow cytometic data had a significant value. In the multivariate analysis, only age, PB leukocytes and minimal gray level were independent prognostic variables. *Conclusions*. in the present study the frequency of gene rearrangements were weaker prognostic factors than the morphometric variables and were excluded from the final model. Variables of classical morphometry showed an important independent prognostic value permitting a retrospective evaluation of the cases. Patients presenting larger and darker nuclei had a worse prognosis. It is worthwhile to validate these results in a larger cohort of patients.

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EFFECTIVENESS OF MEGACHOP PLUS G-CSF AS A PERIPHERAL BLOOD STEM CELLS (PBSC) MOBILIZATION AND HARVEST REGIMEN. COMPARATIVE ANALYSIS OF TWO DIFFERENTS G-CSF DOSE SCHEDULES

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Aims. 1) To assess the effectiveness in mobilizing and harvesting peripheral blood stem cells (PBSC), using Mega-CHOP regimen and G-CSF (filgrastrim), in the autologous stem cell transplantation setting. 2) To analyze two different G-CSF schedule (*low dose* vs *high dose*). *Methods*. We retrospectively studied the results obtained in the PBSC mobilization of 159 patients (87 men and 72 women), with the following diseases: Non Hodgkin Lymphoma (82), Multiple Myeloma (39), Hodgkin Lymphoma (13), Breast Cancer (8), Acute Leukemia (6), Sarcoma (4), Chronic Lymphocytic Leukemia (4), Waldenström Disease (2) and POEMS Syndrome (1). In each case the mobilizing chemotherapy regimen was: Cyclophosphamide 1000 mg/m^2, Doxorrubicin 50 mg/m^2, Vincristin 1,4 mg/m^2 (max. 2 mg), given one day. G-CSF was administred subcutaneously, starting on +10 day, either at 7, 5 µg/kg/day (group 1) or 10 µg/kg/12 hours (group 2) until the completion of the apheresis or mobilization failure evidence. Our aim was to obtain a minimum of 4 $\times10^6$ cells CD34$^+$/Kg for the products that underwent *ex-vivo* purging with an immunomagnetic selection procedure before the final cryopreservation or a minimum of 2 $\times10^6$ cells CD34$^+$/Kg in unmanipulated products. *Results*. The average total CD34$^+$ cells $\times10^6$/kg were 8,07 (0,10 -24,09). 3,14% of the patients (5) didn't reach the CD34$^+$ cells minimum required. From those who achieved the mobilization objectives (150 patients, 93,34%), in 99 cases (66%) only one apheresis process was necessary, in 43 cases (27,77%) two apheresis were required, in 7 cases (4,58%) three processes and just in one case (0,65%) even a fourth one. Comparing both G-CSF dose schedules: 1) Day of apheresis starting: Group 1: 12, 37 (11-14)) vs. Group 2:12, 14 (11-14)) [p= 0.368]. 2) CD34$^+$ $\times10^6$ cells/kg obtained: Group 1: 6,59 (0,19-21,12) vs. Group 2: 8,98 (1.03-24,09) [p=0.045]. *Conclusions*. In our experience, the Mega-CHOP regimen plus G-CSF is a highly effective strategy to perform peripheral blood stem cells (PBSC) mobilization and harvest, with a higher performance reached using a G-CSF high dose schedule and there were statistically significant differences between both group. The first day of apheresis was in both group the +12 day, without any statistically significant differences.

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MONOCYTE AND DENDRITIC CELL ABNORMALITIES IN BONE MARROW OF MYELODYSPLASTIC SYNDROMES

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Background: Recently, a decrease in myeloid and lymphoid precursor dendritic cells (DC) has been described in peripheral blood of patients with MDS. Their origin is controversial, but they are part of the bone marrow stroma (BM) and may have an important role in the pathopysiology of these syndromes. *Aim*. to analyze the production *in vivo* of DC in BM of patients with MDS, and to examine their relation to prognostic factors. *Methods*. BM of newly diagnosed patients with confirmed MDS were examined by multiparametric quantitative flow cytometry (FCM). Cases were classified according to WHO criteria and IPSS. BM from 6 normal donors for allogeneic BM transplantation was used as control. *Results*. 28 cases of MDS entered the study. Median age: 59 years (29-91). By the WHO classification, 9 cases were RA, 1 RARS, 10 were RCMD, 4 were RAEB-1and 4 RAEB-2. The percentage of BM monocytes were significantly increased in MDS (median 2.85%) as compared to controls (median 1.96%) p=0.03. Activated monocytes (CD16$^+$/CD14$^+$) were also increased (median 0.27% vs. 0.09% respectively; p=0.07). The percentage of myeloid DC was also higher in MDS (median 0.56% vs 0.29%; p=0.06). There was a correlation between the number of monocytes and myeloid DCs (r=0.53; p=0.002). No correlation was found between number of these cells and PB cell counts, BM blasts or CD34$^+$ cells, WHO categories and IPSS. Lymphoid DC could also been detected, and were slightly decreased in MDS (median 0.12 vs. 0.16%). Their number, however, showed an inverse correlation with percentage of BM blasts (r=-0.34; p=0.06) and IPSS (r= -0.45; p=0.01). *Conclusion*. in MDS, monocytes are derived from the clonal myelomonocytic precursor. A production of myeloid and lymphoid DC can be detected *in vivo* in BM of patients with MDS. The increase of monocytes and myeloid DCs speaks in favor of their involvement in the inflammatory process occurring in BM in MDS. However, the decrease of lymphoid DCs is related to the progression of the disease.

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Aims. 1) To assess the effectiveness in mobilizing and harvesting peripheral blood stem cells (PBSC), using Mega-CHOP scheme and G-CSF (filgrastrim), in the autologous stem cell transplantation setting. 2) To analyze two different G-CSF schedule (*low dose* vs. *high dose*). *Methods*. We retrospectively studied the results obtained in the PBSC mobilization of 159 patients (87 men and 72 women) with previous diagnosis of: Non Hodgkin Lymphoma (82), Multiple Myeloma (39), Hodgkin´s Lymphoma (13), Breast Cancer (8), Acute Leukemia (6), Sarcoma (4), Chronic Lymphocytic Leukemia (4), Waldenström Disease (2) and POEMS Syndrome (1). Mobilizing chemotherapy regimen was as follows: Cyclophosphamide 1000 mg/m^2, Doxorrubicin 50 mg/m^2, Vincristin 1,4 mg/m^2 (max. 2 mg), given one day. G-CSF was administred subcutaneously, starting on +10 day, either at 7,5 µg/kg/day (Group 1) or 10 µg/kg/12 hours (Group 2) until the completion of the apheresis or mobilization failure evidence. Our aim was to obtain: a) A minimum of 4×10^6 cells CD34$^+$/Kg product, which secondarily underwent *ex-vivo* purging with an immunomagnetic selection procedure, before the final cryopreservation, or b) A minimum of 2 x 106 cells CD34$^+$/Kg in unmanipulated products. *Results*. The average total CD34$^+$ cells ×10^6/kg were 8,07 (0,10 - 24,09). Five patients (3,14%) did not reach the CD34$^+$ cells minimum required. From those who achieved the mobilization objectives (150 patients, 93,34%), a unique apheresis process was necessary in 99 cases (66%), two apheresis in 43 cases (27,77%), three processes in 7 cases (4,58%), and four apheresis in just one case (0,65%). Comparing both G-CSF dose schedules, we observed the following results: 1) Day of apheresis starting: Group 1: 12,37 (11-14) vs. Group 2:12,14 (11-14) [p= 0.368]. 2) CD34$^+$×10^6 cells/kg obtained: Group 1: 6,59 (0,19-21,12) vs. Group 2: 8,98 (1.03-24,09) [p= 0.045] *Conclusions*. In our experience, the Mega-CHOP scheme plus G-CSF is a highly effective strategy to perform peripheral blood stem cells (PBSC) mobilization and harvests. Although the first day of apheresis was in both groups +12 day, a higher performance was reached using a G-CSF high dose schedule and there were statistically significant differences between both groups.

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KIKUCHI-FUJIMOTO DISEASE (KFD) DIAGNOSED SIMULTANEOUSLY WITH SYSTEMIC LUPUS ERYTHEMATOSUS: A CASE REPORT

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Background. Histiocytic necrotizing lymphadenitis (HNL) or Kikuchi-Fujimoto disease (KFD) is a benign and self-limited disorder, characterized by tender lymphadenopathy and is usually accompanied by mild fever and night sweats. The clinical, histopathological and immunohistochemical features appear to point at viral etiology, a hypothesis that still has not been proven. KFD is generally diagnosed on the basis of a biopsy of affected lymph nodes, which shows fragmentation, necrosis and karyorrhexis. The disease mimics, and is associated with, many other diseases, like lymphoma, metastatic carcinoma, systemic lupus erythematosus (SLE), and psoriasis, who can precede, postdate or coincide with the diagnosis of KFD. *Material*. A young woman, aged 25 years presented with prolonged fever, cervical lymphadenopathy and night sweats. Clinical examination revealed cervical lymph nodes with a size ranging from 0,5 to 5 cm, smaller nodes in both axillary areas, and liver and spleen palpable. Laboratory examinations were normal or nonspecific. Complete blood counts showed anemia and raised erythrocyte sedimentation rates. Liver function test were normal but renal function was affected and subsequently progressed, in a couple of days, to nephrotic syndrome with proteinuria and hematuria. The biopsy of the excised lymph node was characteristic of histiocytic necrotizing lymphadenitis with architectural effacement, due to the presence of pale nodular lymphohistiocytic foci with nuclei debris, a few lymphocytes, CD4 and CD8 positive, plasmatocytoid cells, large lymphoid cells (immunoblasts) and absence of granulocytic infiltration. There were no elements of tuberculus lymphadenitis or lymphoma. Serological examinations revealed positivity to antinuclear antibodies and decreased C3 and C4 characteristic for the diagnosis of SLE. The patient was successfully managed with immunosuppressive therapy. *Conclusions*: Although KFD is considered very uncommon, this disorder must be included in the differential diagnosis of *lymph node enlargement* since its course and treatment differs from those of lymphoma, tuberculosis and SLE. The histological differential diagnosis of KFD mainly includes reactive lesions as lymphadenitis associated with SLE, or herpes simplex, non-Hodgkin's lymphoma, plasmacytoid T-cell leukemia, Kawasaki's disease, myeloid tumor and even metastatic adenocarcinoma.

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A NEW P.K404E BCR-ABL MUTATION IN AN PH1 POSITIVE ALL PATIENT

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Background. In patients with Philadelphia acute lymphoblastic leukemia (Ph1ALL), treatment with Imatinib (IM) results in an overall hematologic response rate of about 60%. These results are considerable inferior to those observed in patients with chronic myeloid leukemia (CML)-in fact the responses in Ph1ALL are not sustained and the overwhelming of patients become refractory to treatment after a median of only two months due to the development of resistance.[1] Mutations in BCR-ABL tyrosine kinase (TK) are the most common mechanism of IM resistance. Addition of IM to intensive chemotherapy can produce high-quality complete remission to a majority of newly diagnosed patients, providing them a better chance to receive an allogeneic transplant.[2] More recently, the new TK inhibitors Dasatinib and Nilotinib have produced, in phase I/II clinical trials, very promising hematological and cytogenetic responses in CML and Ph1ALL patients, offering a new tool to circumvent IM resistance.[3] *Aims*. To ascertain if the BCR-ABL variations p.K404E and p.I432I were acquired, and therefore more likely to cause IM resistance, or are constitutional alterations characteristic of the studied patient. *Methods*. BCR-ABL transcript identification and quantification were done according BIOMED-1 and EAC protocols,[4,5] respectively. Mutation screening was done by automatic sequencing of a RT-PCR nested segment including the BCR-ABL TK domain or by automatic sequencing of a PCR segment including the exon of interest. *Results*. A 34 years old female was diagnosed in May 2006 with a Ph1ALL (E1A2 transcript). She began chemotherapy in May 2006, according to hyper-C-VAD protocol, receiving IM in days 1 and 15 of each cycle. She has completed the fourth cycle last December and is now waiting for allogeneic transplant in morphologic, immunologic and cytogenetic remission, although without molecular remission. Table 1 summarizes her follow-up and the molecular studies performed.

Table 1.

Summary/Conclusions. To our knowledge, this is the first time that alterations affecting BCR-ABL amino-acid residues 404 and 432 are described. To ascertain if they were acquired or constitutional, we have sequenced the DNA extracted from the patient oral swab. The absence of p.K404E and the presence of p.I432I in heterozigosity were observed. Therefore, p.K404E (c.1591A>G), which changes the basic lysine (K) to the acidic glutamate (E), is not a constitutional alteration, and is, most probably, an

acquired mutation most prone to cause IM resistance. The heterozigosity of p.I432I (c.1677T>C) in the oral sample, and the fact that it does not cause an amino-acid change, led us to suppose that this should be a polymorphism. We plan, also, to sequence the patient cDNA sample collected at diagnosis. The absence of p.K404E in this sample will be other evidence pointing out to an acquired mutation. Being so, this amino-acid should be taken into account when screening BCR-ABL mutations resistant to IM.

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ANTIBODIES IN THE PATIENTS WITH HAEMOPHILIA EXPOSED TO MULTIPLE TRANSFUSIONS

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Although the indications for transfusion of fresh-frozen plasma and cryoprecipitate are limited, the use of plasma continues to rise in Russia. Residual platelets and RBCs in plasma can provide immunization of the patients. Limited data are available concerning antibodies in multitransfused haemophiliacs. The purpose of our work was the identification of auto- and alloantibodies in the haemophilia patients exposed to multiple transfusions. Auto- and alloantibodies to red cells were identified by direct and indirect antiglobulin test (DiaMed AG, Switzerland). For identification of granulocyte autoantibodies the modified method of agglutination in gel (DiaMed) was used. Platelet auto- and alloantibodies were tested by mixed passive haemagglutination test (Shibata *et. al.*, 1981), which has been modified to avoid HLA antibodies detection. Specificity of HPA antibodies was confirmed by the use of panel HPA genotyped donor platelets. We examined 162 blood samples of patients with haemophilia. Fifteen samples (9,26%) had alloantibodies to red cells (anti-D,-E,-K,-CW). Autoantibodies to red cells were not detected. Blood of 139 patients was investigated for platelet autoantibodies, forty eight (34,53%) of them were positive. Out of 97 analysed blood samples, 41 were positive for platelet alloantibodies (42,27%). The specificity of antibodies was the following: anti- HPA-1a (6 samples), anti- HPA-1b (4 samples), anti- HPA- 2a (2 samples), anti- HPA- 2b (7 samples), anti- HPA- 3a (8 samples), anti- HPA 5a (2 samples). Positive antibody test without detection of specific antibodies was found in 12 patients. 136 patients were investigated for granulocyte auto-immunization, three of them (2,21%) were positive. As a rule, patients with haemophilia receive the transfusions repeatedly, that enables to observe the process of antibodies formation in dynamics. 18 patients were repeatedly investigated for platelet autoantibodies: 10 remained negative, 6 patients developed the autoantibodies, platelet autoantibodies disappeared in 2 patients. Thus, after multiple transfusion of fresh-frozen plasma and cryoprecipitate, platelet auto- and allo-immunization (34,53% and 42,27% accordingly) develops in patients with haemofilia in addition to RBCs alloimmunization.

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RADIOFREQUENCY ABLATION FOR RESIDUAL HEPATIC HODGKINS LYMPHOMA: A CASE REPORT

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Percutaneous radiofrequency (RF) ablation is a well documented treatment for primary and metastatic localized hepatic tumors.[1] Until now few cases of RF in lymphoma patients have been reported.[2] In October 2003 a 33-year-old woman presented with cervical lymphoadenopathies, fever, fatigue and abdominal pain. Hodgkin's lymphoma (HL), sclerono-dular variety, was diagnosed by lymphonode biopsy. CT scan showed mediastinal and para-aortic not bulky lymphoadenopathies and a hepatic lesion suggestive for a disease localization with a maximum diameter of 23 mm. HL was staged as IVB and ABVD treatment was started. After 4 cycles patient achieved a partial response, with complete regression of lymphoadenopathies but a stable size of the hepatic lesion. At the end of 8 ABVD cycles restaging by CT and PET confirmed the complete regression of nodal disease while the hepatic lesion, measuring 16 mm, was still present and metabolically active. Two DHAP cycles were administered and peripheral blood stem cells harvest after the first cycle was successful (20.3×10^6 $CD34^+$/kg). After the second DHAP the hepatic lesion exhibited further reduction, being the unique residual site of HL with a maximum diameter of 14 mm. In January 2005 patient underwent eco-guided percutaneous RF ablation of the hepatic lesion with no significant toxicity. After RF PET was negative and CT still showed the hepatic focal lesion, with a maximum diameter of 14 mm. Patient is now alive and in complete remission after a 26 months follow-up. This case shows that RF, a safe and well known treatment for solid tumors, could be considered as a promising chance for residual HL in the liver, a location not susceptible of external radiation therapy.

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ORGAN DERIVED MATRIX EXTRACTS INDUCE TISSUE SPECIFIC DIFFERENTIATION OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Background. Bone marrow derived mesenchymal stem cells (BMMSC) are rare multipotent cells that can differentiate into multiple lineages such as bone, cartilage and muscle cells among others. Hence, these cells are of potential clinical importance for the repair of damaged tissues. The local microenvironment *in vivo* is critical to support the desired differentiation of stem cells or to sustain the phenotype of the stem cell-derived *in vitro* differentiated cells. This local microenvironment comprises a physical support supplied by the organ matrix as well as tissue specific cytokines. Colloss® and Colloss® E (Ossacur AG) are sterile acellular lyophilizates extracted from bovine and equine bone matrix, respectively. *Method*. We tested the effect of Colloss® and Colloss® E, and cartilage acellular extracts of bovine origin on human BMMSC differentiation. *Results*. BMMSC treated with either Colloss® or Colloss® E exhibited characteristic morphological changes accompanied by the expression of osteoblast specific markers such as alkaline phosphatase activity, osteopontin secretion and calcium deposits, explicitly demonstrating that these bone matrix extracts induce osteoblastic differentiation of BMMSC *in vitro*. Similarly, treatment of BMMSC with either meniscus or joint derived cartilage extracts resulted in the formation of cell aggregates with a central alignment containing pre-hypertrophic cells in the middle suggestive of chondrocytic differentiation. This was accompanied by the expression of chondrocyte specific markers such as Sox9, aggrecan and glycoso-amino-glycans production, hence demonstrating that they induce chondrocytic differentiation of BMMSC *in vitro*. Control cultures, studied at all time points, maintained a spindle shaped morphology typical of bone marrow-derived mesenchymal stem cells. Importantly, Colloss® or Colloss® E did not induce chondrocytic markers, and conversely, cartilage extracts did not induce osteoblastic markers, hence demonstrating organ specificity in BMMSC differentiation phenotype. *Conclusions*. Colloss®, Colloss® E, and cartilage extracts seem to provide stem cells with specific structural and soluble mediators that mimic the *in vivo* microenvironment, and induce bone- or cartilage-specific differentiation of BMMSC, respectively. These results support a novel tissue engineering-based tissue repair, using autologous BMMSC pretreated with organ derived matrix extracts.

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SECONDARY ANTIFUNGAL PROPHYLAXIS (SAP): AN EXPERIENCE OF A BRAZILIAN HEMATOLOGY SERVICE

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Background. Patients undergoing chemotherapy or bone marrow transplantation (BMT) have a great risk to develop invasive fungal infections (IFI). IFI are associated with high mortality in this group of patients and most of them are unable to finish their planned treatment after a diagnosis of IFI. The risk of reactivation of prior fungal infections is prohibitive high in this situation. Therefore, in the last years, the interest in secondary antifungal prophylaxis (SAP) has increased. This strategy may allow the continuation of the treatment of hematological disease with an acceptable protection against IFI relapse. Therefore, it was our objective to relate our experience in SAP. *Methods*. We retrospectively analyzed seven patients who had a proved invasive fungal infection and used SAP. *Results*. The median age was 49 (7-65) years-old. There were four cases of Aspergillus spp., one Fusarium spp., one Mucor spp. invasive infection and one hepatosplenic candidiasis. Three patients had acute myeloid leukemia (AML). AML patients had aspergillosis, candidiasis and zygomycosis. Zygomycosis patient interrupted for 3 months full chemotherapy after diagnosis of IFI. After an accumulated dose of L-Amb higher than 2000 mg, he received a reduced AML induction protocol using amphotericin as SAP. He went well without any sign of reactivation or progression. Two patients developed aspergillosis after BMT. Both used voriconazole as SAP during prednisone and CyA for GVHD treatment. One patient had received the diagnosis of AML and had developed Fusarium spp. pneumonia during first AML induction phase. He received a second induction treatment and used voriconazole for two months until there had been only a small sign in chest CT. During conditioning for allo BMT and during steroid treatment for acute GVHD grade III we maintained him with oral voriconazole. He never showed any sign of relapse or progression of the IFI. There was one patient with mantle cell lymphoma who received HyperCVAD protocol without interruption nevertheless a proven pulmonary aspergillosis was made early during treatment. All patients with Aspergillus spp. and Fusarium spp. infections used voriconazole as SAP. Although some of them used the drug for months no side effects were observed. None of them reported visual hallucination a frequent side effect described for this drug. Mucor spp. invasive infection and hepatosplenic candidiasis cases used amphothericin and fluconazole, respectively. Until now, no patient has any sign of reactivation or progression. *Conclusions*. Prior IFI without prophylaxis carries an unacceptable risk of relapse and death in patients with subsequent immunossupression (Sipsas and Kontoyiannis CID 2006). SAP should be evaluated in further studies but in our cases it was well tolerated and until now, no patient present any sign of fungal reactivation.

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IS THERE A DIFFERENCE IN THERAPY RESULTS BETWEEN ACUTE MYELOID LEUKEMIA PATIENTS WITH MYELOBLASTIC BONE MARROW INFILTRATION WITHIN 20-29% AND =30% AT DIAGNOSIS?

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Definition of acute myeloid leukemia (AML) combines groups of disorders, which differ in pathogenesis, cytogenetics, clinical signs, results of therapy and prognosis. They belongs to cancers of granulocyto-monocytic, megakariocytic or erytroblastic systems and they are characterized by bone marrow, peripheral blood and often internal organs infiltration by clone of abnormal cells deriving from early stages of hematopoiesis. Basic criterion of diagnosis of AML is level of bone marrow infiltration by myeloblasts. Thirty years ago FAB (French-American-British) classification was defined and according to this recommendation AML was diagnosed based on morphocytochemic and immunophenotypic features of myeloblasts with bone marrow infiltration ≥30%. In 2002 the group of WHO investigators published new classification scheme of AML based on cytogenetic and biomolecular features of myeloblastic cells. They decided to decrease level of blastic bone marrow infiltration necessary to diagnose AML to ≥20%. The remaining question was if there is a difference in therapy results between two groups of AML patients: with blastic bone marrow infiltration within 20-29% and ≥30%. We provided retrospective analysis to answer to this question. The analysis was performed on patients population treated according to PALG (Polish Adult Leukemia Group) protocol of DAC vs. DA study. There were 26 cases with myeloblastic bone marrow infiltration within 20-29% (assessed group), and 379 patients with infiltration ≥30% at diagnosis (comparative group) treated within the above mentioned study in years 1999-2002. The patients received as induction DAC-7 regimen: daunorubicin 60 mg/m^2/d iv 1-3; cytarabine 200 mg/m^2/d ci 1-7; cladribine 5 mg/m^2 2h inf. iv d 1-5 or standard DA-7 regimen (the same regimen without cladribine). Patients achieving CR received two courses of subsequent intensive consolidation: 1) HAM (HD AraC, mitoxantrone) 2) HD AraC with or without cladribine in the DAC-7 or DA-7 arm, respectively. In the case of PR after the first induction course the same regimen was repeated. Post-consolidation therapy was in both arms comparable. In 62,5% patients (n=10) of the assessed group AML was preceding by myelodysplastic syndrome. Complete remission (CR) rate was comparable in both populations and reached 73% (n=19) in the assessed group and 70% (n=264) in the comparative one. Simultaneously, there was no statistical difference between overall survival (OS) and leukemia free survival (LFS) in both groups. OS after 5 years reached 34% in the assessed group and 27% in the comparative one and LFS reached 42% and 27%, respectively (p=NS). Our retrospective study did not show any difference in therapy results between AML patients with bone marrow infiltration within 20-29% and ≥30% at diagnosis and it can be an additional argument to join both groups to identical therapeutic proceedings.

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ELEVATED SERUM CONCENTRATIONS OF INTERLEUKIN-6 RECEPTOR AND INTERLEUKIN 1β CORRELATES WITH PCNA PROLIFERATIVE INDEX IN MULTIPLE MYELOMA

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Background. Various cytokines have been involved in the control of growth and progression of multiple myeloma (MM). Among these, Interleukin-6 (IL-6) has been recognized as the major growth factor for human myeloma cells *in vitro*. Soluble IL-6 receptor (sIL-6R) is derived from the extracellular domain of the IL-6 receptor. Increased serum levels of sIL-6R have been found in MM patients in the early or late phase of the disease, while patients in the plateau phase exhibit normal levels of the receptor. Plasma cell nuclear antigen (PCNA) is a proliferative indice whose level of expression varies during the cell cycle, beginning in the late G1 phase and becoming maximal in the S phase. IL-1β is regarded a potent inducer of IL-6 production by stromal cells and monocytes and some studies have suggested an important role of IL-1β in myeloma bone disease. *Aim*. The aim of this study was to determine serum IL-1β and sIL-6R in MM patients at diagnosis and to investigate their correlation with plasma cell infiltration and the proliferative index PCNA. *Materials and Methods*. Forty-four patients with MM (24 male and 20 female, mean age 66 years) were included in the study. At the time of serum collection 12 patients had stage I, 14 had stage II and 18 had stage III according to the criteria of Durie and Salmon's myeloma staging system. Serum samples were collected before initiation of treatment. Ten age-and sex-matched healthy volunteers were used as controls. The measurement of IL-6R and IL-1β in the serum was performed by solid-phase sandwich enzyme-linked immunosorbent assay (ELISA), employing monoclonal human anti IL-6R and anti IL-1β antibodies from commercially available kits (Quantikine® R&D systems). Paramat-embedded bone marrow biopsy was double-immunostained with antibodies to PCNA and CD38 using the DAKO En Vision System. *Results*. The mean concentrations of IL-6r, IL-β, PCNA and infiltration in the entire group of patients were 1350.79±644.92 ng/mL, 2.99±1.16 pg/mL, 26.59±25.30 and 4040.62±2191.68 respectively. SIl-6R, IL-1β and PCNA were significantly different among the three stages of disease p<0.001, p<0.001, p<0.005 respectively. Furthermore in the entire group of patients with MM sIL-6R, IL-1β and PCNA were significantly higher in patients in comparison to controls p<0.0001, p<0.0001, p<0.0004 respectively. A positive correlation was found between IL-6r and IL-1β (r=0.572, p<0.0001), IL-1β and PCNA (r=0.479, p<0.004). *Conclusion*. Serum IL-6r and IL-1β reflect the proliferative activity of malignant cells in multiple myeloma.(393 words)

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ROS GENERATION AND APOPTOSIS IN HUMAN UMBILICAL STEM CELLS BY LOW DOSE RADIATION. PROTECTIVE EFFECT OF IGF1

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Backergound. CD34 cells are sensitive to radiation. Apoptosis may be triggered by alterations in energy production system, translated in ROS generation by the mitochondria of irradiated cells. *Aims*. The aim of our study was to investigate the sensitivity of CD34 cells to low dose radiation and the possible inhibitory/protective role of IGF1 on free radical production by the mitochondria of the irradiated human CD34 umbilical stem cells. *Methods*. Cord blood was collected from normal full-term deliveries and CD34 cells were isolated using MACS system. The purity of isolated cells was evaluated by flow cytometry using a FITC conjugated monoclonal anti-CD34 antibody. CD34 cells were diluted to 25×10^3 cells/mL and cultured with IMDM supplement with 20% BIT. Cells were irradiated with 0, 1, 2 and 5Gy and IGF1 was added to the culture (100 ng/mL) 30 min prior to radiation. Apoptosis was evaluated 6 and 24h after irradiation by flow cytometry using Annexin-V/PI kit. ROS generation was evaluated by FACS analysis 30min and 24h after radiation using a) Hydroethidine staining of cells for O_2. - and b) chloromethyl-H2DCFDA staining (molecular probe) for H_2O_2. For statistical analysis we used two paired t-test. *Results*. 1. Thirty minutes after radiation with 1, 2 and 5Gy, the percentage of irradiated cells that generated O_2.- increased to 5, 11 and 30%, compared to the unirradiated cells innate production. At the same time points, H_2O_2 increased 4, 8 and 20%, respectively. 2. Twenty four hours after radiation, the irradiated cells continued to generate O_2.- relatively to radiation dose 1, 2 and 5Gy, in percentage 11.2, 18.6 and 26% compared to innate production of unirradiated cells, but the fraction of H2O2 producing cells was not affected. 3. The addition of IGF-1 inhibited the ROS production by the irradiated cells significantly at higher doses, at both early and late time points. IGF-1 inhibited percentage of irradiated cells that generated O_2.- 11% and 20% in 30min after 2 and 5Gy, respectively and 8.5% and 8% in 24h. The fraction of H_2O_2 producing cells was inhibited by IGF-1 in 30 min 6% and 13% after 2 and 5Gy, respectively. 4. Six hours after radiation with 1, 2 and 5Gy, apoptosis was increased 31.6%, 37.35% and 42.95% respectively ($p<0.05$). 5. The increase of apoptosis at 24h time point was estimated 58.4%, 67.1% and 77.1% after 1, 2 and 5Gy, respectively ($p<0.05$). *Conclusion*. The generation of free radicals by irradiated $CD34^+$ umbilical stem cells is proportional to radiation dose and triggers apoptosis mechanism. The surviving cells may acquire DNA damage and subsequent chromosomal aberrations. IGF1 may be an inhibitory and protective factor to this procession.

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LOW FREQUENCY OF TEL-AML1 FUSION GENE IN CHILDHOOD B LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA OF TUNISIAN PATIENTS

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Childhood acute lymphoblastic leukaemia (ALL) is clinically heterogenous with prognostically and biologically distinct subtypes. TEL-AML1 fusion gene is the commonest known genetic abnormality in childhood B acute lymphoblastic leukaemia (ALL)occurring in up to 30% of cases. This rearrangement is generated by a reciprocal translocation t(12;21)(p13;q22) which is difficult to detect through conventional cytogenetics and is associated with a favorable prognosis. We used RT-PCR on medullar samples to investigate the incidence of this rearrangement in Tunisian patients. 78 children with newly diagnosed B acute lymphoblastic leukaemia were included in this study .48 female and 30 male .the mean age of the patients was 6.8 years (1-17).median white blood cell counts was $47\ 000\times10^9$/L. Only 3 out of 78 (3%) were positive for this rearrangement. Compared to other pediatric populations TEL-AML1 is underrepresented in Tunisia. Our results confirm that there are indeed significant and important racial differences in the frequency of subtypes of childhood ALL and suggest the existence of geographic /race variations in the genotype of ALL.

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HODGKINS DISEASE IN ELDERLY: CLINICAL PROFILE AND SURVIVAL OVER A 5 CASE REPORT

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Background. Hodgkin's disease occurs most frequently in young adults, although any age group can be affected. *Aims*. Considering that age is a negative prognostic factor for Hogkin's disease, we investigated if Hodgkin's disease in elderly people has a particular clinical picture and outcome. *Methods*. We made a retrospective descriptive analysis on the patients diagnosed and treated for Hodgkin's disease (HD) in our clinic between 2005-2007. *Results*. Among 43 patients with HD, 5 were aged over 65 years. The diagnosis was made by immunochemistry according to WHO Classification. The disease was slightly more frequent in men than in women (sex ratio 3/2), comparable to the general statistic data, and the mean age was 73 years. The most frequent histological subtype was mixed cellularity (2 cases), followed by nodular sclerosis (1 case) and lymphocyte depletion (1 case). According to Ann-Arbor clinical stage, advanced disease III-IV was more frequent (4 cases) than localized disease (stage I-II). All patients experienced B symptoms. Bulky disease was present in 1 patient, with mediastinal involvement in 2 cases and abdominal adenopathies in all 5 cases, 2 cases being with retroperitoneal masses. Pancytopenia was present at diagnosis in 1 case, concordant with the BM involvement. Concerning the prognostic factors, the whole group started with a poor prognosis through the age. Prognosis was also poor through the fact that most of them had an advanced clinical stage at diagnosis-III-IV, with more than 3 nodal groups affected, and 1 with bulky disease, all with signs B, and most of them were males. Histological subtype present in most cases - mixed cellularity, poses an intermediate prognosis, while that with lymphocyte depletion subtype has even a worse prognosis. None of the patients had leukocytosis, one had lymphopenia, and two had hemoglobin under 10 g/dL at diagnosis. ERS and LDH were increased in 4 patients, β2microglobuline could not be performed in any patient at diagnosis and none of them had albuminemia under 4 g/dL. The patients were treated according to combined modality treatment using chemotherapy (mainly ABVD and Stanford V) and involved field radiotherapy. One patient had poor tolerance to chemotherapy, with neuropathy. Response to first line therapy was good in 2 patients in CR+PR, but with 3 patients were resistant. None with abdominal adenophaties experienced a CR. One patient relapsed during the first year with extranodal determination (skin). 2 patients died during this period. *Conclusions*. In our analysis and according to the literature we conclude that HD in elderly people, especially with abdominal node and extranodal involvement had a high rate of resistance and relapses as an aggressive disease. In addition, we noticed a poor tolerance to chemotherapy and therapy with individualized protocol was effective even at low doses.

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THERAPEUTIC PLASMA EXCHANGE IN NEUROLOGICAL DISEASES: SINGLE CENTER EXPERIENCE

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Therapeutic plasma exchange (TPE) has been used for the treatment of neurologic diseases in which autoimmunity plays a major role. We describe our experience in 28 patients treated with TPE between 2002-2007 years. Neurological indications included Guillain-Barre syndrome (GBS) (n=22; 78%), acute disseminated encephalomyelitis (ADEM) (n=3; 10%), myasthenia gravis (MG) (n=2; 6%), and multiple sclerosis (MS) (n=1; 3%). 78% (n=22) of the patients were males; patients median age = 48 years, (range: 17-73). Patients were graded before and after TPE according to Hughe's functional grade scale: 3 patients (%10) with GBS were grade 5,12 (%40) grade 4, 7 (%25) grade 3, all patients with ADEM were grade 5, patient with MS and 2 patients with MG were grade 4. 5 out of 22 patients with GBS were unresponsive to intravenous immunglobulin (IVIG) and 3 were unresponsive to intravenous pulse steroid therapy. TPE was administered as first line treatment for the remaining 14 patients with GBS and other neurological diseases. Continuous flow cell separators were used for TPE. It was generally given every other day for all of the patients and 1-1.5 plasma volume was exchanged for

each cycle. Mean five sessions (range: 3-7) TPE were performed. Although 3 patients with ADEM and 3 patients with GBS did not show any improvement after TPE, all patients with MG and MS and 19 patients with GBS were complete response. In no case was the process terminated because of adverse events. We conclude that TPE is extremely effective in neurological diseases, particularly for GBS which autoimmunity plays an important role in pathogenesis. The cost and effectiveness of TPE need to be further investigated by randomized prospective clinical trials conducted with larger series of patients.

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VINCRISTINE SULFATE LIPOSOMES INJECTION (VSLI) IS SAFE AND EFFECTIVE IN PATIENTS WITH RELAPSED AND REFRACTORY MANTLE CELL LYMPHOMA

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Background. New and more effective agents are needed to improve treatment outcomes for patients with relapsed and refractory mantle cell lymphoma. VSLI is novel formulation of vincristine encapsulated in sphingomyelin liposomes or *sphingosomes*. In preclinical studies, sphingosomal technology has been shown to provide targeted, increased and sustained delivery of vincristine to tumor cells compared to free vincristine or vincristine encapsulated in a conventional liposome. *Aims*. Evaluate safety and efficacy of VSLI in patients with relapsed and refractory mantle cell lymphoma. *Methods*. Mantle cell lymphoma patients from two multicenter phase 2 studies (Study CA99002 and DM97-162) of VSLI in relapsed and refractory lymphoma are integrated for safety and efficacy evaluation. In both studies, after informed consent was obtained, VSLI (2.0 mg/m^2 IV over 60 minutes) was administered to eligible patients every 14 days up to 12 cycles until toxicity or progressive disease was observed. Treatment did not exceed 12 cycles unless a patient might obtain a benefit from continued treatment. The primary efficacy endpoint of the 2 studies was the objective response rate (ORR) defined as the percentage of patients whose best response was complete response (CR), complete response unconfirmed (CRu) or partial response (PR). Best response was determined according to International Workshop Response Criteria. Secondary endpoints included adverse events evaluation, time to progression (TTP) and overall survival (OS). *Results*. Of the patients who received at least one VSLI dose, ten (8M/2F) were diagnosed with mantle cell lymphoma. At baseline the median age was 67 years (range, 54-75), compared to 62 years (range, 20-87) for all patients in the 2 studies. One patient had transformed and nine had *de novo* aggressive disease. Five patients (50%) had bone marrow involvement at baseline. Patients were heavily pretreated. Median number of prior lines of chemotherapy and immunotherapy regimens was 4 (range, 2-8). All patients had prior exposure to at least one of the following class of agents that cause neurotoxicity: platinums (50%), taxanes (12.5%) and vincristine (87.5%). Ninety percent (9/10) achieved a CR or PR to the frontline therapy and 20% (2/10) achieved a CR or PR to their last therapy prior to enrollment. Median duration of exposure to VSLI was 56 days (range, 14-125). The most commonly reported adverse events were constipation and peripheral neuropathy. Two patients experienced Grade 3 peripheral neuropathy. After treatment with VSLI, 2 patients had partial response, 3 patients had stable disease, 3 patients experienced disease progression, and 2 patients were unevaluable. Median OS and TTP were 247 and 132 days, respectively, compared to 260 and 111 days for all intent-to-treat patients from both studies. *Summary/Conclusions*. These preliminary results suggest encouraging activity and tolerability of VSLI in heavily pre-treated relapsed and refractory mantle cell lymphoma patients. Further studies of this investigational agent in this setting are needed.

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COMPARISON OF DENDRITIC CELLS IN HEALTHY VOLUNTEERS AND SUBJECTS WITH MONOCLONAL GAMMOPATHY AND/OR MULTIPLE MYELOMA

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Background. Dendritic cells (DC) are highly specialised antigen-presenting cells, which can be used for immunotherapy trials. Functionally normal DCs play a critical role in the activation and potentiation of tumour antigen-specific responses, but in some cases they can be defective. *Aims*. Finding of differences and/or functional defects in DCs from multiple myeloma (MM) and monoclonal gammopathy of unknown significance (MGUS) subjects in relation to the disease and serum IL-6 levels. *Methods*. Maturation of DCs from 18 healthy donors, 18 subjects with MGUS and 20 MM patients was tested in an *in vitro* study. DCs were generated from an adherent mononuclear precursors of peripheral blood cultured in presence of IL-4 and GM-CSF with human CD40Ligand stimulation. Serum free and an autologous serum conditions were used and expression of antigens CD1a, CD14, CD80, CD86, HLA-DR, CCR6, CCR7 was tested by flowcytometry. Levels of IL-12p70 in supernatant of cultures and also levels of IL-6 in serum of peripheral blood were analysed by ELISA. *Results*. We found no differences in DCs maturation ability between groups of healthy control, MGUS and MM subjects under serum-free conditions with or without CD40L stimulation. Expression of the maturation marker CD83 was not high (healthy control 10,0±6,6%; MGUS 14,6±13,3%; MM 15,7±12,1%) and expression of CD1a was significant lower (p= 0,014) in healthy volunteers when compared with MGUS (healthy control 7,4±5,2%; MGUS 23,0±18,4%; MM 12,1±11,9%). Under autologous condition we found a significant negative effect in MGUS as well as in MM subjects when compared with healthy volunteers. It was manifested by reduction of CD83 (healthy control 36,5%; MGUS 9,5%; MM 13,2%), CD80 (healthy control 63,4%; MGUS 10,3%; MM 12,8%), CCR6 (healthy control 15,1%; MGUS 2,1%; MM 3,3%) and CCR7 (healthy control 14%; MGUS 1,1%; MM 2,5%). Production of IL-12p70 was low in any case when compared immature and mature DCs (healthy control 0,19 vs. 0,78 pg/mL; MGUS 0,3 vs. 0,69 pg/mL; MM 0,25 vs. 0,52 pg/mL) and no difference in IL-6 serum levels between groups was found (median for healthy control 1,7 pg/mL; MGUS 3,1 pg/mL; MM 1,9 pg/mL). *Summary/Conclusions*. There were no differences between healthy volunteers, MGUS and MM subjects under serum free conditions, but stimulation with CD40L did not lead to the full maturation of DCs. Autologous serum had a negative influence on expression of costimulatory and maturation antigens of DCs in MGUS and MM subjects when compared with healthy controls. This influence was not caused by IL-6 alone.

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DETECTION AND QUANTIFICATION OF CYTOMEGALOVIRUS IN BONE MARROW TRANSPLANT RECIPENTS BY REAL TIME PCR AND PP65 ANTIGENEMIA

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Cytomegalovirus (CMV) is the most important pathogen that causes mortality and morbidity in transplant recipients. Detection of CMV antigenemia and viral genome is important in diagnosis of CMV disease. The aim of this study was to compare real time PCR and CMV antigenemia test for detection and quantification of Cytomegalovirus. A total of 181 blood specimens from 42 bone marrow transplant recipients were included in the study. CMV antigenemia was investigated by indirect immunofloresan method (CINA kit Argene, Biosoft, Fransa) for the detection of pp65 in leukocyte. CMV DNA was tested by real-time PCR method (Fluorion Iontek, Turkey). 166 Qualitative results were analyzed with The McNemar's Chi-Square test. Quantitative results were analyzed with Spearman's test. CMV antigen and CMV DNA were found negative in 122 (67.4%), CMV antigen and DNA were found positive in 14 (7.7%) of the specimens. Nine samples (5%) were antigenemia positive but CMV DNA negative, 21 samples (11.6%) were CMV DNA positive but CMV antigenemia negative. Antigenemia test could not evaluated in 15(8.3%) specimens as there were insufficient polymorphonuclear leukocytes. Five of these 15 specimens were found CMV DNA positive. When analyzed the results of pp65 assay and PCR data in 166 specimens statistical significance was found between tests (p<0.05). The quantitative results of two assays were correlated (rho=0.54 p=0.04). CMV antigenemia and CMV DNA PCR assays should be performed together for the diagnosis of CMV disease in bone marrow transplant recipients.

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DIARRHEIC SYNDROME, A CLINICAL SIGN OF INTESTINAL INVOLVEMENT IN PROGRESSING CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. B-chronic lymphocytic leukemia (B-CLL) is characterised by a progressive accumulation of mature and immunoincompetent lymphocytes in hematopoietic organs. Extrahematopoietic infiltration is minimal or absent at diagnosis but can occur in end-stage disease. Lung, pleura, skin, central nervous system and kidney are the most frequent involved organs. B-CLL can also infiltrate gastrointestinal (GI) tract but the frequency is considered to be low (5-17%) even in postmortem studies. *Aims*. 1.To determine the incidence of GI involvement by B-CLL in 28 out of 129 B-CLL patients who underwent a gastroscopy and/or colonoscopy examination by several reasons. 2.To investigate clinical signs that could suggest GI involvement. *Materials and methods*. In 28 patients out of 129 B-CLL patients controlled in our institution, upper (n:15) and lower (n:17) endoscopic procedures were performed, comprising a total of 32 examinations. The reasons to perform a colonoscopy were as follows: active intestinal bleeding (n=4), polyposis (n=1), adenocarcinoma (n=1), ferropenia (n=1), changes in bowel habits (n=4) and chronic diarrhea (n=6). Most of the endoscopic procedures were done in the setting of advance stage and/or progresive disease excluding Richter tranformation. *Results*. Gastroscopy did not show histological evidence of B-CLL infiltration in any patient. The results of colonoscopy exams were the following: villous adenoma (n:2), bowel involvement by B-CLL lymphocytes (n:4) and no abnormalities (n:11). In three patients, GI infiltration involved colonic mucosa, and in one of them sigmoid colonic mucosa was affected , too. The fourth patient presented infiltration only in terminal ileum . Rectum was not involved in any case. Biopsies showed focal (n:3) and diffuse (n:1) infiltration by characteristic B-CLL lymphocytes. Immunohistochemistry was performed in 3 out of 4 and was consistent with B-CLL . Clonality pattern in intestinal mucosa infiltrates was demonstrated in 2 out of 3 cases. *Discussions*. GI involvement in B-CLL is uncommon according to previously reported data. In our experience, no gastric involvement was detected. The incidence of intestinal infiltration was 3.1% in the setting of clinically progressive disease. In all patients with intestinal involvement, the main clinical sign was persistent diarrhea. To summarize, B-CLL lower GI tract involvement shoud be taken into account in those patients who present diarrheic syndrome in the setting of progressive disease.

1474

THE INFLUENCE OF IPI, KI67 AND BCL-2, ON SURVIVAL IN PATIENTS WITH DLBCL TREATED WITH R-CHOP REGIMEN - SERBIAN LYMPHOMA STUDY GROUP EXPERIENCE

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Several clinical parameters including International Prognostic Index (IPI) as well as biological parameters (Ki67, Bcl-2,), are considered to have prognostic relevance in diffuse large B-cell lymphomas (DLBCL). The international prognostic Index based on clinical parameters is strongly predictive of outcome. High proliferative rate has been associated with worse survival in some series while Bcl-2 expression has been associated with poor prognosis in patients with DLBCL. The aim of study was to investigate the correlation between IPI and expression of bcl-2 and Ki-67, and their influence on OS survival. Retrospective analysis was performed on 50 patients (31 male/ 19 female, mean age 48 years, range 17-87) randomly selected from a large group of patients (pts) diagnosed and treated with R-CHOP regimen. Median follow up was five years. Initial IPI was determined in all pts. Staining for bcl-2 and Ki-67 was performed on paraffin- embedded sections using an indirect immunoperoxidase method and a specific monoclonal antibody. We analyzed the percentage of neopastic cells with Ki67+ nuclear staining on 10 different high power microscopy fields (HPF, 400x). The intensity of these stainings was graded as weak (0-30% Ki-67+), moderate (31-60% Ki-67+ cells), and strong (>60% Ki67+ cells). Tumors were considered positive when at least 50% of tumor cells expressed bcl-2 protein. According to the IPI , distribution of DLBCL patients was as follows: 0,1-11pts, 2- 19pts, 3-5 in 20pts. Patients with high IPI had significantly shorter survival comparing to pts in low and intermediate IPI (20,5 vs 52,3 m; $p<0,05$). Patients with low and intermediate proliferative fraction had better survival (OS5y 74%) comparing to patients with high proliferation with OS5y 48% ($p<0.01$). There was a positive correlation between high Ki67+ and the high IPI. Neither positive nor negative bcl-2 had any prognostic value, but in the group of pts with high proliferative fraction, we found positive correlation with bcl-2 positivity. The patients with initial high IPI score associated with high proliferative Ki67+ might be at higher risk of fatal outcome.

1475

CLINICAL COMPLICATIONS AND EVOLUTION OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background. Essential thrombocytemia (ET) and Polycythemia Vera (PV) are Philadelphia negative chronic myeloproliferative syndromes with common biological and clinical manifestations. Recent identification of JAK2 (V617F) mutation open new discussion about pathogenesis and prognosis of PV and ET. Main cause of morbidity and mortality are thrombotics events. Thrombophilia and presence of JAK2) mutation may increase the risk of thrombosis. *Aims*. Purpose of the this study was to: analyze the clinical complicatios and evolution of ET and PV patients form a single institution and explore the relations of clinical evolution with their haematological parameters, thrombophilia, and presence of JAk2 mutation. *Methods*. We have studied 79 patients (58 ET and 21 PV). Sex distribution was: 35 males and 44 women. Age: ranged from 20 to 86 years (mean 59±15). Patients follow up was from six to 253 months. Correlation of haemoglobin, leukocytes and platelets parameters at diagnosis with clinical evolution and with presence of JAk2 mutation was examined. In all patients, the presence of thrombophilia alterations was studied. The studies included: APTT, won Willebrand antigen, Protein C, Protein S, and Antithrombin III, lupic anticoagulant, anticardiolipin antibodies, Protein C Activated Resistance (PCAR), Factor V Leiden, Prothrombin 20210A and MTHFR mutation. Statistical analysis was performed with SPSS software. *Results*. Patients with ET present thrombosis in 29%.(12% at diagnosis 17% on evolution respectively). Patiens with PV showed thrombosis 38% (14% at diagnosis and 24 on evolution). Hemorragic manifestation appear in 3%. 13% of the patients died before 10 years evolution. Dead was associated with secondary neoplasias, thrombosis, mielofibrosis and bacteriemia. Late complications were more frecuent in PV patients (19%). JAK2 mutation was present in 95% of PV and 56% of the ET patients. ET patients with JAK 2 mutation presented higher hematocrit values (44±5 vs 40±3; $p=0.04$), higher white blood cell counts 8951 vs 7304; $p=0.05$) and higher mean number of platelets (908.000 and 685.000 respectively; $p=0.01$). Incidence of thrombosis was not correlated with hematological parameters, presence of thrombophilia or JAK mutation. Incedence of thrombosis was correlated with age. *Conclusions*. 1. Results confirm higher incidence of thrombosis and late complications in PV than ET. 2. Only age remain as significant prognostic factor 3. Prospective studies on young patients are needed.

1476

MYELODYSPLASTIC SYNDROMS IN CHILDREN OF REPUBLIC BELARUS

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Introduction. Myelodysplastic syndromes (MDS) are clonal hematologic stem cell disorders characterized clinically and morphologically by ineffective hematopoiesis. *Methods*. Clinical, morphological, histological, cytogenetic and molecular methods of study were used. *Results*. From 1979 to 2006 we observed at our institution 47 new cases of MDS in children. The male/female ratio was 2,7/1 and age 9 months - 18 years old. The median age was 6,8 years. Prediagnostic period was 2 weeks - 9 months, median - 3,3 months. According to the FAB criteria, 47 patients were classified as follows: refractory anaemia (RA) - 25,5%; RA with ringed sideroblasts (RARS)-2,1%; RA with excess of blasts (RAEB) - 34%; RAEB in transformation-15%; chronic myelomonocytic leukaemia (CMML)-23,4%. According to the WHO classification, the patients were reclassified as follows: RA-8,5%; RARS-2,1%; refractory cytopenia with multilineage dysplasia (RCMD) - 17%; RAEB-34%, myeloproliferative disorder (CMML/JMML)-23,4%; acute leukaemia-15%. The most frequent symptoms were anemia (76,6%), hemorrhagic syndrome (42,6%), large lymphonodes (17%), hepatomegaly (53%) and splenomegaly

(59,5%) and infection (19%). The cariotype of bone marrow cells was detected in 36 children. It was normal in 65,7%, monosomy 7 - 14,3%; the transiocation (8;21)-5,7%, the transiocation (9;11)-2,9%; +14-5,7%; the del 9, inv 9, inv 1, del 13, del 16 in other cases. 57, 4% patients were transformed in acute leukemia. The transformation period lasted from 3 to 27 months (the median age was 7,2 months). The median of transformation was 14 months at RA and 4,6 months at RAEB. The survival rate of children with MDS was 39,4%, a median of survival - 32 months.

1477

GEMCITABINE, DEXAMETHASONE, HIGH DOSE CYTARABINE AND PLATINUM (G-DHAP) IS AN EFFECTIVE SALVAGE IN RELAPSED AND REFRACTORY LYMPHOMA

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DHAP is a standard regimen in relapsed and refractory lymphoma but is associated with a low response rate of around 10-20% in relapsed refractory disease. Gemcitabine (G) has been recently found to be an active agent in this setting. Combining these drugs has theoretical attractions beyond the simple additive effect of the two regimens. Platinum increases the cytotoxicity of Cytarabine. It is well recognized in the treatment of acute leukaemia that purine analogues have synergistic properties with regard to cytotoxicity. We report here three sequential cases with primary refractory and DHAP refractory relapsed disease treated with G-DHAP. *Case 1*: 35 year old female presented with Stage III B lymphocyte rich Hodgkin lymphoma. She progressed through 4 courses of ABVD to stage IVB histiocytic rich diffuse large B-cell lymphoma with bulky abdominal disease, bone marrow involvement and pathological fracture of the acetabulum. She received R-CHOP (Rituximab, cyclophosphamide, Adriamycin, Vincristine and Prednisolone) achieving a partial response after 4 courses but progressed after 3 more and remained avidly PET positive. She received ESHAP as salvage without any response. Gemcitabine (1 gm/m^2 day 1, 8), Cisplatin (100 mg/m^2 day 1-2) and Cytarabine (4 gm/m^2 day3) along with Dexamethasone (40 mg/d days 1-4) was started as salvage with a very good partial response on CT and only one small PET positive site in the liver after one course. *Case 2*: 32 year old male with stage III B nodular sclerosing Hodgkin lymphoma achieved partial response with 5 courses of ABVD with significant residual PET positive disease. He had 4 cycles of G-DHAP with Rituximab as salvage chemotherapy with complete CT and PET response after 2. He relapsed 8 months later and received 2 further courses of the same with complete remission. He underwent high dose therapy and autologous stem cell rescue and remains in remission 2 years later. *Case 3*: 46 year old male with stage IV B mixed cellularity Hodgkin's lymphoma progressed through ABVD and received 7 cycles of escalated BEACOPP chemotherapy having achieved remission after 2. He relapsed 3 years later with stage IVB disease. DHAP as salvage failed but he responded to the addition of Gemcitabine and Rituximab with a complete radiological response and minimal residual PET positivity after one course. He received one more cycle of RG-DHAP followed by BEAM Campath allogeneic stem cell transplant from his sister and is in remission 3 years later. Informed consent was obtained from all patients. The treatment causes grade 3-4 myelosuppression and requires GCSF support and transfusions. Case 2 had a successful peripheral blood stem cell harvest with G-CSF at the back of G-DHAP. We conclude that the addition of Gemcitabine with or without Rituximab to DHAP is an effective salvage of relapsed lymphoma even in cases refractory to DHAP. Further work in the form of a formal Phase II study and subsequent randomized trial will be required to confirm this important finding.

1478

CYCLOOXYGENASE-2 AND TRANSFORMING GROWTH FACTOR-β1 EXPRESSION IN HCV INDUCED CHRONIC LIVER DISEASE: RELEVANCE TO OUTCOME

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Background. Cyclooygenase-2 (COX-2) and transforming growth factor-β1 (TGF-β1) were modulated in a variety of viral infections, but there is a paucity of data about the role of these factors in the pathological process of fibrosis and carcinogenesis in HCV infection. *Aims*. This study aimed to analyze hepatic tissue expression of COX-2 and TGF-β1 in chronic hepatitis C liver disease, and its sequel of cirrhosis and/or hepatocellular carcinoma, in a trial to assess the role of these factors in the multi-step process of fibrosis/carcinogenesis. *Methods*. Hepatic expression of COX-2 and TGF-β1 was assessed using immunoperoxidase staining of liver biopsy specimens of 110 HCV-infected patients, of whom 50, 30 and 30 subjects had chronic active hepatitis (CAH), liver cirrhosis (LC) and hepatocellular carcinoma (HCC), respectively. Histologically-normal livers (n=10) were also assessed as controls. *Results*. Immunoperoxidase staining of normal hepatic tissue revealed faint COX-2 and TGF- β1 immunoreactivity (<10% and <50% of the cells, respectively). COX-2 expression in patients with CAH and LC was comparable to that of controls, yet 80% of positively stained LC sections showed marked staining. In HCC, 67% of the specimens were COX-2 immunostained. In well-differentiated HCC, COX-2 expression as well as intensity of staining were significantly higher than those with CAH, LC and low grades of HCC differentiation (p<0.01). Patients with moderately and poorly differentiated HCC showed up-regulation of COX-2 expression, yet lower scores of marked staining relative to CAH and LC (p<0.01). TGF-β1 was expressed in hepatocytes of all patients with CAH and LC with marked staining in 52% and 93% of the cases, respectively. TGF-β1 was, also, expressed in 67% of cases with HCC and the intensity of staining varied between moderate (30%) and marked (70%). A positive correlation was detected between hepatic expression of COX-2 and TGF-β1 (r= 0.67, p<0.05); however, no correlation was detected between the latter and grade of HCC differentiation (r= 0.33, p>0.05). *Conclusions*. These findings reveal that TGF-β1 expression in patients with severe hepatitis activity and LC may play a role in hepatic cell damage following HCV infection and stress the usefulness of this cytokine as a prognostic marker for liver cell injury. Whereas, the involvement of COX-2 in the early stages of carcinogenesis suggests a predictive role of this enzyme for malignant changes following chronic HCV infection. The combined expression of COX-2 and TGF-β1 in HCV-related HCC suggests a synergistic action of these factors in the pathophysiology of hepatocarcinogenesis process.

1479

CLINICO-HAEMATOLOGIC CHARACTERISTICS, MOLECULAR ANALYSES AND PROGNOSIS OF PHILADELPHIA POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS.A HOSPITAL BASED SUDY

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Background. Philadelphia chromosome (Ph)-positive acute lymphoblastic leukemia (ALL) is a common cytogenetic abnormality in adult ALL and found in 15% to 30% of patients. It is associated with poor outcome both in children & adults. This preliminary study is reported to define the frequency of prevalence of the BCR-ABL fusion subtypes in our ALL patients by reverse transcription-PCR (RT-PCR) and to ascertain the prognostic significance of t9:22translocation along with other characteristics. *Patients and Methods.* Sixty-five adult ALL patients treated at our center between 2004 and 2005 comprised the study group. The diagnosis of patients was based on typical morphological criteria of marrow aspirate and biopsy specimens. ALL patients were treated with induction regimen consisting of a combination of vincristine, prednisone, daunorubicin, L-asparginase and cyclophosphomide followed by courses of intensification and CNS prophylaxis and consolidation I, reinduction, consolidation II, maintenance, and central nervous system (CNS) prophylaxis. *Results*. Among the patients included in this study, Ph-positive ALL was present in 16 of 65 patients (25%). The number of patients within 3 age groups (< 20 years, 20-50 years, > 50 years) differed significantly between BCR-ABL+ and BCR-ABL- patients (p=0.001). The median age was significantly higher in the BCR-ABL+ group (30 versus 15 years; p=0.0001). BCR-ABL+ patients were also characterized by higher median white blood cell (WBC) counts (180000/μL versus 23000//μL p=0.0001). A complete remission (CR) after induction therapy was achieved in 30 of 49 (61%) BCR-ABL- patients and 6 of 16 (38%) BCR-ABL+ patients (p=0.001). Only 10% the BCR-ABL- patients achieved complete continued remission while none of the BCR-ABL+ patients maintained a CR further. The presence of a BCR-ABL fusion predicted (p=0.0001) a lower survival. The estimates of event-free survival and overall survival two years after diagnosis in the combined study group were 20 percent and 30 percent, respectively. Age, initial leukocyte count, were found to have a significant effect on the outcome of treatment. The probability of overall survival at 2 years after diagnosis was 0.30 (± 0.03 SE) in BCR-ABL- (n = 49; median survival, 360 days) versus 0.15 (± 0.03 SE) in BCR-ABL+ patients (n = 16; median survival, 240 days; p=0.0001). The DFS of BCR-ABL+ patients remains markedly low (with CALGB protocol at 2 years, 0.08±0.03 SE). Multivariate analysis confirmed WBC count and BCR-ABL result as an independent prognostic factors. *Conclusion*. This study emphasizes that BCR-ABL

gene fusion is an independent prognostic factor in ALL patients. Identification of this genetic entity in adult ALL at diagnosis is crucial for understanding the nature of adult acute lymphoblastic leukaemia and for deciding optimal treatment

Figure 1. Differences in overall survival of Ph^+ ALL and Ph^-.

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CHRONIC EOSINOPHILIC LEUKAEMIA (ASSOCIATED WITH FIP1L1-PDGFRA)-DRAMATIC RESPONSE TO IMATINIB MESYLATE

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Background. Chronic Eosinophilic Leukaemia (CEL) is a myeloproliferative disorder in which an autonomous clonal proliferation of eosinophilic precursors, results in persistently increased numbers of eosinophils in the blood, bone marrow and peripheral tissues. Organ damage occurs as a result of leukaemic infiltration or due to the release of cytokines and enzymes by eosinophils. CEL along with idiopathic hypereosinophilic syndrome constitute the Hypereosinophilic syndromes (HESs). The recent WHO classification criteria for diagnosis of HES exclude patients with underlying infections, allergic, autoimmune, pulmonary or clonal lymphoid and non myeloid disorders that are known to be associated with secondary eosinophilia in addition to myeloproliferative or myelodysplastic disorders that could give rise to clonal eosinophils. CEL is caused by autonomous proliferation of clonal eosinophilic precursors, whereas idiopathic HES is diagnosed when the criteria for CEL are met but there is no evidence of clonality or neoplastic myeloid cell proliferation. Based on the WHO diagnostic criteria, cases of HES found to be associated with a clonal chromosomal deletion that gives rise to FIP1L1-PDGFRA fusion gene should be reclassified as CEL. *Case report*. We report a 39 year old man who presented with complains of headaches, left earache, rinorrhea, pyrexia and increased sweating.He had a background history of seasonal rhinitis. On examination his left tympanic membrane appeared inflamed.He had no evidence of peripheral lymphadenopathy or splenomegaly. His FBC revealed Hb 13.9, WCC 78.1, Neut 9.6 ,Eosinophils 55.1 and Plat 183. Blood film revealed polychromasia, nucleated RBCs, marked eosinophilia and left shifted neutrophil maturation. Bone marrow- hypercellular marrow with prominent granulopoiesis overwhelmingly along the eosinophilic maturation (85% of the nucleated elements).Cytogeneics-46XX,no abnormal clone detected.CT-Splenomegaly 14 cm.He was commenced on oral Prednisolone.Following 2 weeks of steroids there was no response. Hence Imatinib was commenced and Prednisolone was tapered off. In 1 week's time his eosinophil count was down to 0.4 .His Peripheral blood came back as positive for the FIP1L1-PDGFRA mutation (by single step RT-PCR analysis) Three months after being on Imatinib 100mg once daily, his peripheral blood was negative for the FIP1L1-PDGFRA mRNA(by nested RT PCR), indicating a complete molecular response. After one year of follow up,he is in complete haematological and molecular remission of chronic Eosinophilic leukaemia. *Discussion*. Before the 1990s, lack of evidence for a reactive cause of hypereosinophilia or chronic eosinophilic leukemia (e.g. presence of a clonal cytogenetic abnormality or increased blood or bone marrow blasts) resulted in diagnosticians characterizing such nebulous cases as *idiopathic hypereosinophilic syndrome (HES)*. However, over the last decade, significant advances in our understanding of the molecular pathophysiology of eosinophilic disorders have shifted an increasing proportion of cases from this idiopathic HES *pool* to genetically defined eosinophilic diseases with recurrent molecular abnormalities. The majority of these genetic lesions result in constitutively activated fusion tyrosine kinases, the phenotypic consequence of which is an eosinophilia-associated myeloid disorder. Most notable among these is the recent discovery of the cryptic FIP1L1-PDGFRA gene fusion in karyotypically normal patients with systemic mast cell disease with eosinophilia or idiopathic HES, redefining these diseases as clonal eosinophilias. A unique interstitial deletion on chromosome 4q12 leads to expression of the FIP1L1-PDGFRα fusion tyrosine kinase. Rearrangements involving PDGFRA and PDGFRB in eosinophilic chronic myeloproliferative disorders, and of fibroblast growth factor receptor 1 (FGFR1) in the 8p11 stem cell myeloproliferative syndrome constitute additional examples of specific genetic alterations linked to clonal eosinophilia. The identification of populations of aberrant T-lymphocytes secreting eosinophilopoietic cytokines such as interleukin-5 establish a pathophysiologic basis for cases of lymphocyte-mediated hypereosinophilia. This recent revival in understanding the biologic basis of eosinophilic disorders has permitted more genetic specificity in the classification of these diseases, and has translated into successful therapeutic approaches with targeted agents such as imatinib mesylate and recombinant anti-IL-5 antibody. *Conclusions*. Imatinib, the Tyrosine Kinase Inhibitor specific to Bcr-abl, Kit and PDGFR is an appropriate therapeutic option for CEL with the FLIP1 like 1-PDGFR α. Fusion Gene and often causes dramatic lowering of the eosinophil count.

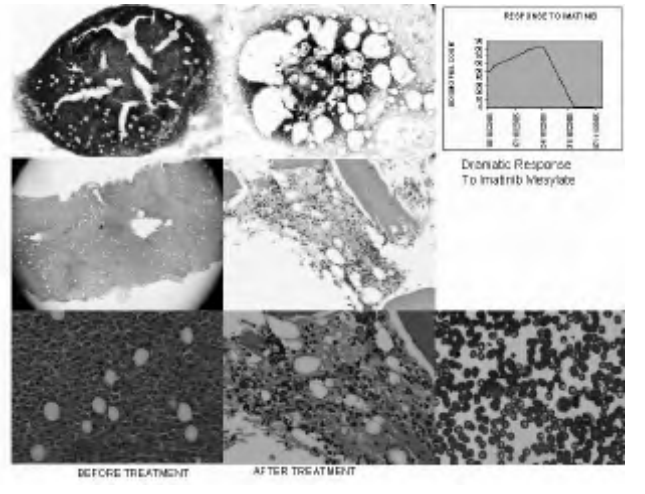

Figure 1. Chronic Eosinophilic leukaemia (Bone Marrow).

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DIAGNOSTIC DIFFICULTIES IN IMPORTED LEISHMANIASIS IN ROMANIA

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The extremely-polymorphous symptoms and signs of visceral Leishmaniasis allow us to state that this infection is part of a grate imitators group together with collagen diseases, systemic vasculitis-polyarteritis, tuberculosis and HIV infection. With no occurrence before 1989, after the liberation of the Romanian frontiers, no less than five visceral Leishmaniasis have we diagnosed only in our clinic: three males and two females ages 24-47. Four of the subject had come from Southern Greece and one of subjects from Southern Italy (Reggio Calabria). The associated symptoms and signs were as follows: prolonged-fever syndrome, consumption syndrome, progressive hepatosplenomegaly, progressive adenomegaly, progressive-aggravating pancytopenia and inflammatory humoral syndrome accompanied by polyclonal hypergammaglobulinemia. The subjects came at our clinic after 8-14 months of suffering, meanwhile having been diagnosed by various medical-attendance unites with the following hypothetic diagnoses: subacute bacterial endocarditis, hepatic cirrhosis HVB^+ with hypersplenism and pancytopenia, idiopathic thrombocytopenic purpura, neoplastic disorders, visceral toxoplas-

mosis, non-Hodgkin's lymphomas (NHLs). The diagnosis in our clinic has been argumented by the occurrence of leishmania amastigotes in the macrophages within the bone marrow grains. We emphasize that we should make a thorough examination considering the following case of a female-patient, who have been performed bone-marrow biopsy in one of the clinics in Reggio Calabria and afterwards in the clinics in Romania (May 2006 and October 2006). At a second examination, the smear obtained in October 2006 evidenced amastigotes leishmania: one infected macrophage per 100 fields. *Conclusions*. visceral leishmania has became part of the pathology that should be taken into account in Romania, the quest *Ubi Vene* and rigorous examination of bone-marrow biopsy being the key to diagnosis.

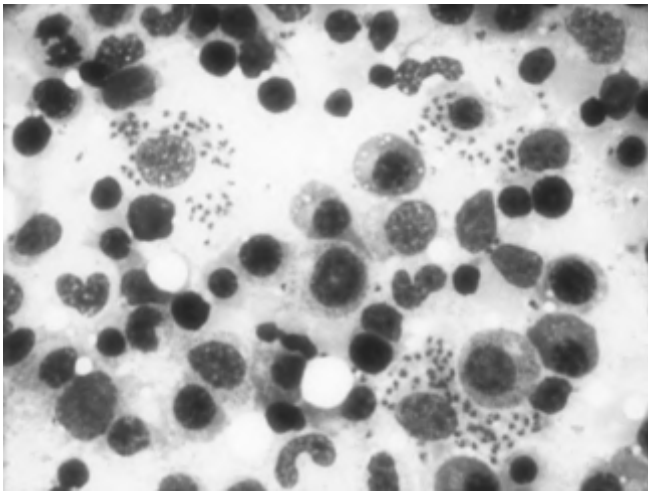

Figure 1.

1482

POLYCYTHEMIA VERA AND SECONDARY ERYTHROCYTOSIS: A COMPARISON IN THE TREATMENT BY TRADITIONAL BLOOD-LETTING AND BY THERAPEUTIC ERYTHROCYTAPHERESIS

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Background. RBC depletion is the first line therapy for polycythemia vera (PV) and secondary erythrocytosis (SE), characterized by increased hematocrit (Hct), with a consequent hyper-viscosity syndrome. In PV, RBC depletion should be preferred to chemotherapy because of the long-term risk of acute leukemia or other secondary malignancies. Blood letting therapy (BL) is an economic and effective medical treatment without complications for patients; while erythrocytapheresis (EA) is an alternative treatment useful in order to quickly remove a large volume of red blood cells, saving plasma, proteins and clotting factors. This difference between EA and BL is very important because long-term survival of these patients is essentially determined by the capacity in reducing the risk of thromboembolic complications linked to the altered blood rheology due to the high RBC mass. *Aims*. In order to evaluate the efficiency and safety of apheresis, we report a retrospective analysis of therapeutic results achieved in the treatment of PV and SE by EA compared to ones achieved with BL. *Methods*. In the period between 2002 and 2006, 95 patients have been studied in Caserta's Hospital and in University. 51 patients, with a mean±SD age of 52.6±13.4 years, were affected with PV, diagnosed according to Polycythemia Vera Study Group's criteria: 15 has been treated by EA, while remaining 36 only by BL. Other 44 patients, with an age of 59.3±16.1 years, were suffering from SE: 21 has been treated by EA, while 23 by BL. Therapeutic targets were to achieve a Hct <45% in men and <42% in women. EA was performed by the discontinuous flow cellular separator Haemonetics MCSplus. Before starting a therapeutic cycle Hb, Hct, platelet count, coagulation test and ECG were verified in every patient. *Results*. In the BL group, a number of phlebotomies has been performed according to initial Hct of each patient with a mean of 4.11±1.64 venesections. Each weekly phlebotomy amounted to about 400±50 mL of whole blood. No side effects, adverse reactions or complications were registered. Retrospective analysis of Hct and Hb, assayed before and after each venesection, showed a reduction of Hb=1.08±0.33 g/dL and of Hct=2.92±0.64%. In EA group, patients were treated with 2.25±0.84 apheresis. At the end of therapeutic cycle, a volume of 576 mL (range 426-830) of concentrated RBC was removed, with final Hb=14.3±0.92 g/dL and Hct=42.4±2.1%; the improvement of symptomatology and hematochemical parameters were maintained on the average for 6 months. All procedures were well tolerated and light side effects (paresthesias citrate-depending in 18 aphaeresis) were easily controlled. All 95 patients resulted iron depleted (Ferritin<50 ng/mL and Transferrin Saturation<20%) and an iron-limited hematopoiesis was obtained in all cases. *Conclusions*. In conclusion, RBC depletion is accomplished much more effectively and rapidly by EA than by repeated phlebotomies, nevertheless blood letting remains the main treatment of PV and SE, because less expensive. The main indication for EA is high risk Hct >55-60%; in fact this value may be reduced to the normal range by only 1-2 apheretic procedures.

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BLEEDING RISK IN ET PATIENTS WITH A PLATELET COUNT OF MORE THAN 1.5 MILLION/L: A MANDATORY CRITERIUM FOR THE INITIATION OF CYTOREDUCTIVE THERAPY?

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Background. The most important limiting factors of patient survival in essential thrombocythemia (ET) are thromboembolic and hemorrhagic complications.[1] It has been postulated that the paradox of hemorrhagic events in a disease with highly elevated platelet counts is caused by an acquired von Willebrand Factor (vWF) deficiency syndrome which is found in ET patients with very high platelet counts.[2] Therefore, cytoreductive therapy is usually recommended by several European expert groups when platelets exceed 1.5 million/µL. *Aims*: To critically examine the concept of cytoreductive therapy in high-platelet ET based upon the postulated increased bleeding risk. *Methods*. In a prospective study we observed 3 patients with a continuously elevated platelet counts of more than 1.5 million/µL and secondary loss of large von Willebrand factor multimers, as shown in SDS electrophoresis, that did not receive cytoreductive therapy, but have been treated with aspirin. *Results*. Patient 1 is a female which was 48 years at the time of diagnosis. She is negative for the JAK2V617F mutation and shows a secondary loss of large vWF multimers. We have now observed her for 6 years 10 months without any bleeding complications on aspirin therapy. During that period she has had more than 1.5 million platelets/µL for more than 46 months with a zenith of 2.27 million/µL. The second patient is a male who was 60 years old at the time of diagnosis. He is JAK2V617F negative and shows a relative decrease in large vWF multimers. He has been bleeding free since his diagnosis 5 years 10 months ago and had platelet levels of more than 1.5 million/µL for a cumulative period of more than 30 months. He has been treated only with aspirin. Patient 3 is a 59 year old male who has been diagnosed with ET 8 months ago. He is heterozygous positive for the JAK2V617F mutation. He also has a relative decrease in large multimers including a complete loss of the largest multimers. His platelet count has been elevated over 1.5 million/µL (maximum: 2.18 million) for a cumulative period of 6 months during which he has been treated only with aspirin. After that period he has been switched to anagrelide due to headaches. He has not shown any hemorrhagic complications except an increased bleeding tendency during shaving. *Conclusions*. Our observations challenge the recommendations by Italian, British, and German/Austrian groups to automatically initiate platelet reducing therapy above a platelet count of 1.5 million/µL. Acquired von Willebrand factor deficiency caused by ET does apparently not lead to hemorrhagic complications in all patients and therapy with aspirin should not be automatically excluded based on this dogma.

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EPIDEMIOLOGY AND RESPONSE TO TREATEMENT IN CHILD ACUTE LYMPHOBLASTIC LEUKEMIA TREATED BY EORTC 58951

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Introduction. Child T cell acute lymphocytic leukaemia has been shown by many studies to have an unfavorable prognosis. But there is a significant improvement in outcome for patients with T-ALL due to the use of intensive rotating pulses of chemotherapy. *Materials and methods*. Between January 2001 and Decembery 2005,120 children with ALL have been treated in our institution (university hospital of Tunis).We reviewed only those with T ALL. 41 patients with T-ALL were treated by pediatric protocol 58951 of EORTC. *Results*. Median age of children was 9.5 years (Quartiles 2 years-20 years). In 14 patients (34%) the age was ″ 10 years. RS: 2.15 (28 boys and 13 girls). Tumoral syndrom was observed in 37 cases (90%). One case of nervous system disease with a facial paralesy. Median presenting WBC was 156000/ mm^3 (quartiles 2600- 760000); WBC ≥50000/mm^3 was noted In 28 patients (68), in 25 WBC≥ 100000/mm^3. karyotyp was normal in 56% of patients. Immunophenotypic presentation: 18 immature T , 12 cortical T, 9 pré T and 2 mature T. Response to treatement at day 8 of induction: age<10 years: 12% were in poor response at day 8, 34% if age ≥10 years, 9% if WBC<50000/mm^3 and 36% if WBC≥ 50000/mm^3. A complete remission was observed in 35 cases (85%). 2 patients were been in non complete remission after induction therapy. Toxic death in 4 patients (9%). Risk group: RM1 : 0 cases, 20 cases of RM2 and 21 of VHR. *Conclusions*. ALL-T is more frequent in our population (34%) than in many studies (10-15%). The frequency of boys 68% similar than in the literature. Tumoral syndrome is usually observed. The complete remission is not correlated neither to the rate of WBC or to the corticosensibility at days 8.

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BURKITT-LIKE LYMPHOMA/LEUKAEMIA WITH DUAL TRANSLOCATION (8; 22) AND (14; 18) A CASE REPORT

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Burkitt's lymphoma is a mature B cell malignant neoplasia with aggressive outcome. The World HealthOrganisation classification recognises several variants of Burkitt's lymphoma with a difficult diagnosis but using the histologic, immunophenotypic and genetic criteria the differential diagnosis may be improved. We report a 63 y old woman patient who presented with leukaemia phase of Burkitt-like lymphoma and Bulky disease. Clinical examination revealed a poor performant status with a left inguinal adenopathy but not hepatosplenomegaly. The CT investigation showed a left pleuresia and retroperitoneal important masse. A lumbar puncture did not revealed evidence of CNS involvement. Laboratory features at presentation were: LDH level at 10000U/L, white blood cell count at 9, 5 10 9/L, haemoglobin level at 9 g/dL, and platelet count at 73 10 9/L. Circulating blasts cells were present (6% malignant cells). HIV, HBV and HCV serology were negative. A bone marrow aspirate showed a diffuse infiltration by malignant cells: a minority of cells revealed a characteristic of the Burkitt cells (the medium-sized cells, chromatin and clear parachromatin, and smaller number of more prominent nuclei) but a majority of the cells are more pleomorphic and overall slightly larger as Burkitt-like morphology features. The immunophenotype study showed an immature profile of the malignant cells with bright expression of CD45, CD19, CD10, CD38, BCL2 and Ki67, moderate expression of CD22, intracellular kappa light chains, but not expression for CD20, sCD79b, cyCD79a and BCL6. Cytogenetic studies showed a complex karyotype with both t (8; 22) (q24; q11) and t (14; 18) (q32; q21). The patient was treated by the standard protocol for Burkitt's lymphoma with adapted doses: Adriblastine, Endoxan, Vincristine, Dexamethasone and Methotrexate . The evolution was aggressive and the patient died 4 months after diagnosis. This interesting case with dual translocation or *double-hit* features has a MYC translocation that enhances cell proliferation and a BCL-2 translocation that inhibits apoptosis and thus prolongs cell survival. This devastating combination leads to a disease with a very poor prognosis. Other reports have confirmed the aggressive behaviour of these lymphomas.

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MINIMAL RESIDUAL DISEASE (MRD) AND CHIMERISM KINETICS IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) AFTER A REDUCED INTENSITY CONDITIONING (RIC) REGIMEN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (ALLO-PBSCT): A CASE REPORT

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Stable mixed chimerism (MC) after RIC allo-PBSCT is probably responsible for a GVL effect in CLL. Some studies in CML suggest that MRD and chimerism kinetics are tightly correlated but can be discordant. We serially analysed MRD and chimerism status in a B-CLL patient who underwent RIC allo-PBSCT. A 43 y old man was diagnosed with stage A Binet B-CLL in Jan 1994. Treatment with Chlorambucil+steroids started in 1997 for progression leading to a VGPR. In 2000, 5 cycles of Fluda were initiated for reccurent progression with a PR. The patient subsequently received from a sex-mismatched related-HLA identical sibling, an allogeneic PBSC graft. Conditioning regimen was 2 Gys TBI+Fluda. GVHD prophylaxis was ciclosporine+MMF until D28, then cyclosporine alone. On D47 the patient developed a grade III cutaneous aGVHD steroid-sensitive. On D100 stable MC and clinical CR were observed. The patient developed limited cGVHD on D120, stabilised by steroids, but the patient relapsed 2 years after transplant, and PB karyotyping analysis revealed an 11q23 deletion. Four cycles of Rituximab induced no response.Escalating doses of DLI were administrated at 3-Mo interval: M29 DLI#1 with 5×10^6 $CD3^+$/kg and M31 DLI#2 with 3.5×10^7 $CD3^+$/kg, with no GVHD and no disease response with progressive MC. Three years later the patient received Campath-1-H as tumoral reduction agent before a 2nd allo-PBSCT, from a 2ndrelated sex mismatched HLA donor, after a RIC regimen: Fluda 5 days+Bus 2 days + ATG 2 days. On D23 the patient presented a cutaneous aGVHD grade III and digestive grade II, steroids sensitive. On D100 he developed a cGVHD with eye sicca syndrome. Chimerism was assessed by STR-PCR and real-time PCR in PB samples from total WBC and from CD3+ T-cells. MRD analysis was performed using the 4-color flow cytometric approach of the CLL specific phenotype $CD5^+CD19^+CD79^{dim}+CD20^{dim}+$. After the 1st transplant, the chimerism and MRD CLL evaluation was difficult to compare because time points were different, the patient was all the time MC. For the 2nd transplant 1 year later MRD and chimerism levels are <1%. In conclusion we demonstrate that there is a direct correlation between the level of chimerism and the level of MRD in a CLL patient after RIC PSBCT transplantation with a follow-up of at least 12 months. This establishes the basis for an immunomodulatory effect of PBSC grafts in B-CLL.

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ANGIOPOIETIN-2 MRNA DETECTED BY REAL-TIME QUANTITATIVE PCR IS DIFFERENTIALLY EXPRESSED IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. Angiogenesis is considered to play an important role in pathogenesis of chronic lymphocytic leukemia (CLL). Angiopoietin-2 (ANG-2) belongs to important cytokines regulating blood vessel formation. Elevated expression of ANG-2 has been found in several hematological malignancies; however, data regarding ANG-2 in CLL are very limited. *Aims*. To develop a method for quantitation of ANG-2 mRNA using real-time quantitative PCR. *Methods*. ANG-2 mRNA levels were analyzed in purified mononuclear cells from 12 CLL patients in different clinical stages and with different prognostic factors (IgVH mutation status, genetic aberrations) by real-time quantitative PCR with specific primers and LNA (locked nucleic acid) hydrolysis probe from Universal Probe Library (Roche, Germany). ANG-2 transcript levels were normalized to reference housekeeping gene porphobilinogen deaminase (PBGD). *Results*. Elevated ANG-2 mRNA concentration was detected in 5 cases (normalized expression > 10-2). On the other hand, 7 patients had very low (normalized expression < 10-2) or undetectable levels of ANG-2 mRNA. So far, no association between ANG-2 levels and prognostic factors was detected. *Conclusion*. We have developed the method for quantitation of ANG-2 mRNA. Our pilot study shows that ANG-2 is expressed at least in some cases of CLL and may play a role in CLL biology. Further studies are necessary to confirm our results and to

define the relevance of ANG-2 for prediction of prognosis in CLL. Supported by research project MZO 00179906 from Ministry of Health, Czech Republic.

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ALEMTUZUMAB TREATMENT IN REFRACTORY LYMPHOPROLIFERATIVE DISORDERS

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Background. CAMPATH-1H is a human immunoglobulin G1 (IgG1) anti-CD52 monoclonal antibody (MAb) that binds to nearly all B- and T-cell lymphomas and leukemias A number of clinical trials have demonstrated the clinical activity of Campath in chronic lymphocytic leukemia (CLL), T-cell malignancies such as T-prolymphocytic leukemia (T-PLL) and cutaneous T-cell lymphoma (CTCL). *Methods*. Fifteen patients wth lymphoproliferative disorders refractory to different chemotherapies or with advanced disease were treated as induction with CHOP-CAMPATH-1H or ATT-Campath-1H for a maximum of 6 cycles, and one patient with CAMPATH-H for 18 weeks and local radiotherapy. The patients who entered only in partial remission were treated with CAMPATH-1H as maintenance treatment. Two patients with CLL, 4 patients with Mycosis fungoide(MF)/ Sesary Syndrome (SS), and 9 patients with Peripheral T Cell Lymphomas were treated The CD4 was measured. RESULTS: Seven patients (46,66%) entered in a complete remission (CR) for 1 year up today (4 NHL, 1 CLL, 1 SS), 6 patients (40%) achieved a partial remission (50-75% PR) (4 LNH, 1 CLL, 3 MF) and one patient did not respond. The most pronounced effects in CLL patient were noted in blood, bone marrow, and spleen in one of the patients with advanced and chemotherapy-resistant CLL. The other patient 82 years old with CLL entered in complete remision after 3 dosis of Campath-1H. Three patients had severe infections : 1 ocular cytomegalovirus, one cristoporidium diarrhea (in these cases Campath-1H was discontinued) and one Herpez zozter. Another patient with NHL died after 3 cycles of CHOP-CAMPATH-1H due to severe pulmonary hypertension. The level of CD4 falled after Campath-1H treatment. *Conclusions*. CAMPATH-1H had significant activity in patients with lymphoproliferative disorders refractories to different chemotherapies so CAMPATH improve survival although it can cause severe infections due partially to CD4 cell count drop, the majority of them can be clinically manageable. One patient died in CR due to severe pulmonary hypertention probably as a complication of Campath treatment.

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IMATINIB DOSE IN INDIAN PATIENTS WITH PH+ CML

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Background. It has been often discussed that the standard dose of 400 mg daily imatinib mesylate (IM) is poorly tolerated by many Asian patients. However, it has remained undocumented. *Aim*: To study the dose of IM tolerated by the Indian patients with Ph^+ CML-CP. *Methods*. A cohort of 182 adult Indian patients (>18 yrs of age) with Ph^+ CML-CP (newly diagnosed or late-CP) and on regular follow-up was included in the analysis. The starting dose of IM was 400 mg daily. The IM dose ingested by the patients (on their own i.e. compliance or on physician's advice) was recorded over the subsequent months. Physicians' recommendations for dose modification was based on hematologic (neutropenia and/or thrombocytopenia, but not anemia) or some severe non-hematologic toxicities (myalgia, muscle cramps, mucositis, GI symptoms or skin rash). *Results*. The consistent IM doses received were - daily 300 mg by 15 patients (8%), 400 mg by 136 (75%), 600 mg by 27 (15%) and 800 mg by 4 (2%). Most patients (except by 3 due to GI intolerance or myalgia/cramps) on a higher dose (raised due to poor response to 400 mg daily) did not reduce the dose and were fully compliant. However, attempts to raise the dose from 300 mg to 400 mg or higher was unsuccessful due both either to hemtologic and non-hematologic toxicities. Non-compliance as regard to 300 mg daily was documented in ~20%of patients (1.1% of the study cohort) receiving this dose. The median dose among these patients was 230 mg daily. This happened as patients were often apprehensive of non-hematologic toxicity of excessive flatulence, nausea, myalgia, mucositis and cramps. Patients who continued with the standard dose of 400 mg, the mean daily dose was 380 mg (IRIS cohort 382±52 mg). Our analysis suggests that Indian patients do tolerate the standard dose of IM as documented in the IRIS study (N Engl J Med 2006; 355: 2408). In reality, in our cohort more patients (6% in the IRIS study) received a higher dose of 600 mg daily. This could be explained by the fact that patients with late CML-CP not responding to 400 mg were advised a higher dose. *Conclusions*. This prospective analysis shows that most Indian patients can tolerate the standard 400 mg daily dose of IM. However, who usually tolerate only 300 mg daily, a good number (~20%) tend to remain somewhat non-compliant and this might have an impact on long-term outcome in them.

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THE INFECTION OF CYTOMEGALOVIRUS IN CHILDREN WITH NEUTROPENIAS

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Background. Cytomegaloviruses (CMV) don't only show activity under the condition of immunodeficiency, but they also have an immunesuppressive effect on the human organism. The pinacle of the diagnostics of neutropenias falls on the early childhooh age, which is the most vulnerable for development of cytomegalovirus infection (CMVI), while during some neutropenic states there is a lack of adequate immune response not only to bacterial agents, but also to virus ones. *Aim*. To find out to which extent children with neutropenias are infected with CMV. *Methods*. A screening examination took place for CMV with the help of the three-phase enzyme immunoassay of blood serum of 269 children in the age 0 to 14 years during their primary visit to the Moscow Hematology Center in the Morozov Child's City Clinical Hospital. The purpose of the assay was to find antibodies of the classes IgM and IgG. The received data were analyzed in 4 age groups: group 1-0 to 6 months (71 children), group 2-6 to 12 months (101 children), group 3-1 to 3 years (97 children), group 4-3 to 14 years (29 children). *Results*. Serologic markers were found in 153 children (51.3%). The percentage of serologically positive children in groups 1,2 , 3 and 4 was 63.4%, 51.3%, 58.8% and 37.9% respectively. The biggest number of presence of the specibic IgM antibodies, which tell about infection prosses activity was noted in children with neutropenias in the age less, than 6 months (12,7% of all the examenees in this age group). *Conclusion*. The high rate of CMV infection in children with neutropenias indicates that there is necessity to examine patients of the group in question for CMVI. The biggest number of the infected was found among children with a debut of neutropenia in the age 6 months to 3 years. This shows that CMV influences the development of neutropenia in children of early age.

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RESULTS OF IMATINIB MESYLATE THERAPY IN CHRONIC MYELOID LEUKEMIA: EXPERIENCE OF A SINGLE CENTER

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Background. Imatinib mesylate, spesific bcr-abl tirosine kinase inhibitor, have been used widely in treatment of chronic myeloid leukemia (CML) recently. Hematologic, cytogenetic and even molecular remission can be achieved by imatinib in the dose of 400 mg/day in chronic phase. Dose adjustment is 600-800 mg/day in accelerated or blastic phase. *Aim*. To evaluated the effectiveness of the imatinib mesylate treatment. *Material and method*. Eighty-seven patients with CML whose followed regularly by department of hematology of Dicle University, Medicine Faculty were included to study. Thirty-nine patients with 44.1 years mean age were female (45%) and 67 patients with 55.2 median age were male (55%). Bcr-abl was detected regulary in every 6 months by PCR. *Results*. Complete hematologic response rate was 82/87 (94.2%). Primary resistance to 400 mg/day imatinib was observed in 5 patients. Dosage increased to 600 mg and subsequently to 800 mg/day. One of 5 resistant patient respond to 800 mg. An another patient who was in blastic phase in 6th months of therapy responsed to 800 mg and regressed to chronic phase. Complete molecular remission achieved in one patient. Superficial edema, vomiting, and grade 3-4 neutropenia or thrombocytopenia were detected in 34 (39%), 37 (42%), and 10 (11%) patients in chronic phase. Myalgia and osteoalgia were observed in 8 patients. Treatment was discontinued in patients with hematologic adverse effects, and subsequent to the recovery of these effects, imatinib mesylate restarted with a dose of 300 mg/day. *Conclusions*. Hematologic response to imatinib mesylate therapy was founded in 94% of patients. Patients should follow regulary for effectiveness of treatment and for the possible advers effects which may be serious.

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FREQUENCY OF MEDITERRANEAN GLUCOSE-6-PHOSPHATE DEHYDROGENASE MUTATION IN SAUDI ARABIA-JEDDAH

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a widespread abnormality of red cell enzyme, which gives rise to hemolysis under oxidative stress. In Saudi Arabia, however, G6PD deficiency has a variable frequency in different regions. The prevalence and genotypes of G6PD deficiency are not known until now in Jeddah province. Accordingly, we design this study to investigate the frequency of the Mediterranean mutation that involves exon 6 and exon 7 of the G6PD gene. 47 samples were collected in a short period from July 2005 to October 2005. Twenty five Saudi males and 22 Saudi females, (their ages ranged between 18 and 45) were screened for G6PD deficiency by quantitative spectrophotometric assay at the Maternity and Children Hospital in Jeddah for premarital screening were found to be deficient in G6PD. The DNA of the G6PD deficient subjects was extracted from whole blood and then amplified by the polymerase chain reaction (PCR). Mutation analysis was performed by using conformation sensitive gel electrophoresis (CSGE), altered CSGE patterns were then sequenced by automated sequencer. All mutations were detected in exon 6 of the G6PD gene, while no mutations were detected in exon 7. In conclusion, Mutations were detected in 7/47 G6PD deficient (6 Mediterranean mutation and one Siberian mutation not been reported in Saudi Arabia). The frequency of Mediterranean mutation in G6PD patients in Saudi Arabia-Jeddah was 10.3%. This can be used as screening method for early detection of G6PD deficiency before marriage. In addition, other G6PD mutations remain to be determined.

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THE EFFICIENCY OF ERYTHROPOIETIN ADMINISTERED AT THE ANEMIC PATIENTS WITH MALIGNANT HEMOPATHIES AND SOLID TUMORS, TREATED WITH CHEMOTHERAPY

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Background. The treatment of the anemia at the patients with malignancies combats the hypoxia, improves the answer to the treatment and ameliorates the quality of life. *Aim*. We proposed ourselves to study the efficiency of the Erythropoietin administration at the anemic patients with malignant hemopathies and solid tumors, treated with chemotherapy. *Method*. We have studied all the 57 patients which were in the evidence of the County Clinical Hospital from Sibiu with malignant hemopathies and solid tumors, treated with chemotherapy, to which we have associated Erythropoietin during 15.09.2006-31.12.2006. For all the patients the informed consent was obtained. We have analyzed the next parameters: age, gender, the doze of Erythropoietin, the treatment's duration and the monthly changes of the hemogram (hemoglobin, hematocrit, leucocytes and platelets). There was analyzed the answer at the treatment with Erythropoietin at the patients with malignant hemopathies compared with those with solid tumors. The variation of the hematological parameters was statistically analyzed using the t Student test. *Results*. The medium age of the group was 60.68±11.64 years. The gender repartition was: 38 women and 19 men. Only 37 patients could have been evaluated after at least one month of treatment. The average of the Erythropoietin dose they have used was 30000 UI weekly. The medium duration of the treatment was 7.54 weeks. After a month of treatment the average increasing of the hemoglobin level was 1.43 g/dL ($p<0.001$), after two months was 2.78 g/dL ($p<0.00001$) and after 3 months was 4.26 ($p<0.001$). The hematocrit increased also in a statistically significant way. The leucocytes and trombocytes did not vary significantly. The need for blood transfusion was of 0.51 units of erythrocyte concentrate/month/patient. The patients with malignant hemopathies had, comparing with those with solid tumors, higher initial values of the hemoglobin level (9,45±2,12 mg/dL versus 8,85±1,12 mg/dL) and hematocrit and higher increasing of the hemoglobin level under the treatment with Erythropoietin (1,67±1,55 mg/dL after a month versus 1,06±1,51 mg/dL), but the results were not statisticaly significant ($p>0.05$).The treatment was well tolerated; no adverse events were reported. *Conclusions*. Erythropoietin incresed significantly the hemoglobin and hematocrite level at the patients with malignant hemopathies and solid tumors, treated with chemotherapy and it decresed the need for blood transfusion. It was well tolerated and it contributed to a better therapeutic efficiency.

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COMPLETE CYTOGENETIC RESPONSE AFTER 12 MONTHS OF TREATMENT AND WITHIN 1 YEAR OF IMATINIB THERAPY IN PATIENTS WITH LATE CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PH+

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The advent of imatinib mesylate (IM), a specific selective inhibitor of the Bcr-Abl tyrosine kinase, has considerably changed treatment of chronic myeloid leukemia (CML). IM induces a high rate cytogenetic remissions especially in first line treatment of the disease. Many patiens with late chronic phase has been given IM following a long period Interferone alfa (IFN alfa) pre-treatment. Cytogenetic response has been also documented in this group, eventhough following a longer treatment as compared to first line therapy. We tried to reveal the type of cytogenetic response achieved in the treatment of late chronic phase CML. From the whole number of 158 treated patients in our institution, we reviewed 82 cases with late chronic phase Ph+CML. All of them had long IFNalfa pre-treatment: some patiens became refractory, others intolerant to therapy. For this reason oral aplication of IM 400 mg/day was initiated. 51 patients whose median age at the time of IM therapy initiation was 48 years, were treated more than 12 months. We described the pattern and rapidity of the response to IM, comparing the cytogenetic and molecular responses and progression-free survivial rates in patients who obtained a complete cytogenetic response (CCyR) within 1 year of treatment (early responders) and in patients where a CCyR was detected after 12 months (late responders).Cytogenetic analysis was performed by standard banding techniques and FISH. Minimal residual disease was detected during follow-up by quantitative realtimeRT-PCR. *Results*. in the group of patients treated with IM for more than 12 months, we observed significant cytogenetic response in 76% (39/51) and complete cytogenetic remission in 53% (27/51). In the group of early responders (complete cytogenetic remission achieved during the first year), CCyR was achieved in 52% (14/27) with median 9 months (6-12). IFN alfa pre-treatment was 13 months (3-48 months). In the group of late responders (IM treatment more than 12 months), CCyR was achieved in 48% with median 22 months (16-33). In 9 cases CCyR was preceeded by significant cytogenetic response (MCyR), in 7 of them following 12 months after therapy initiation. Patiens were IFN alfa pre-treated for 3-52 months (median 24). The reduction in BCR-ABL level was in 46% in late responders and in 36% in early responders. In the group of early responders, CCyR persisted in 86% with median 20 months (6-55), while in the group of late responders, CCyR persisted in 92% with median 25 months (4-32). All of the studied patients are still alive. Eventhough in the group of late responders the time from diagnosis until IM therapy initiation and IFN alfa pre-treatment are significantly longer, the rate of CCyR (48%) is quite similar to the group of early responders (52%). Overall survival without significant progression is also quite similar. The number of persisting CCyR is even higher (92%) in contrast to 86% in early responders. *Conclusions*. Our results suggest that the sensitivity and response to imatinib may require 1 year or more. It could be possible to continue the same dose of IM especially if a CCyR had not been reached within 12 months. This is especially true in patients, as the late responders, who had already a good cytogenetic response within one year, even if not yet complete. For the patients who had not reached CCyR other approaches, for instance the therapy with the new tyrosinkinasis inhibitors or transplantation (HSCT) available could be realized.

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INSIGHTS INTO THE INTERACTIVE FUNCTIONS OF GATA 1S USING GENE EXPRESSION PROFILES

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Background. DNA microarray analysis is a potentially informative approach to understand the pathways that leads to clinical and hematological abnormalities. We applied Affymetrix GeneChip oligonucleotide arrays, a popular platform for the high-throughput analysis of

gene expression in mRNA, in order to study a severe dysfunction in the hematopoietic system. GATA 1 gene transcription represents a crucial transcription factor in erythroid and megakaryocytic differentiation process and the regulation of nonspecific hematopoietic genes. Hollanda *et al*. (2006) identified a hemizygous G-C transition at position 332 at the boundary of exon 2 in the GATA 1 genomic DNA sequence. This GATA 1 mutation leads to splice site changes that prevent the translation of the full-length GATA1 protein and allow the generation of only GATA1s in affected males. This mutation predominantly affects the erythroid lineage and also seems to produce normal or hyperproliferation of morphologically abnormal megakaryocytes and platelets with ultrastructural and functional abnormalities. *Aims*. The aim of this study is to gain new insights into the gene expression profiles of GATA1s in stem and progenitor cells. *Methods*. Total RNA was extracted from cryopreserved bone marrow samples of individual III-16 and one healthy control using RNeasy MiniKit (Quiagen), following the protocol supplied by the manufacturer. These samples were amplified and cRNAs were biotin-labelled and fragmented with the Two-Cycle Target Labelling kit (Affymetrix) in triplicate for hybridization to Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays. Analysis of microarray experiments was performed using GeneChip Operating Software version 1.4 and Array Assist 2.0. Quantification of gene expression by real time quantitative polymerase chain reaction was performed with a GeneAmp 5700 (Applied Biosystems) with Master Mix SYBR green I reagent (Applied Biosystems) in order to confirm the microarray expression results for several genes. *Results*. Our results appear to be in accordance with previous reports, c-myc was increased less than 2-fold in the GATA1s patient, GATA2 has been shown to be up-regulated in the absence of GATA1. Comparative expression data point to several other genes up-regulated in the absence of GATA1; caspases 1, 5, 7, Bcl2, PIAS1, ELOVL5, USP12, in contrast with down-regulated caspases 2 and 6, NCAM1, EPB41L3. *Summary*. The specific expression changes identified must be biologically important in the pathophysiology of this disorder. Understanding of GATA1 regulation and mechanisms of action should be critical for improving the treatment of hematological diseases and leukemias as well as for developing alternative therapeutic approaches, such as differentiation therapy.

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EFFICACY OF THE EXPANDED TREATMENT PROGRAM WITH IMATINIB MESYLATE IN 109 PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML)

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Background. Imatinib mesylate induce complete hematological, cytogenetic response in patients with CML. *Aim*. In the present study we evaluated efficacy of the expanded imatinib treatment program in 109 patients (pts) with different phases CML. *Patients and Methods*. 150 patients are observed since April-2005. 109 patients (52 female, 57 male, median ages 48 years (21-78 years)) received imatinib. 43 patients received imatinib 400 mg/day as a first line therapy in the early chronic phase (The median time from diagnosis CML is no more 12 months). 66 patients had received previous treatment. Among 66 patients 49 were in the late chronic phase, 14 were in the acceleration phase, 3 pts were in blast phase. The median time of illness in the late chronic phase and in the acceleration phase was 4 years (1.5-7 years). For diagnostic and monitoring purposes (in 6 and 12 months of assessment) standard cytogenetics research in bone marrow and FISH method were applied. *Results*. Complete hematological response was achieved in 92% of patients with early chronic phase of CML. 27 pts with the early chronic phases CML received imatinib treatment 6 months (on the moment of analysis). Among them complete cytogenetic response (CCR), partial cytogenetic response (PCR), minor cytogenetic response (MCR) and no response were observed in 6/27 (22.2%), 9/27 (33.3%), 6/27 (22.2%), 6/27 (22.2%) pts respectively; imatinib dose escalation to 600 mg/day occurred in 8 patients. Response to treatment was evaluated after 12 months therapy among 16pts with the early chronic phases CML: CCR was in 9/16 (56.2%) pts, PCR was in 4/16 (25%) pts, no response was showed in 3/16 (18.7%) pts. There is important to emphasize that more than double increase of complete cytogenetic response with regard to treatment duration. In the late chronic phase patients after 6 months treatment CCR was in 12/39 (30.7%) pts, PCR in 3/39 (7.7%) pts, MCR in 9/39 (23%) pts, 15/39 (38.4%) pts were showed no response. Among the late chronic phase patients after 12 months treatment we observed CCR in 12/29 (41.3%), PCR in 2/29 (6.9%), MCR in 6/29 (20.6%), no response in 9/29 (31%) pts respectively. In the acceleration phase patients at 6 months: CCR was in 1/10 (10%), MCR in 1/10 (10%), no response in 8/10 (80%); at 12 months: MCR - 6/10 (60%), no response ' 4/10 (40%). In 1 blast phase patient - MCR was achieved at 6 months and 12 months, PCR was achieved at 18 months. *Conclusions*. The Expanded imatinib treatment program of patients with CML in different phases is highly effective regimen allowing achieving CCR in 56.2% and total major cytogenetic response (CCR+PCR) in 81.2% of patients with early chronic phase of CML after 12 months of treatment with imatinib. 72% of patients with CML in different phases from our hospital database were considered.

1497

SALVAGE THERAPY R-DAOX IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Standard salvage chemotherapy for aggressive non Hodgkin's lymphoma does not exist. 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. *Methods*. Patients relapsed or refractory after first line therapy were treated at three weekly intrevals with rituximab (375 mg/m^2 day 0), oxaliplatin (120 mg/m^2 day 1), cytarabine (2000 mg/m^2 days 2,3) and dexamethasone (40 mg days 1 to 4). *Results*. Twenty-two patients with median age of 57 years (range 33-74) entered this study. Histological subtypes were diffuse large B cell, 19; grade III follicular lymphoma 3 patients. The overall response rate (RR) was 64% (14/22) including 9 complete remission (CR) and 5 partial remission (PR). Sixteen patients were treated with R-DAOx as second line, eleven with the intention to perform autologous stem cells transpantation (ASCT). Only six of these eleven patients obtained a response and were treated with ASCT. Seven patients were primary refractory and only one out seven obtain a partial response with R-DAOx. Six patients were traeted with R-DAOx as third line therapy (4 were relapsed after ASCT) and five obtaine a response to therapy. Grade 3 and 4 toxicity was mainly hematological. All patients were supported with stimulating factors. No grade 4 non-hematological toxicity was reported in particular no significant renal and neurotoxicity was demonstrated. Median survival was 16 months. Probabilities of 1-year progression free survival and overall survival were 20% and 29% respectively. If we consider only chemosensitive patients, after R-DAOx, the PFS and OS were 28% and 43% respectively. *Conclusion*. R-DAOx is a novel combination for the treatment on outpatients basis relapsed or refractory patients. It has a clinically significant activity in chemosensitive patients with an acceptable toxicity profile in particular the lack of renal toxicity and neurotoxicty makes this regimen attractive before high-dose chemotherapy.

1498

HEMOLYTIC ANEMIA WITH POSITIVE DIRECT ANTIGLOBULIN TEST SECONDARY TO CYTOMEGALOVIRUS INFECTION IN INFANT

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We report here a young infant with severe cytomegalovirus (CMV)-associated warm antibody autoimmune hemolytic anemia (AIHA). AIHA is a hematologic disorder that is rarely seen in infants and young children. In contrast to that in adults, childhood AIHA is frequently associated with viral or bacterial infection. A previously healthy 2-month-old infant presented to Hospital with a 4-day history of pallor. The physical examination was remarkable for marked skin and mucosal pallor, trace scleral icterus, mild hepatosplenomegaly, and tachycardia with gallop. Laboratory investigations revealed: hemoglobin 2.2 gr/dL, leukocyte count 35.2×10^9/L, platelet count 263×10^9/L; mean corpuscular volume 90.6 fL; reticulocyte count 17%; and blood type 0-Rh-positive (maternal blood type was same). The direct antiglobulin test (IgG) was positive, consistent with the presence of a warm autoantibody. A diagnosis of AIHA was made, and phenotypically matched erythrocyte transfusion support was initiated. Intravenous methylprednisolone therapy was also started at 4 mg/kg per day. After five days ,as a result of the lack of response to corticosteroid therapy and the continuous need for erythrocyte transfusion support, standard intravenous

immune globulin was administered at a dosage of 5 g/kg, in two divided doses, over the course of 48 hours. Corticosteroid therapy was tapered slowly. Serologic evaluations obtained before the administration of immunoglobulin revealed a positive CMV IgM antibody. Five days after intravenous immune globulin administration, the hemoglobin level improved to 10 g/dL. Hemolytic anemia with positive direct antiglobulin test in association with spontaneous cytomegalovirus (CMV) infection is a rare event in infants and young children. Because CMV serology is not routinely obtained as a part of hemolysis work-up,the true incidence of this complication may be underestimated. We believe that CMV infection should be considered in the differential diagnosi Autoimmune hemolytic anemia s of hemolytic anemia in infants.

1499

EXPRESSION OF COMPLEMENT INHIBITORS CD46, CD55 AND CD59 AND RESPONSE TO RITUXIMAB IN PATIENTS WITH CD20+ NON HODGKINS LYMPHOMA

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Background. Rituximab (R) is an anti-CD20 chimeric monoclonal antibody widely used for the treatment of B-cell non-Hodgkin's lymphomas (NHLs). There are several data suggesting that different pattern of the response to rituximab therapy may be related with complement function-complement mediated cytotoxicity (CDC). Complement inhibitors CD46-MCP (membrane cofactor protein), CD55-DAF (decay accelerating factor) and CD59-HRF20 (homologous restriction factor 20) are important regulators of complement mediated cytoxicity. The main function of the complement system is to lyse virally infected cells and tumour cells. CD46, CD55 and CD59 are the regulators of the classical, alternative and terminal complement pathways. *Aim*. The aim of this study was to analyze expression of complement inhibitors CD46, CD55 and CD59 in patients with CD20+ non Hodgkin's lymphoma treated with R-CHOP. *Material and methods*. 23 patients with CD20+ non Hodgkin's lymphoma were evaluated (11 females and 12 males). The median age of patients was 56 years (range: 41-74 years). There were 14 patients with diffuse large B-cell lymphoma (DLBCL) and 9 patients with follicular lymphoma (FL). By the Ann Arbor Lymphoma Staging Classification 4 patients (17%) had stage II, 3 patients (13%) had stage III and 16 patients (70%) had stage IV. 7 patients (30%) had bulky disease. All patients were examinated before treatment with rituximab. Expression of CD46, CD55 and CD59 was determined by 2-color flow cytometry. FITC labeled monoclonal antibodies against CD46, CD55 and CD59 (Becton Dickinson) were used. For statistical analysis U-Mann-Whithey Test and ANOVA-rang Kruskal-Wallis test were used. P≤0,05 was considered statistically significant. *Results*. All patients were treated with standard dose R-CHOP (4-8 courses). 13 patients (57%) achieved complete response (CR), 3 patients (13%) achieved partial response (PR) and 7 (30%) patients had no or minimal response (NR) after rituximab/CHOP therapy. We compared level expression of CD46, CD55 and CD59 in 3 groups: patients with CR, patients with PR and patients with NR after rituximab/CHOP therapy. The mean expression of CD59 was statistically significant higher in patients with NR than in patients with CR (p<0,05). We observed that expression of CD46 and CD55 were higher in patients with PR and NR than in patients with CR but these differences were no statistically significant. Results are shown in Table 1. *Conclusion*. Our preliminary study suggests that levels of CD 59 but not CD46 and CD55 may be important to predict response to rituximab.

Table 1. Meam expression of CD46, CD55 and CD59 in patients with CR, PR and NR (SD-standard deviation).

	Mean expression of CD46 [SD]	Mean expression of CD55 [SD]	Mean expression of CD59 [SD]
CR	71,41 [±5,77]	70,63 [±6,02]	75,74 [±6,60]
PR	77,25 [±7,13]	76,24 [±7,79]	75,74 [±8,86]
NR	77,57 [±1,18]	77,46 [±1,04]	80,17 [±2,28]

1500

TWO CASES OF REFRACTORY AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) TREATED WITH EPOETIN ALFA (EPO)

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Background. The treatment of AIHA consists in corticosteroids, splenectomy and other immunosuppressive drugs like rituximab. However, in elderly patients the risk of splenectomy may be high owing to other concomitant diseases. *Aims*. We describe two elderly patients with AIHA, who responded to EPO and became independent of transfusions. Both were refractory to corticosteroids and could avoid splenectomy. *Methods*. Study of the two cases. *Results*. 1. A 78-year-old woman was referred to us because of anemia that had required 2 units of red blood cells (RBC). Her hemoglobin (Hb) was 68 g/L and his reticulocyte count 121 $\times 10^9$/L. Biochemical determinations established an increased hemolytic activity. The serologic study demonstrated a direct antiglobulin test (DAT) positive and a warm IgG antibody. A bone marrow (BM) film revealed an erythroid hyperplasia. The BM inmunophenotype exhibited a clonal expansion of CD19 lymphocytes with kappa chain restriction. She had a splenomegaly of 17,6 cm. A diagnosis of AIHA was made. Prednisone therapy at daily dose of 1,5 mg/kg was started to maintain an Hb between 70 and 85 g/L during 3 weeks. A descompensated diabetes prompted us to reduce the dose of prednisone, and to commenced EPO, 10.000 U three days a week. The Hb raised to 115 g/dL one month later. The prednisone was withdrawn progressively and a low dose of chlorambucil was began. We discontinued the EPO 7 months later. After 31 months the patient is keeping in remission. 2. A 82-year-old man with pancytopenia was referred to us. He had needed 4 RBC units last 2 weeks. His initial Hb was 77 g/L and his WBC count 2.1×10^9/L with a normal blood smear; his platelet count was 106× 109/L and his reticulocyte count 220×10^9/L. Biochemical determinations established an increased hemolytic activity, with DAT negative. Anti-DNA antibodies were positive. A BM film revealed an erythroid hyperplasia. The BM inmunophenotype exhibited a clonal expansion of CD3+CD5+CD8+CD56- lymphocytes. Monoclonal rearrangements of T-cell-receptor genes were detected in gamma-1 chain, using polymerase chain reaction. A paroxystic nocturnal hemoglobinuria was ruled out by means of CD55 and CD59 100% positivity in erythrocytes and granulocytes by flow cytometry. A splenomegaly of 16 cm was detected. A diagnosis of pancytopenia and AIHA associated to a T-lymphocyte clonal proliferation was made. The patient didn't respond to high dose of prednisone and required 6 more RBC units the next two months. We began cyclosporin A, but we had to withdrawn it because of hepatic and renal toxicity. Then, we started methotrexate and additionally 4 cycles of Rituximab at 375 mg/m^2. The patient reached a partial remission requiring 2 units every month. Thereafter, we tried EPO 40.000 U each week and the patient became independent of transfusions attaining an Hb of 111 g/L one month after the starting treatment with EPO. *Conclusions*. These two cases reported support the use of EPO in AIHA to manage refractory patients who are not candidates for esplenectomy, and could aid to maintain them until other treatments had effect.

1501

ACHIEVEMENT OF BCL-2/IGH NEGATIVITY IN PERIPHERAL BLOOD/BONE MARROW IS ASSOCIATED WITH BETTER CLINICAL OUTCOME IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Background. Bcl-2/IgH rearrangement is the molecular hallmark of follicular lymphoma with up to 90% of positive cases at the time of diagnosis. Howewer, its clinical significance remains controversial. *Aims and methods*. The aim of this study was to evaluate Bcl-2/IgH rearrangement in 50 patients with follicular lymphoma before treatment by nested PCR and to assess the prognostic impact of molecular remission on clinical course. *Results*. Twenty six out of fifty patients (52%) were positive for Bcl-2/IgH rearrangements before treatment, 24 in major breakpoint region (MBR) and 2 in minor cluster region (mcr). Twenty-seven patients (54%) were treated with chemotherapy (CHT) alone; remaining other 23 patients (46%) with chemotherapy in combination with anti- CD20

monoclonal antibody-rituximab (R-CHT). Molecular remission has been achieved more likely after R-CHT (64%) than after CHT (14%), p=0.037). Seventeen out of twenty seven patients treated with CHT alone who did not achieve complete remission underwent consolidation therapy with rituximab (375 mg/m^2, four weekly infusions) in order to eradicate residual disease. 12/17 patients (71%) were Bcl-2/IgH positive before consolidation. Five out of twelve patients (58%) became negative 1 month after rituximab consolidation therapy, others 5 patients were followed every 3 months for Bcl-2/IgH rearrangement. In two of them molecular remission has been achieved after 3 and 6 months, respectively. Complete molecular response in this group was 75%. By univariate analysis, Bcl-2/IgH negativity during treatment was achieved more likely in patients under 65 years of age (p=0.02), with ECOG performance status 0/1 (p=0.02) and with FLIPI score 0/1 (p=0.05). Patients in molecular genetic remission had lower risk of relapse/progression (27% vs. 75%, p=0.03), better 2-year progression-free survival (81% vs. 38%, p=0.004), event-free survival (74% vs. 38%, p=0.01) and borderline overall survival (87% vs.74%, p=0.05) in comparison with those who remained Bcl-2/IgH positive. *Summary*. In conclusion, achievement of molecular remission in our study was associated with better clinical outcome of patients with follicular lymphoma. Consolidation treatment with four doses of rituximab after chemotherapy alone was very effective in eradication of residual lymphoma activity.

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1502

THE FACILITATING FACTORS IN THE TRANSFORMATION OF MULTIPLE MIELOMA IN PLASMOCYTE LEUKEMIA

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In a 10 year interval (July 1997- February 2007) a lot of 154 patients diagnosed with multiple mieloma with ages from 49 to 78 were followed. The sex ratio was 1.6/1. Out of all the patients, 17 developed plasmocyte leukemia, during a 3 months to 7 years interval from the initial diagnosis of multiple mieloma. All the patients that developed plasmocyte leukemia were investigated for: - the type of multiple mieloma, - the amplitude of the monoclonal behavior and it's dynamic evolution, - the initial infiltration of mieloma cells in the bone marrow and its dynamic evolution under treatment, - the presence or absence of the plateau phase post therapy, - the presence or absence of bad initial prognostic factors especially: PCLI; C-reactive protein; the concentration of β2-microglobulin. - the presence of Trysomy 12. The results were as follows: - 9% of the followed patients developed plasmocyte leukemia within 3 to 84 months from the initial diagnosis. -52.9% were initially diagnosed with IgA type mieloma -17.7% with light chain mieloma. - 29.4% were IgG3 mieloma. - the initial infiltration with mieloma cells varied between 26 and 56%. Sixteen of the seventeen cases mieloma unresponsive to classic treatment we use (Melfalan+ Prednison, Dexametazona in high dosage and VAD type sessions). The infiltration with mieloma cells varied with + or-15% compared with the initial infiltration without a plateau phase. The monoclonal component was relatively stable despite the treatment (+ or-19% compared to initial values). 76.4% of the patients had values of more than/ equal to 3% of PCLI and values of β2-microglobulin higher than 6 mg. The initial values of the C- reactive protein were higher than 6 mg in 38.2% of the cases. Trisomy 12 was initially discovered at 9 patients (52.9%) including the patient that quickly plasmocyte leukemia. To conclude plasmocyte leukemia appeared at the great survivors of reluctant multiple mieloma with generally moderate infiltration of mieloma cells, having 2 to 4 facilitating factors. The most frequent cases of plasmocyte leukemia were recorded at IgA mieloma, light chain mieloma, IgG3 mieloma. After 39 to 52 months, citopenia appeared no matter the treatment utilized, 4 patients having 1 citopenia, 4 patients having 2 citopenias, 9 patients being pancitopenics. 52.9% from the transformed cases developed plasmoblastic leukemia, all of the 9 patients presenting trisomy 12. Although the appearance of secondary plasmocyte leukemia can not be foreseen, we believe that the initialization of classical therapy in patients with reluctant mieloma is not a recommendable alternative. In our cases the classical treatment did not induce a plateau phase, although the percent of long time survival till the moment of transformation is relatively satisfactory. In Most of the cases we monitored, the timing of the first signs of plasmocyte leukemia came as a natural step in the evolution of the disease, precipitated by many adverse factors.

1503

THE EFFECTS OF HYDROXYUREA TREATMENT IN BAHRAINI PATIENTS WITH SICKLE CELL ANEMIA AND SICKLE CELL THALASSEMIAS

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Background. Induction of fetal hemoglobin (HbF) by hydroxyurea (HU) ameliorates the morbidity of sickle cell disease (SCD) and is part of standard therapy for this disorder. The majority of reports have described the results of HU treatment in patients with homozygous sickle cell anemia (SCA) and a few have studied patients with double-heterozygous sickle-β thalassemia (S-β thal). The sickle cell mutation and its interactions with α- and β- thalassemias are relatively common in the Arabian Gulf region. However no previous study has compared the effects of HU in these subsets of patients with SCD. *Aims*. The objectives of the study were to evaluate the effects of HU therapy on laboratory and clinical parameters in SCD and to compare the extent of these changes among sub-groups of these patients. *Methods*. Fifty-one consecutive cases of SCD who were being followed up during HU therapy at Salmaniya Medical Complex, Bahrain, were included in the study. The availability of a pre-treatment history and post-treatment follow-up of at least one year respectively were the inclusion criteria. Hematologic variables included hemoglobin, hematocrit, red cell indices and hemoglobin fractions obtained by HPLC at the time of presentation and one year post-therapy. Patients were stratified into four groups: 1. SCA (n=24) 2. SCA with possible α-thalassemia (MCV <80 fl, negative for Hb H/Hb Barts/HbH inclusions, n=16) 3. SCA with α-thalassemia (positive for Hb H/Hb Barts/HbH inclusions, n=7) 4. S-β thal (high HbA2 and positive family-study or genotype, n=4). HU therapy was initiated after assessment of disease severity according to standard practice guidelines. The initial dose was 15 mg/kg and was increased to 30 mg/kg depending on response. Neutropenia was the dose-limiting factor. Documented clinical response variables included the number of hospital admissions, days spent in hospital, pain crises requiring hospital admission, pain crises that did not require admission and blood transfusion episodes during the year preceding and the year following initiation of HU. Data were analysed using SPSS 14.0 software. Analysis included non-parametric tests for inter-group comparisons (Kruskal-Wallis and Mann-Whitney tests), t-test for paired-samples and Pearson correlation test for significance of association between laboratory and clinical variables. *Results*. HU treatment resulted in highly significant changes in almost all clinical and hematological indices that were considered. Significantly increased hemoglobin, hematocrit, MCV, MCH, and HbF and reduced HbS and RDW were observed a year after starting treatment. All cases considered, the mean increase of hemoglobin was 0.86 g/dL and that of HbF was 5.2%. Highly significant improvement of morbidity indicators in the pre-treatment versus post-treatment phases was also observed. These included reduction of hospital admissions/year (mean 2.80 versus 0.69), hospital stay (mean 19.08 versus 3.57 days/year) and blood transfusion episodes/year (mean 2.04 versus 0.12). No significant inter-group differences of response were observed. *Conclusions*. HU therapy resulted in highly significant hematological changes that correlated with reduction in severity of all indicators of disease morbidity. There does not appear to be any significant difference in the magnitudes of response of subgroups of patients with or without co-inheritance of sickle cell and α- or β- thalassemia.

1504

IMATINIB EXACERBATES HYPOTHYROIDISM IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA

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Imatinib a specific small molecule inhibitor of BCR-ABL has become the standard treatment regime for Chronic Myeloid Leukaemia. Willem *et al.* 2000 reported that post thyroidectomy patients taking Imatinib had markedly elevated TSH levels and reported clinically hypothyroid. We would like to report a female patient aged 54 with a known medical history of hypothyroidism for two years, who remained euthyroid and asymptomatic on a maintenance dose of Levothyroxine 125 micrograms. The patient was diagnosed with Philadelphia positive CML (t (9, 22) confirmed by PCR) in 2005 with a white cell count of 226.30×10^9/L. She was commenced on Imatinib 800 mg for 11 days, reducing to 400 mg daily. Since her WCC was slow to respond the dose was further increased to 600mg daily. Subsequently the WCC stabilised, and she was

maintained on Imatinib 400 mg. After one year on imatinib, she presented with tiredness and weight gain and a raised TSH level of 42.1 miu/L. The dose of Levothyroxine was increased to 150 micrograms daily, with a reduction in the TSH levels to 12.7 miu/L and improvement in her symptoms. Three months later, the TSH levels elevated to 25.1 miu/L. She was also found to have Thyroglobulin antibodies of 152 units. The dose of Levothyroxine was further increased to 175 micrograms daily, two months later to 200 micrograms daily but her hypothyroidism remains increasingly difficult to treat. Meanwhile, she continues on Imatinib 400mg daily. This is the first case of hypothyroidism following Imatinib treatment in a patient who had not undergone a thyroidectomy. It has been suggested by Willem *et al.* 2000 that the most likely mechanism that contributes to Imatinib-induced hypothyroidism involves stimulation of T4 and tri-iodothyroxine (T3) clearance. We would recommend regular thyroid function tests in patients taking Imatinib with or without thyroidectomy.

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1505

TREATMENT OUTCOME OF PATIENTS RECEIVING IMATINIB FOR CHRONIC MYELOID LEUKEMIA AS FIRST OR SECOND LINE THERAPY

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Background. Imatinib is currently the first line treatment for CML patients. In Brazil until recently patients have used imatinib after Interferon failure or intolerance. *Methods*. retrospective analysis of 139 patients with CML that received imatinib as first or second line therapy from December 2000 to November 2006. Patients were evaluated with overall and event-free survival; progression to accelerated-phase CML or blast crisis; hematologic, cytogenetic, and molecular responses and resistance. *Results*. sixty-one patients (44%) were previously treated with Hydroxiurea (HU), 65 with HU+IFN (47%) and 10 with other treatments (three with autologous bone marrow transplantation). When patients started imatinib, 90 were in CP, 31 in AP and 18 in BC. 115 patients (83%) achieved hematologic response, 46 of 91 (50,5%) achieved complete cytogenetic response (CCR) and 19 of 91 (20,9%) partial cytogenetic response (PCR). Molecular response was accessed in 73 patients and 25 (34,2%) achieved major molecular response. Sokal and Hasford score were associated with achievement of cytogenetic response (p=0,001 and p<0,0001, respectively). The median follow-up was 11 months. Kaplan-Meier estimates of cumulative best rates of complete cytogenetic response among patients receiving imatinib were 75% by 18 months. Overall survival and disease free survival according disease phase was 92% and 62% for patients in CP, 62% and 35% for patients in AP and 15% and 16% for patients in BC (both, p<0,0001). The estimated overall survival of patients which started imatinib before one year from diagnosis was 88% and 65% for patients which started imatinib after one year from diagnosis (p=0,007). Overall survival was significantly better in patients with low risk Hasford score (low=100%, intermediate=71% and high=70%) (p=0,03). Resistance occurred in 60/135 (44%) patients most in advanced phases. 11 out of 18 patients presented bcr-abl mutations at time of resistance. *Conclusions*. Treatment with imatinib in the first 12 months from diagnosis and in early chronic phase CML induced durable responses in a high proportion of patients and was related to better overall and disease free survival. Patients treated in advanced phases had poor outcome and most of them developed resistance.

1506

GENDER AND BIRTH WEIGHT INFLUENCE ON CORD BLOOD CELLS COUNT

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Background. Investigation of cord blood specific features is prognostically important for determination of efficacy of volume reuction that is obligatory in cord blood banking. Antropometric characteristics are also important for selection of voluntary donors for cord blood donations. The aim of the study was to determine the influence of gender and birth weight on the cell counts of cord blood. *Materials and methods*. Cord blood of 1004 newborns (age of gestation 37-41 weeks) was collected in utero by venepuncture of umbilical vein in closed system (250 ml, containing 35 ml CPDA as anticoagulant) under gravity 5-15 minutes after intersection of cord. Blood analysis was performed by two methods ' automated screening (AS) by ABX Pentra 60C+ and light microscopy (LM) of smears. Counts of WBC, red cells, platelets and hemoglobin were corrected to exclude the deviations due to different proportion of anticoagulant in samples. From 1004 newborns 524 (52,2%) were boys. *Results*. We found that girls have significantly higher level of leukocytes (17,74±0,24×10^9/L) than boys (16,8±0,23×10^9/L) (p=0,005). At the same time girls have higher percentage of neutrophils, and lower percentage of lymphocytes, monocytes and eosinophils. Girls have higher absolute counts of neutrophils and lower counts of eosinophils. Red cell counts (4,33±0,02×10^{12}/L), hemoglobin (155,1±0,07g/L) and hematocrit (31,79±0,2%) are lower in girls than in boys (4,46±0,02 ×10^{12}/L; 159,5±0,06; 32,76±0,19%; p<0,0001, respectively). Red cell indexes (MPV, MCH, MCHC, RDW) were independent of gender and birth weight. Platelet counts were higher in girls (314,87±2,98×10^9/L) than in boys (300,72±2,62×10^9/L; p<0,0001). Birth weight was higher in boys (3629,24±18,25 g, range 2270-5000g) than in girls (3467,23±18,17 g, range 2300-4650g, p<0,0001). There are more boys in group with newborn weight > 4000 (p=0,001) than girls and girls in group with newborn weight ~2500-3000 (p=0,008). However, differences in cord blood cells count in boys and girls were similar in identical newborn weight groups. Hemoglobin level and red blood cell count are not different in compared body weight groups of newborns. Thus, we demonstrated significantly higher neutrophyl count and significantly lower lymphocyte, monocyte and eosinophile counts in newborn girls, that is not related to newborn weight and gestational age.

1507

FACTORS THAT INFLUENCE THE EFFICIENCY OF CORD VOLUME REDUCTION

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Background. Cord blood banks are making efforts to create and improve technologies for cord blood volume reduction and cryostorage. Investigation of factors that influence the efficiency cord blood volume reduction is very important in public cord blood banking. *Aim*. To evaluate factors that may influence on the efficiency of cord blood volume reduction. *Materials and methods*. Cord blood of 1095 term newborns (age of gestation: 37-42 weeks). was collected in utero by venepuncture of umbilical vein using a closed 250 ml system, containing 35 ml CDPA as an anticoagulant (5-15 minutes after cord dissection).Volume reduction procedures were done using method of 1) gradient centrifugation and plasma extraction established in New York cord blood bank (n = 539) and 2) using Sepax (BioSafe, Switzerland) (n=554). Cells composition was analyzed using automated cell counter ABX Pentra 60C$^+$ and light microscopy of smears. Number of WBC, red cells, platelets, and hemoglobin were corrected to exclude the deviations due to different proportion of anticoagulant in samples. *Results*. Average leukocyte recovery was 71,45±0,35%, recovery of total nucleated cells-74,0±0,45%, lymphocyte recovery- 76,48±0,45%. Average number of residual red cells per sample was 28,65±0,43%. Efficiency of volume reduction was independent of delivery, birth weight and gender, and twin delivery. The average recovery of WBC decreased with longer gestational age from 75,85± 3,53% on 37th week to 67±2,46% on 41st week (p=0,043). Maximal recovery was on the39th gestational week-80,11±0,95% (WBC) and 82,46±1,01% (lymphocyte) and it decreases with shorter and longer gestational age (p=0,025). WBC count before volume reduction is associated with the recovery: if WBC count is <10 ×10^9/l (n=354) recovery rate is 73,19±0,52%, lymphocyte recovery- 76,55±0,71%; if WBC count is 10-20 ×10^9/l (n=694) recovery rate is 70,81±0,44%, lymphocyte recovery' 76,57±0,5%; if WBC is >20 ×10^9/L (n=44) recovery rate is 63,61±1,86%, lymphocyte recovery -68,15 ±2,39% (p<0,0001). Recovery rate also depends on anticoagulant amount: leukocyte yield increases from 67,67±1,2% when of anticoagulant content is less than 20% up to 74,99±1,18% when anticoagulant amount is more than 40%. Mononuclear fraction and lymphocyte yield is maximal when anticoagulant rate is 20-40% and decreases when amount of anticoagulant

decreases or increases (p<0,0001). Our results demonstrate that recovery of cord blood WBC depends on gestational age, leukocyte count before procedure and cord blood and anticoagulant ratio.

1508

RITUXIMAB MAINTENANCE THERAPY: AN EFFECTIVE AND TOLERABLE STRATEGY TO IMPROVE AND PROLONG RESPONSE IN CD20+ B-CELL LYMPHOPROLIPHERATIVE DISORDERS

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The sinergistic effect of rituximab on first line and salvage chemotherapy in CD20+ NHL has improved OR, PFS and OS. However, still a constant pattern of relapse is observed after such an optimized therapy. Rituximab maintenance prolongs PFS and OS compared to observation alone. The best schedule, its role in erradicating minimal residual disease, and concerns like the efficacy of R-maintenance after induction and salvage rituximab-containing therapy and the efficacy of rituximab-containing salvage regimens in relapses after R-maintenance remain unanswered issues. *Aims*. To evaluate efficacy and safety of R-maintenance in CD20+ lymphoprolipherative disorders (LPD). Patients (pts) with CD20+ LPD (indolent NHL and selected cases of high-risk aggressive NHL and CLL) were eligible if only they achieved CR or CR with persistent minimal residual disease (MRD) -detected by PCR and/or flow citometry- after first-line or salvage therapy, either combining rituximab or not. Rituximab (375 mg/m^2) was given by 2 schedules: 4 weekly doses/6 months in high-risk factors or MRD+ pts, or 1 dose/3 months in the rest of them. Total duration: 2 years. Clinical performance, MRD and biochemical and hematological profiles were assessed every 3 months, CT/PET at 6/12 months, unless relapse was suspected. Patients were evaluable if less than 3 months of initiating R-maintenance. *Results*. From January'01 to February'07, 49 pts (24 male:25 female; age (M[R]): 59 [34-76]) diagnosed of FL(28), Marginal Zone NHL (11), Waldeström´s macroglobulinemia (3), Mantle Cell Lymphoma (2), DLBCL (3) and B-CLL (2); stages III-IV: 42 (89,3%), were prospectively included in this observational, single arm study. Number of previous treatments [M(R)]: 2(1-5). Thirty-three of 49 pts had received R-containing therapy. Forty pts had achieved CR MRD- before R-maintenance, and the remaining 9 had CR with persistent MRD. Efficacy: Thirty-eight (95%) of 40 pts in CR MRD- currently remain in CR, 1 not evaluable (<3 months follow-up), 1 relapsed. Five (55%) of 9 CR MRD+ achieved negativity of MRD- and remain CR; 2 remain stable CR MRD+ and 2 relapsed. Median follow-up: 12 m (3-79), 10 pts having completed 2 years and still in CR. The 3 relapsing pts were successfully treated with local radiotherapy (1 DLBCL) and R-chemo (1 FL, 1 Mantle). No differences in efficacy were seen either between both schedules or with/without R in previous treatments. Toxicity: 4 episodes of neutropenia grade 3/4 (all with previous myelotoxicity during induction/salvage QT), 4 fever with different infectious focus, 2 diarrhoea, 5 lymphopenia with hipogammaglobulinemia (not associated infections), 1 anemia grade 3 and 1 hipertransaminasemia in HCV+ pt were recorded. All episodes were successfully controlled, requiring interruption of R only the HCV+ patient. *Conclusions*. 1. Rituximab maintenance is effective improving CR (eradicating MRD) and prolonging TTF in first-line and relapsing CD20+ LPD, either with or without R-containing previous regimens. The 3 relapsing patients favourably responded to salvage strategies, whether they included rituximab or not. 2. R-maintenance is safe and manageable. Cases with neutropenia are probable due to myelotoxicity produced by previous QT. 3. No differences were seen between both schedules regarding efficacy or associated toxicity.

1509

STEROID REFRACTORY GRADE IV ACUTE GASTROINTESTINAL GRAFT VERSUS HOST DISEASE (GVHD): A DIFFICULT PROCESS FOR A TRANSPLANT PYSICIAN

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Introduction. Steroid refractory severe gastrointestinal (GI) GvHD is one of the important complications in patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) and is associated with very high mortality and morbidity, despite the improvements in cGVHD prophylaxis and treatment. Although various treatments including high dose steroid, anti-thymocyte globulin (ATG), infliximab and daclizumab have been used as the salvage regimens on these patients, the results are not satisfying. In recent years, several studies were shown that application of steroid directly into arteries supplying blood to the liver and intestines could be an effective and safe treatment modality. In this study, we presented a case with steroid refractory grade IV GI-GVHD, which exhibited a prominent recovery by steroid injection into superior and inferior mesenteric artery. *Case*: The patient, 44-year old male, with 1st complete remission-AML underwent allo-peripheral HSCT from an HLA identical sibling donor on 17.06.2006 following a myeloablative conditioning regimen including cyclophosphamide plus total body irradiation (Cy-TBI). After the successful engraftment, skin (GrIII) and gastrointestinal (Gr IV) GVHD developed on day +30 and it was confirmed by histopathological examination. Skin findings resolved after systemic methylprednisole (MP) at the dose of 2mg/kg/day i.v. treatment. However, GI GVHD did not respond to the steroid treatment. The volume of diarrhea reached 6 L daily. On day +35 ATG-Fresenius (5 mgr/kg for 5 days i.v.) was administered. During ATG treatment, severe abdominal pain and GI bleeding and also liver GVHD were added. Infliximab, 10 mg/kg weakly for 4 weeks was initiated together with ursodeoxycolic acide on day +41 and intravenous immune globuline replacement terapy for severe hypogamaglobulinemia. His diarrhea volume decreased only 25% after infliximab. Therefore, extracorporeal photoimmunotherapy (ECP) was applied on day +46 after the Health Ministry Turkish Republic-approval. Taking into a consideration that the response of ECP would be late and the patient's status was getting progression, MP at the dose of 1mgr/kg was applied through the superior and inferior mesentery artery on day +54. The patient's diarrhea volume decreased 60% (1L/day) two days after applying of intra arterial steroid. On day +90 his symptoms and findings completely resolved and was discharged. He currently (on day 180) has been continuing to use only tacrolimus (po 6 mg/day, bid) as a immunsuppressive therapy with no evidence of GI GVHD. *Discussion*. Our case showed us that the applying of intraarterial steroid for the treatment of severe GI GvHD might be considered in early period due to the fact that such a type approach seemed easy, reliable and effective without causing systemic immunosupression.

1510

LANGHERHANS CELL HISTIOCYTOSIS ASSOCIATED WITH FOLLICULAR LYMPHOMA: A CASE REPORT

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Introduction. Langerhans cells histiocytosis (LCH) may present in a variety of clinical situations, ranging from a solitary lesion of bone to a multisystem, life-threatening disorder. The association of LCH with a malignant neoplasm is rare and generally it has been related to Hodgkin's disease. *Case*. A 27-year-old man was admitted for evaluation of peripheral lymph nodes. There were no systemic symptoms. On physical examination, he presented local adenopaties in all the peripheral areas of 1-3 cm in diameter and two subcutaneous nodes in the back. Initial laboratory tests were normal. Peripheral blood smears and bone marrow examinations were unremarkable. A computer tomography (CT) of the thorax and abdomen showed thoracic lymph nodes and an important retroperitoneal adenopatic mass. A lymph node biopsy was performed revealing infiltration by follicular lymphoma cells. The patient was diagnosed of follicular lymphoma grade III stage IV-A, IPI 1/5, FLIPI 2/5. He received chemotherapy with CHOP-Rituximab for eight cycles and local radiotherapy in abdominal bulky mass. The patient achieved a complete remission and he initiated maintenance therapy with Rituximab. Three and a half years after remission, the patient presented a painful lesion on left parieto-temporal zone, without apparent skin lesion. A cranial radiography showed a radiolucent lesion on left parietal zone and a CT cranial scan described a litic lesion of 12 mm in diameter, with edema of subcutaneous tissue. In order to rule out a progessive disease, a CT scan of the thorax and abdomen was done, which revealed no significant changes. Bone marrow biopsy was normal. A bone scintigraphy and FDG-PET showed a focal uptake of 2x2 cm along the left parietal bone, with extension to soft tissue, with no other alterations. A surgical excision of the lesion was made, showing an infiltration by Langerhans cells (CD1a+, S-100+, CD79A-, CD20-, CD3-, CD5-, Ki-67<5%), confirming a LCH. A new FDG-PET was performed after surgery showing no abnormalities. No further treatment was administered. *Discussions*. LCH has been described in association with a variety of neoplasms, preceding, being synchronous or appearing after the other tumor. The association with

Hodgkin's disease is well known, but association with other lymphoproliferative disorders is rare. To our knowledge, this is the second case of follicular lymphoma associated with LCH. In the first case, LCH was diagnosed simultaneously with the lymphoma. By contrast, in our case, LCH was diagnosed 3 years after the lymphoproliferative disease, when there were no evidence of follicular lymphoma progression. The origin of LCH is not clear, and there is controversy as to whether it represents a true independent clonal neoplasm or a reactive and/or therapy-related phenomenon. Reviewing the literature, FDG-PET study appears to have higher sensitivity than bone scintigraphy, radiography and MR imaging for the identification of active osseous lesions in LCH. However, it also has a limited spatial resolution and requires complementary CT or MR imaging to define the area of increased glucose metabolism. In summary, FDG-PET atypical uptakes appearing in a patient with lymphoproliferative disease in complete remission should not be considered as lymphoma progression without confirmation by histologic examination.

1511

FLUDARABINE PLUS CYCLOPHOSPHAMIDE (FC) IN PATIENTS WITH PREVIOUSLY UNTREATED LOW-GRADE LYMPHOMA

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Background. In the last years, fludarabine alone or in combination with other drugs has been reported to be effective in the treatment of previously treated low-grade non-Hodgkin's lymphomas (LG-NHL). The aim of this study was to define the therapeutic efficacy and toxicity of a combination of fludarabine and cyclophosphamide (FC regimen) in untreated LG-NHL. *Patients and Methods*. Between January 2002 and June 2006, forty-five previously untreated patients with LG-NHL, were enrolled in the study. All patients (M/F: 28/17, median age 62 years) vere treated with three-day courses of fludarabine 25 mg/m^2/day, cyclophosphamide 300 mg/m2/day, every four weeks for a maximum of six courses. Granulocyte colony-stimulating factor and Pneumocystis Carinii prophylaxis was given. Among 45 patients, 21 (46%) were diagnosed with small lymphocytic, 9 (20%) with malt gastric, 8 (18%) follicular grade I, and 7 (16%) with immunocytoma subtypes. *Results*. Of the 45 patients, 43 (94%) achieved complete response (CR), 1 (3%) partial response, while the remaining 1 (3%) showed no benefit from the treatment. Regarding histology, in the follicular and malt gastric subtype we observed an overall response (OR) rate and CR rate of 100%. Median duration of follow-up was more than 27 months. Overall survival and disease-free survival rates were 93% and 88%. Hematologic grade 3-4 toxicity was seen in only five (11%) patients; no opportunistic infections or deaths were associated with the administration of the FC regimen *Conclusion*. These preliminary data show that the FC regimen is a high level of activity, with prolonged CR, well-tolerated combination chemotherapy for untreated patients with LG-NHL.

1512

MANAGEMENT OF SEVERE MUSCULOSKELETAL PAIN CAUSED BY IMATINIBE MESYLATE WITH DULOXETINE HYDROCHLORIDE IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA (CML). A CASE REPORT

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Imatinibe mesylate is an orally administered inhibitor of the breakpoint cluster region-Abl tyrosine kinase, which is capable of blocking proliferation and inducing apoptosis in chronic myeloid leukemia cell lines. Its introduction in everyday clinical practice led to an advantageous hematological and cytogenetic response in patients suffering from CML, even though mild or more severe adverse effects of the drug may present, affecting patients' compliance or even calling for treatment withdrawal. We present the case of a 23 year old patient with chronic myeloid leukemia under imatinibe mesylate (Gleevec 400 mg once daily) who developed severe musculoskeletal pain located at the lower extremities (due to the above treatment) and its management with Duloxetine Hydrochloride. After the patient was diagnosed to suffer from CML (G-banding karyotype showed 46,XX,t(9;22)(q34;q11) and a BCR-ABL transcript was demonstrated by reverse transcription-polymerase chain reaction (RT-PCR) on bone marrow), a treatment with Gleevec begun. After two weeks of treatment (400 mg once daily) the patient complained for acute dysaesthesias and paraesthesias of the legs as long as for severe pain on the same area. The above symptoms had the typical distribution of an acute polyneuropathy. Despite that and the findings of the clinical examination a full laboratory investigation (including X Rays of the area, biochemical analysis and an electromyogramm) didn't prove the existence of polyneuropathy. Because of the severity of pain the patient received large doses of paracetamol plus codeine or even dextropropoxyphene hydrochloride without satisfactory analgetic results. subsequently, duloxetine hydrochloride (Xeristar) was administered initially at doses of 30 mg once daily for the first four days and continuing with 60 mg once daily. The patient responded to the treatment. One week later she needed no more opiates while after two weeks the symptoms were fully relieved. Duloxetine Hydrochloride is a new SSRI approved by FDA both for the treatment of depression as along as for the management of diabetic polyneuropathy. As far as we know it is the first time for this drug to be used successfully in the management of acute musculoskeletal pains due to imatinibe mesylate. It is certain that prospective studies are needed to evaluate the efficacy and safety of the drug under conditions similar to the above. However, duloxetine hydrochloride offers a new alternative choise for pain alleviation in patients receiving Gleevec which seems to be much more attractive than treatment withdrawal.

1513

GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND ITS PREDICTION BY PROTEOMIC APPROACHES

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Background. Acute graft-versus-host disease (GvHD) is a frequent complication of allogeneic stem cells transplantation (allo-SCT). The rapid diagnosis of acute GvHD following allo-SCT is important for optimizing the management of this life-threatening complication. Differentially expressed or excreted polypeptides and proteins have potential for early and accurate diagnosis of GvHD and other complications of allo-SCT. *Aims*. Search for blood plasma proteins useful as potential biomarkers for early detection of GvHD. *Methods*. 2-dimensional gel electrophoresis (2-DE) was used for separation of blood plasma proteins. Proteins considered as potential biomarkers for early prediction of GvHD were selected by image analysis. Protein spots with different expression levels were characterized by matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF-MS) using peptide mass fingerprinting. Samples of blood plasma, urine and lymphocytes, collected from the same patient before and after allo-SCT were analyzed. Analysed samples were collected at three time points: (1) approx. 10 days before GvHD manifestation, (2) approx. 2 days before GvDH manifestation and (3) during GvHD manifestation. *Results*. Proteomic analysis revealed several proteins differentially expressed during GvHD development. These potential biomarkers included serum natural proteinases (macroglobulin α2 and α1 anti-trypsin), components of complement (complement 4 binding protein and complement factor I), proteins of acute phase (haptoglobin, C-reactive protein and amyloid related serum protein (SAA)) and other proteins such as fibrin, fibrinogen, inter-α (globulin) inhibitor H4 and hemopexin precursor. Concentration of serum natural proteinases, haptoglobin, inter-α (globulin) inhibitor H4 and hemopexin precursor in blood plasma were significantly decreased in both samples collected prior to GvHD manifestation. In these samples was detected also slightly decreased expression of analyzed components of complement in comparison to samples collected during GvHD manifestation. On the other hand, expression of C-reactive protein and SAA was detected only 14 days before GvHD manifestation and these proteins completely disappeared 1 day before onset of disease and during its manifestation. *Summary/Conclusions*. These potential biomarkers could improve early prediction and treatment of GvHD and thereby reduce GvHD incidence and complications.

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HER-2/NEU MRNA IN PERIPHERAL BLOOD & BONE MARROW IN FEMALE BREAST CANCER PATIENTS

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Background. The prognostic significance of the bone marrow

micrometastasis in breast cancer patients has been emphasized, as it will improve tumor staging and subsequent plan of management. It also helps in monitoring the effectiveness of chemotherapy and the detection of contaminated bone marrow grafts to be re-infused after high dose chemotherapy. *Aim*. To assess the HER-2/neu mRNA expression in bone marrow, peripheral blood in HER-2/neu mRNA positive breast tissues of female breast cancer patients. To correlate between the level of the HER-2/neu mRNA over-expression in bone marrow and the established risk factors for metastatic breast cancer. Type of the study: a cross sectional study. Study population: 38 female breast cancer patients, diagnosed with fine needle histo-pathological assessment were included. A control group of 5 female patients with benign breast conditions were included. *Methods*. All individuals included in the study were subjected to: breast tissue fine needle aspiration biopsy & grading according to Scarf, Bloom and Richardson, Chest X-ray, abd. ultrasonography, bony scan, complete blood count, assessment of stained bone marrow smear. Molecular assessment: For detection of HER-2/neu mRNA over-expression in breast tissue specimens, bone marrow and peripheral blood. After RNA extraction carried out using the MagNA Pure LC RNA and isolation Kit for blood/bone marrow. Quantitative assessment of HER-2/neu mRNA was carried out using the Light-Cycler and the HER-2/neu mRNA quantification kit from Roch-Molecular diagnostics. *Results*. HER-2/neu mRNA over-expression positivity was detected in: 16/38 (42.1%) breast cancer tissues cases, in 7/16 (43.7%) bone marrow & and in 6/16 (35.5%) peripheral blood samples of the breast cancer HER-2/neu mRNA tissues positive cases. A significant difference was detected between HER-2/neu mRNA tissue positivity and tumor size (P value: =0.032) & lymph node metastasis (P value =0.014), between HER-2/neu mRNA bone marrow positivity and lymph node status (P value = 0.036), between HER-2/neu mRNA marrow positivity and tumor grade. (P value = 0.011), between bone marrow and peripheral blood HER-2/neu mRNA positivity in the tissue positive patients (P value = 0.001): highly significant. Recommendations: assessment of HER-2/neu mRNA in bone marrow and/or peripheral blood for the detection of disseminated tumor cells should be carried out for all patients with HER-2/neu mRNA tissue positive breast cancer patients either for assessment or for follow up. The Bone marrow microscopic examination is valueless for the detection of micrometastasis, as all cases that showed HER-2/neu mRNA bone marrow positivity were free of any malignant disseminated cells on microscopic examination.

1515

BIOLOGICAL PROFILE OF BLAST CRISIS OF CHRONIC MYELOID LEUKEMIA: ANALYSIS OF CLINICAL RELEVANCE AND PROGNOSTIC SIGNIFICANCE

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Background. The blast crisis (BC) of chronic myeloid leukemia (CML) represents terminal and infaust phase of CML, with average duration of 3-9 months. The mechanisms responsible for progression of CML to BC are still unknown. Genomic instability, i.e. presentation of new cytogenetic abnormalities other than Ph chromosome is significant pathogenetic factor, as well, as activation of some oncogenes. *Aim*. To define and correlate clinical, immunophenotypic, cytogenetic and laboratory characteristics of BC in CML with duration of BC phase. *Methods*. The study included 31 patients (pts) with BC of CML (median age 51; range 19-72 years; M/F ratio 18/13). The median duration of BC was 4 months (range 1-12 m). The immunophenotypic analysis of bone marrow (b.m.) aspirates of 26 pts was performed by flow citometry (EPICS-C, Counter of FACScalibur, Becton, Dickinson). In 5 pts, b.m. biopsies were analyzed by immunohystochemistry in order to define a type of blast cells. The kariotype of pts was analyzed on metaphases from b.m. aspirates by conventional cytogenetic analysis. Furthermore, the study included analysis of laboratory data (Hb, WBC, pletelet count, percentage of blast and basophills, LDH) as well as degree of splenomegaly, and degree of reticulin fibrosis in the b.m. biopsies. *Results*. The type of BC was as follows: in 16 pts myeloblastic, in 7 pts lymphoblastic, in 6 pts megakarioblastic and in 2 pts biphenotypic. Thrombocytosis was found in 4/31pts, 6/31 pts had normal platelets, and in 21/31 pts existed low platelet count. Twenty-six pts were anemic; and in 20pts leukocytosis upper than 20×10^9/l was detected. Twelve pts evolve additional karyotypic abnormalities; 9/12 pts had one or two additional abnormalities and 3/12 pts show myltiple karyotypic anomalies. A grade III or IV myelofibrosis was present in 17/31 pts. The serum activity of LDH was elevated in all pts (med 1159U/L). Splenomegaly more than 5cm below left costal margin was found in 19/31 pts. There was no correlation between clinical, immunophenotypic, cytogenetic features, type of BC and duration of disease. *Conclusions*. The studies performed on a molecular level in the large cohort of patients are need to define basic biologic event that lead CML from chronic to BC phase, which is refractory to all known type of therapy.

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PREDICTIVE VALUE OF DISCRIMINATION INDICES IN DIFFERENTIAL DIAGNOSIS OF IRON DEFICIENCY ANEMIA AND β-THALASSEMIA TRAIT

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Background. Iron deficiency anemia (IDA) and β-thalassemia trait (B-TT) are most common causes of hipochromic microcytic anemias. Many indices have been defined to quickly discriminate these similar entities via parameters obtained from automated blood count analyzers. *Aims*. The purpose of the study is to evaluate the predictive value of these indices in differential diagnosis of IDA and B-TT in adult cases. *Methods*. This study consists of 45 IDA cases, 36 women and 9 men, whose mean age is 33,87±11,59 (mean±SD) (range 17-57 years) and 66 B-TT cases, 41 women and 25 men, whose mean age is 33,26±13,36 (mean±SD) (range 14-74 years). IDA cases with Hb value <8,7 g/dL have been excluded because these cases are not confused with B-TT cases in practice. Patient groups have been evaluated according to red blood cell (RBC), red blood cell distribution width (RDW), Mentzer index, Shine and Lal indices, England and Fraser indices, Srivastava index, Green and King indices, RDW index and Ricerca index. Sensitivity, specificity, positive and negative predictive values and Youden's index have been calculated. *Results*. None of these different formulations are superior to RBC value obtained from automated analyzers in adult cases with IDA and B-TT. RBC is the only index with both sensitivity and specificity more than 80%. If it is accepted that RBC value more than 5 x10e12/l is in favor of B-TT and a value less than 5×10^{12}/L is in favor of IDA, this method of discrimination has more power than all of the defined indices for adult cases. However, Youden's index of this parameter is only 73,7%. *Conclusions*. Finally, total body iron status and hemoglobin A2 level should be obtained for accurate differential diagnosis of IDA and B-TT until more efficient tools develop.

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HCV-RELATED THROMBOCYTOPENIA : PREVALENCE, CLINICAL CHARACTERISTICS, AND RESPONSE TO STEROID THERAPY

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Background. Hepatitis C virus is very common in Egypt. Various studies suggested the association between HCV infection and some immunological disorders including immune thrombocytopenic purpura (ITP). *Aim*. detecting the prevalence of HCV infection among ITP patients in our locality and if this association has any impact upon the clinical characteristics of ITP or its response to steroid therapy. *Methods*. Studying 42 cases of ITP patients regarding their HCV status, clinical characteristics and response to steroid therapy. *Results*. we found that about 43% were HCV +ve as detected by ELIZA and PCR and that the high female/male ratio in HCV-ve patients was less clear in HCV+ve patients. Only about one third of HCV+ve patients were steroid sensitive compared to more than 90% of HCV-ve ITP patients. The difference was highly significant ($p<0.001$). *Conclusions*. HCV infection could be one of the important causes ITP in Upper Egypt. ITP patients who were HCV+ve had had a more severe form of thrombocytopenia and were, in general, resistant to steroid therapy. We recommend check the hepatitis status in patients with ITP. Modalities of treatment other than steroids should be studied for those patients.

1518

THE CARRIER FREQUENCY AND MUTATION PROFILE OF ?ETA-THALASSEMIA IN TAIWAN

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Background. Thalassemia is a public health problem in Taiwan. Since 1993, the government has carried out a National Thalassemia Screening Program to decrease the number of affected patients. *Aims*. The pro-

gram provided a unique chance to determine the β-thalassemia carrier frequency and mutational burden. Hence, reporting the current carrier frequency of β-thalassemia and understanding all the mutations found in Taiwan were the objectives of the present study. *Methods*. Although the screening procedure was operated in 1993, the monitoring system did not function well until 1999. Only the data after 1999 was reliable. The Hardy-Weinberg equation was used to calculate the carrier frequency. The β-thalassemia mutations that had ever been found were searched through literature review and the database of the Taiwan Thalassemia Association. *Results*. The carrier rate was between 1.45 and 2.01%, higher than the previously reported 1.1%. Twenty-five mutations have been encountered, of which 19 were detected in patients with β-thalassemia major. Three new mutations which had never been reported in Taiwan, including Poly A (AAA→AAG), Poly A (AAT→AAC) and CD 95 (+A), were identified in this study. Poly A (AAT→AAC) and CD 95 (+A) were found in female immigrants from Vietnam. *Conclusions*. The higher β-thalassemia carrier rate may be attributed to small sample size in previous studies, and recent population movement from Southeast Asian countries via interracial marriages. Becoming more familiar with the mutations encountered, and the advances in the methodology of genetic examination, will be helpful in future prenatal diagnosis.

1519

RED CELL INDICES AND SERUM FERRITIN IN ANAEMIA OF PREGNANCY

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Background. Anaemia is a common haematological condition in developing countries which is particularly more pronounced in pregnancy. *Objective*. In this study we studied the hypothesis that iron deficiency is more likely in multigravidas than primigravidas. *Methods*. Pregnant women in second trimester of gestation were recruited into the study. Following baseline haemoglobin estimation patients were subdivided into aneamic and non-anaemic primigravidas and anaemic multigravidas. Haemoglobin concentration (Hb) was estimated using standard methods and red cell indices were determined using an auto-analyzer. Serum ferritin was estimated using Spectro ferritin an enzyme immunoassay method for the quantification of serum ferritin supplied by Ram co laboratory Texas (lot no: 3-080) *Results*. a total of 90 pregnant women were studied comprising of 30 anaemic pimigravidas (Hb <10 g/dL), 30 anaemic multigravidas, 15 apparently healthy primigravidas and 15 healthy multigravidas as controls. There were no significant differences in Hb, mean corpuscular volume(MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration(MCHC) and serum ferritin in anaemic primigravidas and anaemic multigravidas ($p>0.05$). There were highly significant differences in Hb, MCV, MCH, MCHC and serum ferritin between anaemic primigravidas and non-anaemic primigravidas ($p<0.0001$). The differences in red cell indices and serum ferritin between the anaemic multigravidas and non-anaemic multigravidas were also significant ($p<0.0001$). A positive correlation was obtained between Hb and serum ferritin in anaemic pimigravidas and non-anaemic primigravidas ($r = 9193$, $p<0.0001$ and $r = 0.6201$, $p=0.003$ respectively.) *Conclusions*. It was concluded that iron deficiency anaemia is the main type of anaemia in Nigerian pregnant women despite iron supplements in pregnancy and lactation. It was also observed that iron status was not influenced by the number of previous pregnancies.

1520

COMPARATION AMONG SYSMEX XE-2100, CELL-DYN SAPPHIRE AND MICROSCOPIC COUNTING IN ERYTHROBLASTS DETECTION

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Background. Erythroblasts are only found physiologically in the foetus and the newborn. The presence of erythroblasts outside these two conditions is an indication of an altered haematopoiesis and interferes with the white cell counting. Microscopic counting of erythroblasts is tedious and time consuming. Both accuracy and precision are limited by the number of cells counted. Autoanalysers which are able to determine automatically the number of erythroblasts, have become available in these last years. *Aims*. To evaluate the accuracy of erythroblast counting using two autoanalysers: Sysmex XE-2100 and Cell-Dyn Sapphire, and to compare it with the microscopic counting. *Methods*. We have evaluated 44.600 blood samples processed over three months in our hospital. There were 74 samples in which erythroblasts were detected by any of the two counters. There were then processed by the other counter and a peripheral blood smear was also evaluated microscopically, counting 400 leucocytes. Data were evaluated with the statistical package SPSS-12. *Results*. A summary of the data is shown in the attach Table 1. The smears of 29 of the 74 samples showed a number of erythroblasts ≈ 1:100 white cells. Five samples did not show any erythroblasts even after a systematic search. Intraclass correlation coefficient (ICC) between the two counters is 0.968 (consistency) and 0.965 (absolute concordance). *Conclusions*. 1. Both autoanalysers show high sensitivity and specificity in the detection of erythroblasts, being even higher in the Sysmex counter, when compared to microscopic counting. 2. The Sysmex counter give higher figures than the Cell-Dyn Sapphire. 3. There is an excellent correlation between both counters.

Table 1.

	Number	Mean (SD)	Median	Area under the curve COR
Sysmex	74	6.28 (11.59)	1.90	0.867 (0.781-0.953)
Cell-Dyn	74	5.54 (10.24)	1.09	0.688 (0.568-0.809)
Microsc	74	4.95 (9.26)	1	

Mean and median: number of erythroblasts:100 leucocytes.
DS: standard deviation

1521

LARGE HIATAL HERNIA IN PATIENTS WITH IRON DEFICIENCY ANAEMIA

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Iron deficiency anaemia in postmenopausal women is mostly due to chronic gastrointestinal blood loss. One of the most common missed lesions while performing upper endoscopy in the work-up of IDA, located at the neck of a large hiatal hernia. *Aims*. Description of the bio-clinical and endoscopic findings of a large hiatal hernia, diagnosed in patients presenting with iron deficiency anaemia. Furthermore, a review of the literature concerning the diagnostic and therapeutic management of these patients will be outlined. *Study*. We retrospectively evaluated 106 patients, presenting with IDA (hemoglobin < 10 g/dL) associated with a large hiatal hernia. *Results*. Cardiopulmonary complications of anaemia were the presenting symptoms, rather than gastrointestinal related complaints or bleeding. The lesions were visualized only in 53 (50%) of our patients at their first presentation. Initially, almost all of our patients were treated medically. Seven underwent surgical repair of the hiatal hernia and all remained asymptomatic afterwards. *Conclusion*. We conclude that a hiatal hernia, with or without visible lesions, is a real and maybe underestimated cause of IDA. Finding a large hiatal hernia on upper endoscopy, together with a negative colonoscopy, completes the diagnostic work-up of IDA in most of these elderly patients. Therapy may depend upon the need of transfusion, the efficiency of medical treatment, the risks of surgery and the preference and general condition of the patient.

1522

THE ROLE OF FLOW CYTOMETRY AND THE PROGNOSTIC IMPORTANCE OF CD 87 (UPAR) AND CD 45 EXPRESSION IN MULTIPLE MYELOMA

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Flow cytometry is important for diagnosis and for estimating the prognosis of multiple myeloma. With flow cytometry an antibody panel including cytoplasmic Ig light chain, CD38, CD138, CD45, CD56, CD19, CD20 and Ig heavy chain should be examined. CD 87 (uPAR) has many regulator effects on cell migration, leukocyte adhesion, chemotaxis and signal conduction. Previous studies have demonstrated that malignant plasma cells express uPA and uPAR (CD 87) and CD 87 is related with

the pathogenesis and the prognosis of the disease. In our study including 29 patients, we checked CD 38 and CD 138 in order to support the diagnosis of multiple myeloma. In our study CD87 was negative in 8 patients (27.5%), low possibly positive in 9 patients (31.1%) and bright positive in 12 patients (41.4%). Extramedullary involvement rate was meaningfully high in patients with high CD 87 expression. However we couldn't find any relationship with other prognostic factors. CD87 expression was meaningfully high in CD45 negative patients. CD45 negativity and CD 87 (uPAR) positivity correlated with extramedullary relapse, short lifetime, and bad prognosis in other studies. Flow cytometry is important in diagnosis and estimating the prognosis of MM. CD 56, CD45 and CD 87 are important for estimating prognosis. However more prospective studies should be done to understand especially the effect of CD 87 positivity in prognosis of MM patients.

1523

EFFECT OF GLP-PRO-ARG-GLY ON RAT PLATELET AGGREGATION

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Background. It has been shown that small glycine-containing peptides cause an inhibition of blood coagulation, have fibrinolytic and anticoagulant effects. Besides, various glycine-containing peptides inhibit the activity of thrombin and platelet aggregation. But mechanisms of peptides action on haemostasis system, in particular on platelet aggregation, remain poorly understood. *Aims*. The aim of the present investigation was to study the effect of glycine-containing peptide Glp-Pro-Arg-Gly on ADP-induced platelet aggregation (AIA) and to reveal possible mechanisms of this action using inhibitor of NMDA- receptors. *Methods*. The experiments were carried out on white rats. *In vivo* experiment AIA were monitored after intravenous injection of peptide (50 or 200 mkg/kg) or after peptide on a background of preliminary blockade NMDA-receptors by amantadine (2 mg/kg). *In vitro* experiment amantadine was added to pool rat plasma before ADP (1 mkM). *Results*. Our experiments on normal rats demonstrate that intravenous injection of Glp-Pro-Arg-Gly lead to AIA decrease on 15-28% from preadministration value. Amantadine effectively blocked the influence of peptide on AIA. On a background of preliminary blockade NMDA- receptors by amantadine Glp-Pro-Arg-Gly lost thus effects on platelet aggregation. In this case AIA was increased as compared with normal rats. *In vitro* experiment amantadine didn't change AIA. *Conclusions*. Thus we conclude that glycine-containing peptide Glp-Pro-Arg-Gly inhibits the blood platelet aggregation and NMDA-receptors participate in realization of peptide anti-platelet activity.

1524

EVALUATION OF NEONATAL SCREENING FOR HEMOGLOBINOPATHIES IN PICARDIE FROM 1999 TO 2006

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Background. According to the « Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant » (AFDPHE), neonatal selective screening of sickle cell diseases (SCD) and other hemoglobinopathies has been implemented in metropolitan France since 1999. Related to its proximity to Ile-de-France, prevalence of SCD is on the rise in Picardie due to the increasing of people immigration from African countries and French Antilles. Benefits of early diagnosis is well-demonstrated as far as SCD are concerned but it also raises some ethical questions regarding detection of heterozygous babies. *Aims*. We report the incidence of hemoglobinopathies and hemoglobinopathy carriers in Picardie over a 8-year period, from 1999 to 2006. *Methods*. The targeted screening concerned neonates whose parents came from high-risk areas. Blood samples were obtained either from a heel prick or from venous puncture after 72 hours of life and spotted onto Guthrie cards. Isoelectric focusing was performed as a primary screening method, completed by high performance liquid chromatography if necessary. *Results*. The number of investigated newborns in Picardie has increased from 738 in 1999 to 4243 in 2006. The overall incidence of SCD among the 22605 screened neonates was 0,13% (n=29 of which 21 S/S and 8 S/C) and the frequency of sickle cell trait (A/S) was 2,35% overall (n=532). The frequency of variant hemoglobin C (HbC) gene was 0,69% overall including S/C profiles (0,04%), HbC homozygosity (0,02%) and HbC heterozygosity (0,63%). We found 0,04% of variant hemoglobin D (HbD) carriers and 0,01% of hemoglobin O Arab heterozygotes. Parents of all SCD diagnosed neonates received information about the disease during the first announcement consultation and patients care is assessed by medical SCD experts. But, less than 1% of heterozygous newborns parents could be given the information. *Discussions*. The increasing number of detected constitutional hemoglobinopathies in Picardie is related to the increasing number of tested newborns. Although neonatal screening has resulted in a better follow up of SCD affected children, the management of hemoglobinopathy carriers neonates needs to be improved. Recently, we have proposed a specific consultation to disclose heterozygosity in order to identify high-risk families and try to give adequate counselling considering the cultural and religious beliefs.

1525

PLATELET ACTIVATION AND ACUTE CORONARY SYNDROME (ACS): EFFECT OF ANTIPLATELET AGENTS ANALIZED BY FLOW CYTOMETRY

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Background. Platelet (plt) activity is crucial to the pathogenesis of atherosclerosis and arterial thrombosis. These are the principal contributors to the development of ACS. *Aim*. To study the parameters of platelet activation, variability of responsiveness to an agonist and response to an antiplatelet agent such as clopidogrel, in patients with ACS. *Patients and Methods*. 26 patients admited to hospital with the diagnosis of ACS undergoling percutaneous coronary intervention (PCI) were included in the study. Clinical and therapeutic parameters were evaluated. All patients had started aspirin intake before the study. Samples were obtained before PCI. To detect plt response to an agonist (activation) we added a 100 ¿M ADP concentration. The surface expression of plt receptors was determined by flow cytometry using: anti-CD41 (Gp IIb/IIIa) FITC and PE, anti-CD62p (P-selectin) PE, anti-CD11b FITC, anti-CD14 PC5 (Immunotech, Marseille, France) and PAC-1 (activated Gp IIb/IIIa) FITC (BD Biosciences, San Jose, CA, USA).

Table 1.

Control			Patients		
	Median	Range		Median	Range
PAC-1 (MFI)	0.4	0.3 0.7	PAC-1 (MFI)	0.4	0.3 1.10
PAC-1 ADP (MFI)	2.3	0.7 5.7	PAC-1 ADP (MFI)	0.95	0.4 5.50
ΔPAC (MFI)	1.8	0.3 5.2	ΔPAC (MFI)	0.4	0.1 4.90
CD62p (%)	11.2	4.4 18.0	CD62p (%)	4.95	1.21 42.5
CD62p ADP (%)	26	11.3 57.4	CD62p ADP (%)	1.42	3.34 68.87
ΔCD62p (%)	14	1.7 44.1	ΔCD62p (%)	4.87	1.33 44.3
Plt-Mon (%)	25	9.7 51.9	Plt-Mon (%)	20.4	14.15 40.38
Plt-Mon ADP (%)	50.1	16.4 96.7	Plt-Mon ADP (%)	37.61	24.71 65.38
ΔPlt-Mon ADP (%)	23.7	0.8 68.9	ΔPlt-Mon ADP (%)	17.01	6.88 37.67
Plt-Nt ADP (%)	17.3	8.5 58.5	Plt-Nt ADP (%)	17.42	6.83 33.58

The samples were analized on a Coulter® EPICS XL-MCLTM. PAC-1 was expressed in mean fluorescence units (MFI) of total plt population. CD62p and leukocytes positive for plts were expressed in percentage (%). Descriptive statistics and correlation test were also studied. Results were compared to a control group of 25 healthy donors. *Results*. Blood samples from 4 females and 22 males with a median age of 66 years (range: 44-82) were studied. 11 (42.3%) patients were current smokers, 6 (23.07%) presented hypercholesterolemia, 10 (38.4%) hypertension and 1 (3,8%) Diabetes mellitus. 9 patients received clopidogrel previous study were done. See descriptive statistics in Table 1. We found a differences with statistical significance between controls and patients for P-selectin with and without agonist (p= 0.032 and p=0.020 respectively) and for PAC-1 whith agonist (p=0.001). When analized patients with or without clopidogrel treatment we found statistical significance between both groups for PAC-1 (p=0.016) and the percentage of neutrophil-platelet conjugates (p=0.033). *Conclusions*. a) In our series expression of P-selectin (alpha granules release) is correlated to the binding of PAC-1 (conformational change) according to the results of other series,

even when ADP-induced plt activation varies considerably from one individual to one, as observed by other groups. b) Patients with ACS present less response to plt agonist showing less degree of plt activation due to antiplatelet drugs administration. c) Flow cytometry represents an useful method to measure effect of antiplatelet drugs according to our results, altough more studies are nedeed.

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SECONDARY HEMOSIDEROSIS, IRON DEPLETION AND HEPATITIS C VIRAL LOAD

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Background. Iron overload is observed in about 30% of patients affected with chronic hepatitis HCV-related. Although relations between hemosiderosis, HCV genotype and viral replication do not seem to exist, some studies have suggested that plasmatic and tissual iron levels may be related with hepatic inflammation and with fibrogenesis. *Aims*. Aim of this study has been to detect an eventual reduction in viral replication consequently to modification in hematological parameters after a therapeutic cycle of blood letting, performed, in order to reduce the secondary iron overload, in patients affected with HCV-RNA+ chronic hepatitis. *Methods*. In 19 patients, enrolled and treated in University, the iron overload has been evaluated 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. The patients have been treated by phlebotomy, taking away 400-450 mL 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. The statistical analysis has been performed by the Wilcoxon test. *Results*. At time 0, patients showed these values (mean±SD): Ferritin=399±244 ng/mL, Iron=146±27 µg/mL, TIBC=350±48 µg/mL, TS=43±6%. Genetic mutations for C282Y were absent either in homozygosis either in heterozygosis, while 4 patients were heterozygote for H63D. The patients have been submitted to a mean of 5±2 blood letting. At sequent controls, Ferritin levels significantly decreased (51 at time 1, p=0.0002; 39 at time 2, p=0.0004); Iron also significantly decreased at time 1 (=63, p=0.0008), but not at time 2 (87, p=0.06); TIBC showed no statistical significant variations; moreover TS significantly decreased between time 0 and 1 (p=0.001) and it did not significantly go up at time 2 (p=0.059). Viral typing resulted: genotype 1 for 16 viruses, genotype 2 in 1 case, as genotype 3 and 4. Viral load has not been significantly influenced by phlebotomy (time 0: 657.500 UI/mL and time 2: 655.805, p=0.47). *Conclusions*. Blood letting results effective in the treatment of hemosiderosis, permitting a rapid iron depletion; this is not limited to period of therapeutic cycle, but it is maintained, on the basis of our data, at least 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 only 4 subjects carried a minor HFE mutation, but this was unable alone to determine hemosiderosis because present in heterozygosis. 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 pathology, linked at active viral replication.

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A PHASE II STUDY OF THALIDOMIDE PLUS BOLUS VINCRISTINE/ADRIAMYCIN AND MODIFIED DEXAMETHASONE COMBINATION THERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: PRELIMINARY DATA

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Background. High-dose chemotherapy supported by autologous stem cell transplantation (ASCT) after the combined chemotherapy with vincristine, doxorubicin and dexamethasone (VAD) for initial cytoreduction is effective therapy for newly diagnosed, symptomatic multiple myeloma (MM). Bolus vincristine/doxorubin intravenous infusion as an outpatient route is convenient and is acceptable efficacy and toxicity. Thalidomide has recently shown significant antimyeloma activity. *Aims*. We studied the efficacy and toxicity of the combination of bolus administrating vincristine/doxorubicin and reduced-dose of dexamethaxone with thalidomide, administered on an outpatient basis, as initial cytoreductive treatment in previously untreated patients with symptomatic myeloma. *Methods*. Twenty-six myeloma patients were treated with vincristine 0.4 mg intravenously (i.v.), doxorubicin 9 mg/m^2 (i.v.) administered as single dose on day 1, and dexamethasone 20 mg per os daily for 4 days. Dexamethasone was also given on days 8-11, 15-18 of the each cycle of treatment. The regimen was administered every 4 weeks for three courses. Thalidomide was given daily at a dose of 200 mg at bedtime. Response to treatment was evaluated after each cycles of treatment. After completion of three cycles, the patients were allowed to proceed to high-dose chemotherapy with ASCT or to receive further chemotherapy including changed regimen. *Results*. On an intention-to-treat basis, 23 of the 26 patients (88.4%) responded to treatment. Sixteen patients (61.5%) achieved complete and seven (26.9%) partial response. Only two (7.6%) were rated as non-responders. In total 78 cycles, major grade 3 or 4 toxicities consisted of neutropenia (8.9%), neutropenic fever (6.4%), anemia (7.6%), thrombocytopenia (3.8%) without significant nonhematologic event including cardiomyopathy and peripheral neuropathy. But, ten patients (38.4%) experienced pneumonia and one patient (3.8%) deep vein thrombosis during this regimen. In twelve patients who received ASCT, the event free survival was 67% and the overall survival was 100% for one year. *Conclusions*. In initial cytoreductive treatment, the combination of bolus administrating vincristine/doxorubicin and reduced-dose of dexamethaxone with thalidomide would be expected to have exellent efficacy and be relatively well-tolerated. Now this study for previously untreated MM is going to enroll more number of patients.

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THE IMPACT OF AGE AND SEX ON GAMMA-DELTA T LYMPHOCYTE PREVALENCE IN PERIPHERAL BLOOD OF MULTIPLE MYELOMA (MM) AND LYMPHOMA MALIGNUM (NHL) PATIENTS

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Background. Gamma-delta T lymphocytes (gd T) constitute average 4% of all T lymphocytes population in peripheral blood. Activated gd T cells express antigens CD 25$^+$ (late activator) and CD69$^+$ (early activator) on their surface. They appear to posses an intrinsic cytolytic activity against tumor cells in carcinomas, sarcomas, acute lymphoblastic leukemia and lymphomas. Statistically, higher multiple myeloma (MM) morbidity among men and higher lymphoma malignum (NHL) morbidity in women was documented. *Aims*. Comparison of gd T cell mean percentage (%) in peripheral blood of MM and NHL patients (pts) regarding age and sex discrepancies. Material and *Methods*. A total of 59 pts: 35 of MM and 24 of NHL, treated in Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of WrocΠaw Medical University and 14 healthy controls were included into analysis. 35 MM pts: 8 pts were in stage I, 6 in II and 21 in III stages, according to Durie-Salomon classification; sex: F/M- 23/12, age: 42-77, Me=62 years. 24 NHL pts: 2 were in I, 2 in II, 2 in III and 18 in IV stages according to Ann-Arbor classification; sex: F/M-11/13, age: 20-85, Me= 65 years. Samples of blood were taken at the time of MM and NHL diagnosis. gd T cells were estimated by flow-cytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies: MoAbs:Ab-anti TCRgamma1-FITC (Becton-Dickinson), anti CD14-RPE, anti CD-45-FITC, anti CD25-RPE, anti-CD69-RPE and Ab IgG1- RPE as negative controls (DAKO). *Results*. gd T lymphocytes mean% in peripheral blood of MM pts was higher than in NHL pts (4,33 vs 2,73, p=0,05); moreover in both pts groups is lower than in control healthy group: MM: 4,33 vs 5,63, p=NS, NHL: 2,73 vs 5,63, p=0,0003. In 23 MM women gd T % in blood was higher than in 12 MM men: 4,44 vs 4,14, whereas in 11 NHL women it was lower than in 13 NHL men: 2,48 vs 2,93. In MM higher activated gd T CD25$^+$ mean % in female than in male sex was observed: 0,19 vs 0,05, p=0,07. There were no differences in activated gdT CD25$^+$ lymphocytes number in blood between male and female sex in NHL and in healthy individuals: 0,08 vs 0,11 and 0,07 vs 0,06,respectively. Activated gd T CD69$^+$ mean % in MM women was higher than in MM men: 0,65 vs 0,38, whereas in NHL women mean % of these cells was lower than in men 0,48 vs 0,7. Statistically significant negative correlation between gd T cells % and NHL pts age was found, r=-0,42, p=0,04. *Conclusions*. Lower gd T lymphocyte number in peripheral blood in MM and NHL pts, compared to healthy individuals, is probably due to gd T cells migration from peripheral blood to other tissues included in tumor process, where these cells directly eliminate tumor cells. Higher multiple myeloma morbidity among men and higher lymphoma malignum morbidity in women may be result from dissimilar gd T lymphocyte antitumour cytolytic activity disorders, occurred in men and women, predispose given sex to MM or NHL development.

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EXTRAMEDULLARY RELAPSE AFTER ALLOGENIC BONE MARROW TRANSPLANTATION IN THREE PATIENTS DIAGNOSED OF ACUTE MYELOBLASTIC LEUKEMIA

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Background. Extramedullary relapses of tumors composed of malignant myeloid cells after allogenic bone marrow transplantation are uncommon. In fact, there is little known about biology, treatment or prognosis of this kind of tumors. *Aims*. Due to the fact of being exceptional, we expose three cases of Acute Myeloblastic Leukemia diagnosed patients, who suffered this type of relapse after allogenic bone marrow transplantation. *Methods*. We studied three patients diagnosed of acute myeloblastic leukemia (M6, M1 and M4 FAB classification). Two of them showed complex cariotype (45,XX,-7 and 47,XY,inv(16)(p13q22)) and all of them were in molecular complete chimerism. They suffered extramedullary relapse (granulocytic sarcoma) after allogenic bone marrow transplantation. *Results*. In our experience and reviewing the bibliography, extramedullary relapses after allogenic bone marrow transplantation in acute myeloblastic leukemia are scarce. This kind of relapse is usually related to M2 subtype, but it has also been described in M3, M4 and M5 FAB subtypes. In this case, one of our patients was diagnosed of M4 acute myeloblastic leukemia. As a matter of fact, two of the patients showed cytogenetics alterations known to be linked to this type of tumors: monosomy of chromosome 7 and inversion of chromosome 16. *Conclusions*. Generally speaking, extramedullary granulocytic sarcoma is very rare as a way of relapse after allogenic bone marrow transplantation in acute myeloblastic leukemia. In most cases, it appears at diagnosis of acute myeloblastic leukemia or during its follow-up. We describe three cases with this unusual presentation, not only because of the timing but also because of the location(central nervous system and breasts). It is also important to note the different evolutions that patients have followed; two of them in complete remission and one exitus.

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CONTINUOUS INFUSION OF FLAG AND IDARUBICIN FOR PATIENTS YOUNGER THAN 60 YEARS WITH RESISTANT ACUTE MYELOID LEUKEMIA

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Continuous infusion (CI) of cytarabien and fludarabine can accentuate drug activities. We studied the feasibility and the efficacy of fludarabine and cytarabin as a continuous infusion plus idarubicin for resistant AML under 60 years old. Inclusion criteria were AML except for acute promyelocytic leukemia, patients with resistant acute myeloid leukemia (failure to achieve CR after initial induction chemotherapy including standard dose cytarabine; early relapse, occurring after a first CR lasting less than 12 months; relapse after allogeneic hematopoietic stem cell transplantation [SCT]; relapses more than 2 times). Induction chemotherapy consists of idarubicin 12 mg/m^2 iv infusion (day 1- 3), fludarabine 30 mg/m^2/d and Cytarabine 1000 mg/m^2/d (day 1-5) as a 24-hour CI. G-CSF was added on day 1-5. More 3 cycles of FLAG as CI were followed for consolidation. All 24 patients were enrolled. Median age was 38.5 (18-57). Disease status were primary refractory in 5 (20.8%), early relapse in 17 (70.8%), multiple relapse in 1 (4.2%), and relapse after SCT in 1 (4.2%). Translocation (8;21) and variants (8, 33.3%) and 2 or 3 abnormalities (5, 20.8%) were most common chromosomal abnormalities. Twenty two patients (91.7%) could complete induction. Hematological recoveries were follows: ANC > 500/mm^3 in 11 (45.8%, median 29 days); ANC >1000/mm^3 in 11 (45.8%, median 32 days); platelet >20K/mm^3 in 9 (37.5%, median 29 days); platelet >100K/mm^3 in 4 (16.7%, median 43 days). Response for induction were CR in 7 (29.2%), CRp in 1 (4.2%), and treatment failure in 16 (66.7%, aplasia in 13, indeterminate course in 3). Median days required for CR was 42 (37-63) days. Consolidation stopped in 7/8: 5 patients underwent SCT; 1 patient died during consolidation; 1 patient stopped due to toxicity. Six patients are alive. Seventeen patients died of induction aplasia (13), toxicity during consolidation (1), relapse after SCT (3). Autolous SCT performed in 2 and allogeneic SCT in 4. Median overall survival in all and CR patients were 2.47 (0.67-4.26) and 11.38 (3.12-19.64) months, respectively. Relapse after autologous SCT in 2/2 and after allogeneic SCT in 2/4. Median event-free and disease-free survival were 2.47 (0.66-4.28) and 8.32 months, respectively. Continuous infusion of FLAG plus idarubicin showed high toxic deaths during induction chemotherapy. Conventional infusion schedule will be more suitable for resistant AML.

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HEMATOPOIETIC COLONY STIMULATING FACTORS CAN CAUSE CATARACT

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Background. Granulocyte-colony stimulating factor (G-CSF) is a lineage-restricted hematopoietic growth factor as it induces proliferation and maturation of neutrophilic precursors and progenitors, mobilizes myeloid progenitors into peripheral blood and activates neutrophil functions. Other cells like stromal cells and endothelial cells are activated too. It has been used to ameliorate or prevent profound neutropenia and its consequences. Congenital neutropenia such as Kotsmann's or Schwachmann-Diamond syndromes are associated with severe neutropenia, which causes serious infectious complications that may be life threatening. CSF therapy in these disorders showed increased number of circulating neutrophils and improvement of infectious complications. *Aim*. Awareness of the uncommon side effect of high dose G-CSF. *Method*. Two-years-old boy who had repeated admission to the Hospital due to febrile illnesses and infection, associated with severe neutropenia in the range of 0.1-0.3×10^9/L since early infancy. Bone marrow aspiration was consistent with the diagnosis of Kotsmann's syndrome disease. Other reasons of neutropenia and immune deficiency were excluded. The patient received granulocyte-colony stimulating factor (G-CSF) and three times granulocytes monocytes-colony stimulating factor (GM-CSF) in a dose of subcutaneous 5 microgram/kilogram body weight (mcg/kg per day) during admission then once weekly for 7 months and neutrophil count was maintained in the range of 0.3 ' 0.6 ×10^9/L. This dose was increased to 15 mcg/kg per day weekly but no increase in neutrophil count was observed. After 3 months of the high dose of the G-CSF, the patient developed bilateral eye cataract more at the left eye that required left lensectomy and its histological evaluation revealed chronic inflammation, many macrophages and some fibrosis and there were no positive cultures for the lens tissues. *Discussion and Results*. G-CSF can raise the granulocytes count and its usage is usually with febrile neutropenia and infections. The dose required for congenital neutropenia is usually 5-12 microgram/kg/day, the dose can be escalated until adequate response. Small number of patients experience bone pain during therapy. High dose of G-CSF can cause fever, rashes, pericarditis and pleural effusion. In chronic sitting, thrombocytopenia, splenomegaly, and vasculitis may be also seen at increased doses. GM-CSF is a potent hemopoietic cytokine that stimulates stem cell proliferation in the bone marrow and inhibits apoptotic cell death in leukocytes. Transgenic mice expressing a hemopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. *Summary*. High dose of G-CSF can cause cataract. Regular opthalmological examination is needed. Higher dose need close follow-up.

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DIAGNOSTIC UTILITY OF 18-FDG PET IN THE ASSESSMENT OF PATIENTS WITH MULTIPLE MYELOMA

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Objective. The role of whole-body PET with 18-FDG in detection of bone marrow involvement in patients with multiple myeloma was evaluated. The presence of extramedullary plasmocytomas and distribution of diffuse or focal lesions in the bones was also detected. *Materials and methods*. Between November 2006 and February 2007 the whole-body FDG PET scans (60 min after intravenous administration of 370-555 MBq FDG) were performed in 15 patients (age 51-71, median 59 years, 5 males) with multiple myeloma. Five patients were referred before therapy and ten patients were referred for evaluation of therapy response (chemotherapy, radiation therapy, bone marrow transplant). Standardized uptake values were obtained to quantify FDG uptake. Results of other imaging examinations (MRI, CT, radiography), laboratory data, bone marrow biopsies and the clinical course were used for verification of detected lesions. *Results*. FDG PET was able to detect

medullary involvement of multiple myeloma and was helpful in differentiating between post therapeutic changes and residual/recurrent tumor cells also in assessing response to therapy. In six patients PET demonstrated a favorable treatment response by showing a decline in lesion metabolic activity. In another patients PET showed progression of disease, by demonstrating diffuse or focal bone lesions or higher lesion glucose metabolism, concordant with the clinical evaluation. *Conclusions*. FDG PET is able to detect bone marrow involvement in patients with multiple myeloma. FDG PET is useful in assessing extent of disease at time of initial diagnosis, contributing to more accurate staging. FDG PET is also useful for evaluating therapy response. PET can detect the extent of marrow involvement in multiple myeloma patients and is very useful in monitoring of the treatment results.

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LIPOSOMAL ANTHRACYCLINES IN THE TREATMENT OF MULTIPLE MYELOMA

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Backgrounds. Not pegylated liposomal doxorubicine (Myocet) shows a better pharmacokinetic profile and a much lower cardiological toxicity than conventional anthracyclines. *Methods.* In Our Department, from October 2003 to October 2006, we have treated 24 patients affected by Multiple Myeloma (MM), either at a first line approach (n=13) or in resistant and relapsed disease (n=11). At admission diagnosis concerned patients with IgG Myeloma (n=20), IgA Myeloma (n=3) and micro-molecular Myeloma (n=1). All the patients have been treated with one or more cycles according to chemiotherapic scheme: VCR 1mg iv at day 1 + Myocet 25mg/sm iv at day 2 + CTX 100mg/sm iv days 1-4 + PDN 60mg/sm os days 1-4. In all patients Monoclonal Component (MC) and Left Ventricular Ejection Fraction (LVEF) have been assessed in order to evaluate treatment efficacy and cardiotoxicity of liposomal anthracycline administered. *Results*. We have evaluated 24 patients (12 M, 12 F), mean age 68.71±7.96 years (50 min., 82 max.), mean baseline MC 2488.00±1791.74 and mean baseline LVEF 56.04±6.4%. Evaluation of treatment efficacy has been made in 24 patients: MC has been reduced from 2488.00±1791.74 to 1981.30±1240.86 (CR 49%, PR 17%, NR 13%, PD 21%). The possible Myocet cardiotoxicity has been evaluated by assessment of LVEF: before therapy, the mean value was 56.04±6.4% and slightly lowered to 54.08±5.90% (p=ns) as a sign of cardiac function maintenance. *Conclusions*. Treatment with liposomal anthracycline (Myocet) lowers MC in patients with MM and leaves cardiac function unaltered.

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PROGNOSTIC IMPACT OF NORMAL KARYOTYPE IN CHRONIC LYMPHOCYTIC LEUKEMIA. CLASSICAL CYTOGENETIC ANALYSIS OR FISH?

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Cytogenetic analysis of chronic lymphocytic leukemia (CLL) patients, despite inherent technical limitations, has provided important information on disease biology and clinical outcome. FISH analysis is more sensitive; however, it only covers regions often involved in numerical or structural rearrangements and, therefore, does not allow assessment of the entire karyotype. We evaluated 86 CLL patients with normal karyotype by interphase FISH and explored possible additional prognostic information. Study group: 55 males/ 31 females, median age: 62,5 years, Binet stage-A/B/C: 65/17/4/ median time from diagnosis to cytogenetic testing: 4,8 months/ median follow-up time: 36.8 months. CD38 expression: 21/86 cases (24.5%)/ unmutated IGHV genes (U-IGHV): 28/86 cases (33%; 17/28: Binet-A). The FISH panel included probes for detection of trisomy 12 and deletions of 13q14/11q22.3/17p13. Deletion 13q14 was detected in 33/86 cases (38,4%); 12/33 carried biallelic deletions. Trisomy 12 (+12) was detected in 4/86 cases. Deletions 11q22.3/17p13 were identified in, respectively, 2/86 and 1/86 cases, who also carried del(13)(q14). Forty-nine cases (57%) were not found to carry cytogenetic aberrations on FISH analysis. Patients were divided in four subgroups: (i) normal karyotype/FISH; (ii) +12; (iii) isolated del13q14; (iv) 11q-17p aberrations. The four subgroups were not significantly different with regard to age/sex/clinical stage at diagnosis. On univariate analysis, advanced clinical stage, CD38 expression and U-IGHV genes were associated with a shorter progression-free survival (PFS). CD38 expression and U-IGHV genes were significantly more frequent in +12 cases (p=0.0001/ p=0.002, respectively). Patients with +12/11q-17p had significantly shorter PFS (Log rank test=0.0007). Biallelic del(13)(q14) did not affect prognosis. On multivariate analysis, only U-IGHV genes and advanced clinical stage were found to adversely affect prognosis (p=0.001/ p=0.0001, respectively). In conclusion, interphase FISH analysis of CLL patients significantly increases the detection rate of cytogenetic aberrations. The most frequent abnormality on FISH analysis in normal karyotype cases was del(13)(q14), which did not affect prognosis. This finding suggests that del(13)(q14) could very likely represent a secondary event associated with disease evolution.

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HUMAN HEART-TYPE FATTY ACID-BINDING PROTEIN AS AN EARLY DIAGNOSTIC MARKER OF DOXORUBICIN CARDIAC TOXICITY

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Background. Late cardiac toxicity following treatment with doxorubicin in patient with non-Hodgkin's Lymphoma may lead to latent cardiomyopathy. So, early prediction of toxicity can lead to prevent the occurrence of heart failure. *Aim*. the aim of this work was to investigate the clinical applicability of H-FABP as early diagnostic marker of chemotherapy induced cardiac toxicity. *Patients and Methods*. This study enrolled 10 normal controls and 40 patients with non-Hodgkin's lymphoma (NHL) received chemotherapy based doxorubicin for 6 cycles (one day CHOP) not exceeded total allowed dose. Human heart-type fatty acid-binding protein (H-FABP) was assessed 24 hours post first cycle of chemotherapy. Plasmatic levels of brain natriuretic peptide (BNP) were measured at the end of chemotherapy cycles. Resting echocardiography was done before starting chemotherapy and after last cycle. *Results*. follow up examination echocardiography showed that ejection fraction (EF) of nine patients decreased below 50% while eighteen patients demonstrated diastolic dysfunction. Elevated levels of both H-FABP and BNP were found in all patients with decreased EF and diastolic dysfunction, positive correlation was found between them. *Conclusion*. our study showed that H-FABP may serve as early prediction reliable marker for latent myocardial damage secondary to doxorubicin chemotherapy which may be of practical importance in preventing heart failure in future with subsequent planning for alternative chemotherapy modalities with no cardiac toxicity.

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THE PROGNOSTIC SIGNIFICANCE OF THE EXPRESSION OF UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (UPAR) ON ACUTE MYELOID LEUKEMIA CELLS

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Introduction. The receptor for urokinase plasminogen activator (uPAR; CD87) expressed by many normal and malignant cells is an important component of cell migration, adhesion, chemotaxis and tumor-cell invasion. Over-expression and negative prognostic role of uPAR has been reported in many malignant tumors. There are less reports on the role of uPAR in hematologic malignancies, especially in acute leukemias. The aim of this study was to determine the prognostic significance of the expression of uPAR on acute myeloid leukemia (AML) cells. *Material and methods*. Thirty seven patients (pts: 20 males and 17 females) were included in the study. They were treated with induction therapy acc. Polish Adult Leukemia Group (PALG) programme. The uPAR (CD87) expression was measured by labelling fresh viable bone marrow cells with anti-CD87 monoclonal antibody (Chemicon) and analysis in FACSCalibur flow cytometer. *Results*. The mean expression of uPAR on bone marrow blast cells was 44.67% (from 10.1% to 94.4%; SD=26.77%). There was no correlation between uPAR expression and sex, age of pts and type of AML. We found positive correlation between uPAR expression and the expression of CD4 (p=0.049), VCAM-1 (p=0.035) and MDR1 (p=0.002). Twenty one pts obtained CR after one or more induction therapy, but 16 pts never had CR. There was no statistical significant difference in uPAR expression between pts with CR and pts without CR (p=0.87). We found correlation between uPAR expression and disease free survival (DFS), but without statistical significance (p=0.056). There were statistical significant correlation between uPAR expression and overall survival (OS) (p=0.028). *Conclusions*. The correlation between uPAR and CD4 and also VCAM-1 expression confirm the role of uPAR in blast cells migration, adhesion and invasion. The unfavorable prognostic importance of uPAR expression is suggested by correlation between uPAR and MDR1 expression and statistical significant influence on OS.

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DETECTION OF MOLECULAR RELAPSE OF AML BY VERY FREQUENT QUANTITATIVE MONITORING OF DIFFERENT MOLECULAR MARKERS IN DIFFERENT COMPARTMENTS AND ITS TREATMENT WITH CHEMOTHERAPY OR GEMTUZUMAB OZOGAMICIN

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Background. AML is a disease organised as a system based on the cellular population called leukemia stem cells (LSCs), which have fundamental importance in the origin and maintenance of the disease. We hypothesized, that by monitoring of minimal residual disease (MRD) and its dynamics in different compartments (peripheral blood, PB; bone marrow, BM; and sorted CD34+ BM cells) it would be possible to find some patterns reliably predicting clinical relapse. *Aims*. To find which compartment is the best for MRD monitoring and whether it would be possible to treat the disease in the phase of molecular relapse in order to prevent the hematological relapse. *Methods*. MRD monitoring, in average once or twice per month, was performed in all phases of treatment, and was done even more frequently in the cases of unstable MRD. RQ PCR for fusion transcripts (CBFB/MYH11, AML1/ETO or involving MLL gene) and WT1 gene was used. Molecular relapse was defined as reappearance of the fusion transcript detection or its 10-fold increase, repeatedly detected. Some patients with already known MRD dynamics and high probability of imminent hematological relapse were treated at the time of molecular relapse. *Results*. In 67 AML patients and 6 healthy volunteers, 2352 BM or PB samples were examined, including 265 samples from CD34+ BM cells. Follow up was 31-287 weeks (median: 113 w). The correlation between the fusion transcripts levels in BM and PB was excellent (r=0,9676). The correlation between WT1 PB and BM levels was far less satisfactory. Since the WT1values were frequently >0 even if the level of fusion transcript = 0, we wanted to find some *normal* value for WT1. Using the ROC curves, however, we were not able to find any WT1 level being a confidential marker of molecular remission in either compartment (PB, BM or CD34+). The time from molecular to haematological relapse was 8-79 days (median: 25 d). In the cases of subsequent development of haematological relapses, the levels of fusion transcript in CD34+ BM cells were one order of magnitude higher than in the BM or PB, even in the case of CD34- blasts. Nine patients were treated for 17 molecular relapses with following results: chemotherapy, CR=2, PR=3, NR=1; gemtuzumab ozogamicin, CR=3, PR=1, NR=3; IL-2±DLI, CR=3, NR=1 (PR was defined as a decrease in fusion transcript level at least 10-fold). Patients with CD33- blasts at diagnosis did not respond to gentuzumab ozogamicin. Non-responsiveness to one treatment option did not mean non-responsiveness to another treatment. *Conclusion*: Frequent quantitative monitoring (especially in CD34+ BM cells) of fusion transcripts (in contrast to WT1) is useful for reliable prediction of haematological relapse in AML patients. PB seems to be sufficient for frequent outpatient MRD monitoring. Efficient targeting of LSCs will be essential for AML cure, however, the best method is currently not known. Some now available procedures are sometimes surprisingly successful. Supported by Research Grant MSM 0021622430.

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ATRA + CHEMOTHERAPY CAN CURE MOST APL PATIENTS: LONG TERM RESULTS OF A SINGLE CENTER EXPERIENCE

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Background and aims. since ATRA was introduced in induction therapy, prognosis of APL patients has dramatically improved. We performed a retrospective study on 72 consecutive, newly diagnosed APL patients who have been treated and followed -up in a period of 15 years at the Department of Hematology and Oncology of the S. Martino Hospital (Genova, Italy). *Patients and Methods*. Cytogenetic demonstration of t(15;17) and or detection by RT-PCR of PML-RARalfa was required for the confirmation of diagnosis. The median age of patients was 43 years (range 21-83). FAB subtypes were M3 in 64 patients (88%) and M3v in 8 patients (12%). The vast majority of patients had *de novo* APL. One patient developed APL after NHL, while two other patients after radiochemotherapy for breast carcinoma. According to the PETHEMA scoring system, the risk was low in 16 patients (22%), intermediate in 38 (53%) and high in 18 (25%). Two patients died of brain haemorrhage before the beginning of therapy. Nine patients were not treated initially with ATRA as the drug was not available yet. Four of them were treated with ATRA containing regimens due to relapse or refractory disease. Sixty-one patients were enrolled in multicenter GIMEMA trials, all of which included ATRA. *Results*. Among the 61 consecutive induction courses there were 4 deaths during the first period of therapy (7%), which were caused by haemorrhagic and thrombotic complications (n. 3) or infection (n. 1). Symptoms of retinoic acid syndrome were reported in 3 patients (5%). Fifty-seven of 61 patients (92%) were evaluated for response and all achieved CR. Forty-five patients underwent molecular evaluation of response at the end of induction, and 35 (78%) of them achieved molecular remission. Forty-one (67%) patients completed the 3 consolidation courses. Following consolidation 48/49 patients were found to have achieved molecular remission (98%). Thirty-seven patients (60%) completed maintenance therapy and maintained molecular remission. Nine patients (17%) relapsed, 2 with associated central nervous system involvement. Among the relapsed patients, 2 (22%) had the M3 variant, 4 (44.5%) belonged to the high risk group, only 4 had completed consolidation course for varying reasons (44.5%) and 3 (33%) had received maintenance therapy. Seventy-eight % of patients are expected to be alive at 14 years from diagnosis. After a median follow up of 96 months (4-159), median survival is 68 months (range 1-159) and median length of CR is 60 months (range 3-157). The expected survival at 14 years is 90% and 48% in patients with intermediate-low risk and high risk at presentation, respectively (p= 0.0009), thus highlighting the prognostic relevance of the PETHEMA score Eighty-nine percent of patients who received maintenance therapy are alive and disease free at 14 years. *Conclusions*. a review of our long term results in the treatment of APL in the ATRA era largely confirms that this *targeted* therapy has profoundly modified the clinical outcome of this severe disease, even though several problems still persist and need to be specifically addressed with more tailored therapeutic strategies.

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SEVERE ACUTE RENAL FAILURE AS A RESULT OF TUMOR LYSIS SYNDROME AFTER BORTEZOMIB THERAPY IN A CASE OF THERAPY-RESISTANT MULTIPLE MYELOMA

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Background. The proteasome inhibitor bortezomib, has antimyeloma activity even in myeloma cells refractory to multiple prior treatments. Frequently described side effects are gastrointestinal symptoms, fatigue, neuropathy and thrombocytopenia. Clinical trials have not indicated severe nephrotoxicity with this agent. *Aims*. To report our experience with bortezomib and a very severe, unexpected side effect after the administration of this medication. *Methods*. A 62-year old male patient with a therapy resistant IgG type multiple myeloma, stage III-A, was treated with bortezomib. For the past 6 years he had been treated with melphalan-containing protocols (VMPC, VBMCP and ABCM), then with thalidomide, and had a refractory disease. Prior to treatment with bortezomib the patient had documented increments of serum monoclonal component IgG 38.4g/l, bone marrow infiltration with 45% plasma cells and normal renal function. *Results*. The patient was treated with bortezomib started as a single-agent therapy planning the 3-weekly regimen (1.3mg/m (2) at days 1, 4, 8 and 11, followed by rest for 10 days). We noticed a slight increase in serum creatinine value after the administration of the first dose of bortezopmib when coricosteroids and hidratation were started. But, serum creatinine and BUN (blood urea nitrogen) levels continued to increase with the next three doses of bortezomib (creatinine 165, 420 and 588 micromol/l and BUN 12.9, 15.5 and 23.8mmol/l respectively). There was a prompt deterioration of renal function with oliguria, hyperkalemia (5.6 mmol/l) hypocalcemia (1.8mmol/l) and hyperphosphatemia (2.4 mmol/l). The patient was admitted to the Department of nephrology where dialyses 3 times weekly were started. Markers of tubular damage (NAG, AAP and gamaGT) were also elevated. The clinical condition of the patient had been worsening from day to day with an extreme prostration and paroxysmal tachycardia and the patient died within one month. *Conclusions*. To our knowledge this is the first report of life-threatening renal failure after bortezomib in a patient with refractory myeloma. Even more, there are several reports of reversal of renal failure at patients with myeloma and renal failure after the treatment with bortezomib. These findings suggest that a bortezomib-indused rapid reduction in tumor burden may lead to tumor lysis syndrome so that, caution is always needed when threatening myeloma patients with this very effective agent.

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ADMINISTRATION OF G-CSF AND CHEMOTHERAPY IN PATIENTS WITH LYMPHOMA AND MYELOMA OPTIMIZED SUCCESSFUL MOBILIZATION OF HEMATOPOETIC PROGENITOR CELLS FOR AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION (SINGLE-CENTRE EXPERIENCE)

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Introduction. Hematopoetic stem cell mobilization and collection have been optimized in numerous clinical trials, but significant proportion of patients mobilize an insufficient number of hematopoetic stem cell, resulting in an inadequate graft. Classical strategies for peripheral blood stem cell mobilization include administration of growth factors, mainly G-CSF alone or in combination with marrow suppressive chemotherapy. The administration of a combination of chemotherapy and cytokines G-CSF is associated with a significantly increased efficacy of stem cell mobilization compared with either modality alone. *Method*. The aim of this study was to evaluate the efficacy of G-CSF preceded by chemotherapy (cyclophosphamide 4g/m sq for 1 dose) for hematopoetic progenitor cell mobilization for lymphoma and myeloma patients. We started G-CSF as a fixed dose 480MU SQ every day as soon as the leukocyte counts began to rise after chemotherapy induced myelosupression. Leukapheresis was commenced at the time when leukocyte count rose up to 1000/uL, and repeated for 2-4 consecutive days until target number of $CD34^+$ cell, at least 2×10^6/kg was collected. *Results.* 39 (male to female, 21:18, age range 21-65, lymphoma 25, myeloma 14) underwent a total of 86 courses of leukapheresis for hematopoetic progenitor cell collection prior to autologous transplantation from April 2002 through October 2006. The target amount of marrow was harvest in all patients. All the patients achieved good engraftment after autologous transplantation. The mean days required for WBC count to be over 1,000/uL was 8-16 days. Patient's age, sex, underlying malignancy, exposure to chemotherapy before mobilization did not show any statistically significant correlation. *Conclusion*. We can conclude that chemotherapy followed by G-CSF administration is an effective way for mobilization of hematopoetic progenitor cell and verified itself as a good mobilization method.

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WITHDRAWN

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PREDICTION OF THE OUTCOME OF THE TREATMENT IN THE ACUTE MYELOID LEUKEMIA PATIENTS ON THE BASIS OF THE THYMIDINE KINASE LEVEL IN BLOOD SERUM AND LIQUOR

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Background. The thymidine kinase (TK) is a enzyme that convert deoxythymidine (Thd) to thymidine monophosphate (TMP). This phosphorylation is the only pathway to introduse Thd into DNA metabolism. TK is an essential enzyme which is expressed in cell division activity. An increased level of cell division is linked to malignant tumor diseases, such as leukemia and lymphomas. *Aims*. The purpose of the current investigation is determination the prognostic value of TK activity in blood serum and CSF in acute myeloid leukemia (AML) patients. *Methods*. In our study the activity of TK was measured in blood serum and liquor by radioimmunoassay using 5-125 I-iodedeoxy uridine as a substrate. TK levels were observed in 79 AML patients in the time of pretreatment and after finishing of induction chemotherapy. All patients were treated by standard chemotherapy ('7+3'). In three patients who developed CNS-relapse, TK level was measured in liquor in the time of intrathecal therapy (methotrecsate, cytoranisbe, dexamethasone). The patients were grouped as follows: 1) those, who achieved positive treatment result (complete remission) after the first course of induction chemotherapy - 19 patients (24%); 2) patients with positive results after 2 chemotherapy courses-28 (35.5%); 3) patients, who were resistant to the treatment-19 (24%); 4) patients, who died in 6 weeks period after diagnosis (the early death)-13 (16,5%). *Results*. In the time of pre-treatment the average TK levels were observed the next way: in the first group-7,1±1,672 U/L, in the second-13,89±1,679 U/L, in the third-34,33±5,287 U/L and in the fourth-53,95±8,46 U/L. After finishing of induction chemotherapy TK level significantly decreased up to the normal range (0-6 U/L) in the patients who achieved positive treatment results (to the 4,08±0,498 U/L in the first group and to the 4,78±0,71 U/L in the second), but remained increased in the patients, who were resistant (20,82±2,95 U/L) and with the early death (52,45±8,77 U/L). The TK level in the liquor was increased during all treatment period (from 28,6 U/L to 36,6 U/L). All patients died due to neurological complication or subsequent bone marrow relapse. In conclusion, TK level is independence prognosis factor in chemotherapy results in AML patients. The lower TK level in blood serum at the time of pretreatment can predict the better outcome.

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MEDIASTINAL GRANULOCYTIC SARCOMA (CHLOROMA) INVOLVING THE SUPERIOR VENA CAVA. CASE REPORT OF A PATIENT WITH DIAGNOSTIC DIFFICULTIES AND COMPLETE REMISSION AFTER CHEMOTHERAPY AND ADJUVANT RADIOTHERAPY DOCUMENTED WITH PET-SCAN

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Backgraound. Granulocytic sarcoma or chloroma is a rare extramedullary mass consisting of immature myeloblasts. This disease usually arises during the course of acute myelogenous leukemia, although it also occurs in chronic myelogenous leukemia and other myeloproliferative disorders *Aims*. To report a case of a patient affected of a rare mediastinal hematological malignancy known as granulocytic sarcoma or chloroma and in that opportunity to review the literature. *Methods*. A 40 years old man was referred to our Department affected of mediastinal chloroma firstly diagnosed approximately a year before. The patient initially presented a mass on the right cervical region and 3 months later in the left cervical region, initially considered of inflammatory origin and faced with antibiotics and consequent diminution of the masses. Because of new enlargement of the masses, the patient underwent thorax and cervical CT which revealed cervical lymph nodes with central necrosis and a compact mass in the mediastinum which invaded the superior vena cava and almost complete obstruction. A mediastinoscopy was performed and the histological specimen confirmed a mediastinal chloroma. A PET-CT scan confirmed active disease in the left cervical region, in the upper mediastinum and in the right submandibular region. *Results*. The patient received chemotherapy and partial response was confirmed by a thorax CT scan which referred a diminution of the mass (diameter: 5.5cm) and finally was referred to our Department with the purpose to receive adjuvant radiotherapy. The patient received adjuvant radiotherapy of the mediastinum (41.4 Gy in 23 fractions using a 6 MV Electa Linear accelerator). Complete remission of the disease was documented with a PET scan. The use of PET scan was of extreme usefulness because revealed no active cells, although the mediastinal mass was still present on thorax CT after the completion of radiotherapy. *Conclusions*. The mediastinum is rarely involved by granulocytic sarcoma and superior vena cava obstruction is an even rarer presentation (3 cases out of 11 patients with prominent mediastinal chloroma in the litterature) Treating superior vena cava syndrome regardless the underlying pathology is criticized. Complete remission of the disease documented with PET scan can be achieved with radiotherapy. PET scan is of extreme importance, by adding important information regarding active cells metabolism and helping phycisians on taking further therapeutic decisions.

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REAL-TIME PCR INCREASES THE DETECTION RATE OF THE JAK2 V617F MUTATION IN CHRONIC MYELOPROLIFERATIVE DISEASES

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Background. The diagnosis of bcr-abl negative chronic myeloproliferative diseases (CMPDs) is based on clinical and biological criteria. However, a single acquired mutation of the Janus kinase 2 (JAK2 V617F) has been described in the majority of patients with CMPD, hence sensitive detection methods are needed. *Aims*. Two different PCR methods for the

detection of the JAK2 V617F mutation were compared by testing blood samples from 130 patients (115 CMPD, 15 non-CMPD) after informed consent according to local clinical legacy had been taken. CMPD-patients were classified as essential thrombocythemia (ET, n=56), osteomyelofibrosis (MF, n=10), polycythemia vera (PV, n=47) and two patients with unclassified CMPD. *Methods*. DNA was prepared out of 200 µL total blood and up to 200ng DNA were tested. On the one hand, a qualitative PCR was used (Baxter EJ: Lancet 365, 2005) whereby mutated as well as wildtyp JAK2 sequences are amplified in parallel. PCR-products were separated on agarose-gels. On the other hand, samples were tested with a quantitative real-time PCR (Kroger N: Blood 109, 2007). In this method only mutated JAK2 molecules are detectable with specific fluorescence-labelled TaqMan probes. The proportion of JAK2 V617F was calculated by a correlation with GAPDH . *Results* Corresponding results with both assays were achieved in 34 negative patients (15 ET, 4 MF, 1 PV, 14 non-CMPD) and 76 positive patients (25 ET, 5 MF, 45 PV, 1 unclassified CMPD). In patients with ET the median proportion of mutated JAK2 was 15,7% (2,3%-52,7%), in MF 45,3% (28,8%-77%), in PV 42% (4,4%-66,2%) and 17% in one patient with unclassified CMPD. In addition, 10 patients (8 ET, 1 RARS-T, 1 unclassified CMPD) with only weak mutated JAK2 DNA-bands on agarose-gels were clearly positive in real-time PCR with a median proportion of mutated JAK2 of 4% (1,8%-8%). Finally, 10 qualitative PCR negative patients (8 ET, 1 MF, 1 PV) were positive in real-time PCR with a median proportion of 2,8% (1,1%-28,8%). Homozygosity was indicated in 10 Patients (8 PV, 1 ET, 1 MF) by JAK2 V617F proportions of equal or higher than 50%. Overall, 41/56 (73%) patients with ET, 6/10 (60%) with MF and 46/47 (98%) with PV were JAK2 V617F positive. *Conclusions*. The quantitative real-time PCR is more sensitive than qualitative PCR since only mutated JAK2 DNA sequences are detectable. Ten of 44 (23%) qualitative PCR negative patients were positive in real-time PCR. Differences in the incidences of JAK2 V617F positive patients reported may, at least in part, be caused by the detection-methods in use. In addition, the proportion of mutated JAK2 differs in CMPD subgroups, indicating a possible diagnostic value of the V617F quantification.

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THERAPY OF ANTIBODY-MEDIATED FACTOR VIII DEFICIENCY BY IMMUNOSUPPRESSIVE THERAPY CONTAINING CYCLOPHOSPHAMIDE, DEXAMETHASONE AND RITUXIMAB: A CASE REPORT

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Background. Aquired factor VIII deficiency is a rare coagulation disorder with an incidence of 1.34 cases per million population per year with malignancy, post-partal status and autoimmune disease as most commonly associated diseases. Besides control of active bleeding by substitution with coagulation factor concentrates, immunosuppressive therapy is the backbone of therapy. We report succesfull therapy of aquired factor VIII deficiency with cyclophosphamide, dexamethasone and rituximab in a patient with aquired factor VIII deficiency. *Case*. A 64 year old male patient presented at our hospital with spontaneous hematoma of the gluteal region and of both feet. Initial laboratory analysis revealed prolonged partial thromboplastin time (ptt) (65 seconds), enhanced fibrinogen activity (539 mg/dL) with normal prothrombin time and ATIII level. Detailed analysis of coagulation parameters showed decreased factor VIII activity (8.1%), increased von Willebrand factor antigen (> 250%) and factor VIII inhibitor (35 Bethesda Unit, BU). Autoimmune diseases, underlying malignant conditions, pulmonary diseases as well as inducing concomitant medications were ruled out clinically. Thus the diagnosis of aquired idiopathic antibody-mediated factor VIII deficiency was established. The patient was treated with intravenous cyclophosphamide 750 mg/m^2 day 1, oral dexamethasone 20 mg day 1 to day 7 and weekly intravenous rituximab 375 mg/m^2 day for 4 consecutive weeks. The initial hematoma in the gluteal region as well as in both feet started disappearing 2 days after initiating of therapy. Normalization of factor VIII activity was observed on day 49 after start of therapy. Concentration of factor VIII inhibitor parallely decreased over time. New hematoma or other signs of active bleeding did not occur. Significant toxicities observed were an abscess in the gluteal region, which could be managed by surgical means as well as systemic antibiotics; additionally, the patient developed dyspnea with an interstitial pulmonary infiltration as underlying condition. Further diagnostic work up revealed non-infectious pneumonitis, that was judged to be rituximab-related. Administration of steroids lead to improvement of the symptoms as well as CT-scan and functional analysis based findings. One year after diagnosis the patient remained stable. Especially new symptoms suggesting relapse of disease have not occurred. *Conclusion*. We report a case of aquired factor VIII deficiency that was successfully treated with an immunosuppressive regimen composed of cyclophospamide, dexamethason and rituximab. The symptoms of coagulopathy as well as the initially pathological laboratory findings were released relativily fast, however toxicity of the regimen lead to 2 consecutive hospitalisation of the patient, but could be resolved.

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THE IMPACT OF HIGH-DOSE SODIUM SELENITE THERAPY ON BCL-2 EXPRESSION IN ADULT NON-HODGKIN`S LYMPHOMA PATIENTS :CORRELATION WITH RESPONSE AND SURVIVAL

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The present study was undertaken to explore the effect of administration of high doses of sodium selenite on the expression of Bcl2 in patients with non-Hodgkin's Lymphoma. Fifty patients with newly diagnosed non-hodgkin's lymphoma were randomly divided into two groups, Group A-1 received standard chemotherapy while group A-2 received adjuvant sodium selenite 0.2 mg/kg/d orally for thirty days in addition to chemotherapy after giving informed consent. Enzyme linked immunosorbent assay(ELISA)was used ta assess BCL2 at the time of diagnosis and after therapy in the two groups. Sodium selenite administration resulted in significant decline of BCL2 level after therapy in group A-2(8.6+ or - 6.9 ng/mL vs 6.9+ or - 7.9 ng/mL, p<0.05). Also,complete response reached 60% in group A-2 compared to 40% in group A-1.Significant increase in CD4/CD8 ratio was noticed in group A-2 compared to group A-1 after therapy (1.45+ or - 0.36 vs 1.10 + or - 0.28 p 0.04). Overall survival time (OS) in months was significantly longer in complete remission patients in groupA-2(21.87+ or -1.41)compared to group A-1 (19.70 + or -1.95)(p 0.01). It is concluded that sodium selenite administration at the dosage and duration chosen acts as a down regulator of BCL2 and improves clinical outcome.

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VEGF AND SURVIVIN MRNA EXPRESSION IN AML PATIENTS WITH LONG-TERM COMPLETE REMISSION

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Background. Some recent studies have shown the importance of angiogenesis and apoptosis in the biology of AML. VEGF plays an important role in angiogenesis by acting as a potent inducer of vascular permeability and serving as a specific endothelial cell mitogen. Overexpession of VEGF is associated with increased angiogenesis, growth and invasion in haematologic malignancies. Furthermore, VEGF induces the expression of anti-apoptotic protein Survivin. Survivin is a unique member of the inhibitors of apoptosis protein (IAP) family, which is involved in both control of cell division and inhibition of apoptosis. Due to its bifunctional role, it has been hypothesized that Survivin plays a central role in cancer progression and resistance to therapy, in diverse tumour types. The AIM of this study is to examine the mRNA expression of VEGF and Survivin in AML patients with long-term complete remission. *Methods*. Total RNA was isolated from bone marrow cells of 16 AML patients (median age 54.4±6.7 years, 7 men and 9 women) in complete remission (<5% bone marrow blasts) and 10 individuals, of the same age, with normal haemopoiesis. All our patients were in complete remission for more than 5 years (mean 8 years, range 6-14 years). Real Time Semi-Quantitative RT-PCR assay was performed in order to detect the mRNA levels of VEGF and Survivin. Abl was used as a reference gene. The regulation of the target genes was estimated as an expression rate. *Results*. In our study, we detected mRNA expression of VEGF and Survivin, in all control samples. Furthermore, VEGF and Survivin were both down-regulated in our patients compared to control group, by the factor 2.28 (p>0.05) and 1.65 (p>0.05), respectively. Down-regulation is not statistically significant and therefore, the expression of each gene ranges within the same values, either we examine AML patients with long-term complete remission, or control individuals. *Conclusion*. Since our findings suggest correlation between angiogenesis and apoptosis, additional work is now desired to understand the role of VEGF and Survivin in the pathophysiology of haematologic malignancies and the progression of AML. Further studies are needed in order to determine whether VEGF and Survivin could be used as prognostic markers, minimal residual disease (MRD) indicators or promising cancer therapeutic targets.

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QUALITY ANALYSIS OF THE MULTIDISCIPLINAR PROGRAM DESIGNED FOR THE APLICATION OF RADIOINMUNOTHERAPY IN NON-HODGKIN LYMPHOMA

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Introduction. 90Y ibritumomab tiuxetan (90Y-IT) is not only an effective treatment for refractory follicular non-Hodgkin lymphoma (NHLF), but also, it is well-tolerated and safe in an outpatient regimen, nevertheless it requires a coordinating multidisciplinary team who follows a sequential and organized protocol. *Methods*. 18 refractory/relapsed patients with NHLF were treated with 90Y-IT according to protocol, within September 2005-December 2006, in an university hospital, which is the referral centre of the remaining haematology departments of the Aragon. Adult patients with NHLF who met the inclusion criteria were selected. A sequential schedulle was planned following a programmed track, the activity and the participation of each member of the group were registered. -Selection of each patient for the responsible clinical haematologist- Inform the patient, obtain the Informed consent (IC) and give the patient warming information regarding post-treatment- After 24hours coordinating meeting within haematology/Nuclear medicine/ Clinical Pharmacy was arranged, at this time, date of treatment as well as Rituximab application procedures are fixed. The date (days -7 and 0) and time for the 90Y Ibritumomab tiuxetan infusion is also reserved in the out patient clinic- Registration in the agenda of Nuclear Medicine and in the out patient clinic-Graphic calendar performance in which you can fix the steps and also define at any moment the process tradability. Weekly patient visits are scheduled until recover the normal haematology values and at 12 weeks, the response is also assessed. A satisfaction survey is given to the patients as well as mailbox for suggestion. For the analysis, the following indicators have been used: compliance % of the IC, time from indication to treatment, agenda fulfilment grade and drug application, quality control of 90Y-IT dose, satisfaction survey. *Results*. We have had success in the performance of the process. 100% had been signed the IC. Quality control of dose adjustment 100%. Patient visits performed in the programmed dates 95%. High grade of patients and professionals satisfaction. *Conclusions*. The compliance of the multidisciplinary operation program has been evaluated, which has been designed for the use of the radioinmunotherapy in the regular practice and the established quality indicators compliance.

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COST OF TRANSFUSION-DEPENDENT MYELODYSPLASTIC SYNDROMES (MDS) IN GERMANY

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Background. Only few studies assess the health economic burden of myelodysplastic syndromes (MDS) to payers, society or patients. So far, in Germany no such cost study has been conducted. The annual incidence of MDS is estimated to be about 5 in 100,000 persons overall with a peak of 20-30 in 100,000 persons among over 70 year old. About two-thirds of MDS patients are estimated to also suffer from anaemia and require red blood cell transfusion, costing approximately €140 per unit. However, treatment of MDS and its side effects as well as MDS-related co-morbidities lead to intensive resource use, which increases with duration of illness. *Aims*. The objective of this study is to assess the costs of transfusion-dependent low/intermediate-1 risk MDS in Germany from a payers' perspective. *Methods*. 100 low/intermediate-1 risk transfusion-dependent MDS patients from 6 outpatient facilities and 25 low/intermediate-1 risk transfusion-dependent deletion 5q MDS patients from a hospital-based MDS registry were identified. Claims data and patient records of the previous five years are used to collect health care utilization data of these patients retrospectively. Publicly available tariff books and remuneration schemes are applied using e.g. EBM 2000+, GOÄ, G-DRG to evaluate mean costs p.a. in 2006 EUROS. *Results*. This is an ongoing study where the collection of data is almost finalized and data analyses just started. Results will be presented at the conference explaining cohort characteristics such as age, gender, time since diagnosis, disease state, co-morbidities and health insurance. The average annual cost per patient will be shown in total and in detail for the following categories: inpatient services, outpatient services, oncological and other medication, transfusion cost, cost according to age groups, and disease states. Sensitivity analyses will be conducted on the unit cost of medical services, cost of co-morbidities and resource usage versus remunerated services. Further, the results will be shown in comparison to published cost of MDS in other countries.

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CORRELATION BETWEEN LDH LEVELS AND MUTATIONAL STATUS OF JAK2 GENE IN ESSENTIAL THROMOCYTHEMIA

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Background. Essential Thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPDs) relatively indolent and often asymptomatic, characterized mainly by a sustained elevation in platelets count (>600 x 109/L), proliferating enlarged and hyperlobated megakaryocytes, and minimal or absent bone marrow fibrosis. The prevalence in general population is approximately 30/100.000. The median age at diagnosis is from 65 to 70 years old, but the disease may occur at any age. The clinical feature is dominated by a predisposition to vascular occlusive events (involving the cerebrovascular, coronary and peripheral circulation) and haemorrhages. Recently, a mutation in the Janus Kinase 2 (JAK 2) gene has been found in a significant number of cases of ET and other CMPDs. In the majority of ET cases, a 57% of them, the mutation in the JAK 2 gene can be detected. Data on the JAK 2 mutation status of ET patients cannot be used at present for prognosis assessment. Correlations between JAK 2 status and the vascular risk and, eventuality, of clone progression remains unknown. For the same reasons, a therapeutic approach based on the positive or negative status JAK 2 of an individual patient does not present clinical relevance at the moment. However, the presence of a mutation in an important signalling way as JAK STAT could provide some key to differentiate between negative and/or positive JAK2 trombocythemias *Aims*. The aim of this work was to analyse the presence or not of the JAK2 mutation by ARMS procedure and its relation with other haematological and/or bioquimical parameters in ET patients. *Methods*. In order to reach this objective, 30 patients fulfilling the criteria of ET have been studied (sixteen women and fourteen men). The features at diagnosis as haemoglobin levels, leukocyte differential counts, platelet counts, LDH, β2 microglobulin, creatinine and uric acid were analyzed. *Results and discussion*. Seventeen cases (56.6%) were detected as negative, and 13 (44.4%) were positives for the mutation. After statistical analyses, only the LDH level was significantly correlated with the mutational status. The majority of cases JAK 2 negatives presented LDH normal values. There was not other parameter statistically significant including sex, age, platelets count, and hematocrit or haemoglobin level. A known fact is that the increase of LDH is an indicator of tumoral activity. This activity could explain the difference between positive and negative cases and that could means more clinical aggressiveness in the JAK-2 positive cases, because one major activity, of the tumoral clone. Explanation of this fact is unclear at the moment and further studies are needed in the future to value these differences.

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JAK2 V617F MUTATION AND VEGF LEVELS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

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Background. The pathogenesis of bcr/abl negative myeloproliferative disorders (MPDs) has been recently associated with the V617F somatic mutation of the Janus (JAK) 2 kinase. This mutation leads to increased activation of the single transducer and activator of transcription (STAT) molecules and further, to inhibition of apoptosis and augmented proliferation of the progenitor myeloid cells. On the other hand, the vascular endothelial growth factor (VEGF), a promoter of vasolidation and angiogenesis, is increased in solid tumors and haematology malignancies including MPDs. *Aims-Methods*. In this study we investigated the impact of JAK 2 V617F mutation in the expression of VEGF in patients with

MPDs. We studied 17 patients with MPDs, 9 of them experienced ET, 5 PV and 3 IMF. *Results*. 7 out of 9 patients with ET, 1 out of 2 patients with PV and 2 out of 3 patients with IMF were JAK 2 V617F positive. We found that the patients who were positive for the V617F mutation had also increased levels of VEGF in both serum and immunohistochemical proportions of bone marrow, compared to the MPD patients with the JAK2 wild type. Higher levels of VEGF in both the serum and bone marrow were found in MPD patients than in controls. The statistical analysis showed that, in patients with JAK2 wild type, the mean±SD level of serum VEGF was calculated 639±594.3 pg/mL, whereas in JAK2 V617F patients, the serum VEGF level was 1001±528.4 pg/mL. The medial levels for VEGF in serum were 539 pg/mL for the JAK2 wild type patients and 866 pg/mL for the JAK2 V617F patients. Regarding the immunohistochemical VEGF expression in bone marrow, the mean±SD level was counted 23.75± 10.3% for the patients with JAK2 wild type and in JAK2 V617F patients the mean level was 37.14± 9.9%. The median levels were 20% for the JAK2 wild type patients and 30% for the JAK2 V617F patients. *Conclusions*. These results are suggestive of an important role of angiogenesis and apoptosis in MPDs. Those two procedures are likely to influence each other and we are not yet sure which of those events is the promoter for the other. It also seems that they play a crucial role in the pathogenesis and prognosis as well as in the duration of the diseases and transformation to acute leukemia. They probably are two of the key points towards the therapeutic options of MPDs. This finding puts forward the use of targeted therapies, such as anti-VEGF antibodies in MPD patients.

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RECLASSIFICATION OF HAEMATOLOGICAL MALIGNANCIES ACCORDING MORPHOLOGY DATA

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Background and Aims. Related to the HAEMACARE project we pretend revise the codes of Zaragoza population cancer registry in order to increase availability and standardisation of morphology data, ensuring a strict adherence to ICD-O classification assess the distribution of the haematological malignancies as lymphoid neoplasms, acute and chronic myeloid leukaemias and multiple myeloma. *Patients and Method.* From January 1996 to December 2000, 2,852 patients diagnosed with haematological malignancies were recruited from different sources: the population-based Cancer Registry of Zaragoza, Registry of haematological malignancies of FEHHA and Miguel Servet Hospital records. Former pathological and hematological diagnoses were reviewed and some were prospectively reclassified following the latest WHO classification and their histological subtypes in accordance with the World Health Organization (WHO) classification. We have estimate survival according to specific morphology groups. *Results.* Following criteria established by WHO classification the distribution of lymphoid neoplasms was as follows: B-cell neoplasm 64.5%, T-cell neoplasm 3.3%, Hodgkin lymphoma 4.1%; Myelodisplastic syndromes 9.9%, acute lymphoblastic leukaemia 1.9%, acute myeloid leukaemia 4.0%, chronic myeloid leukaemia 2.3% and multiple myeloma 10.0%. Diffuse large B-cell lymphoma (13.5%) and chronic B lymphoid leukaemia (9.3%) showed the highest incidence rate in adults. *Conclusions*. A higher incidence rate of lymphoid neoplasms was found in men in our area. We have observed in the last period of study a tendency of survival differences.

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