

001

**TRANSCRIPTIONAL ANALYSIS OF FOLLICULAR LYMPHOMA AND THE TRANSFORMATION INTO DIFFUSE LARGE B CELL LYMPHOMA**

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B cell lymphoma is a malignancy consisting of different subtypes of lymphomas showing diverse clinical progression. Follicular lymphoma (FL), an indolent growing lymphoma with few symptoms, and diffuse large B cell lymphoma (DLBCL), a more aggressive lymphoma with severe symptoms, are two of the most common B cell lymphomas. The main treatment of these lymphomas today is chemotherapy solely, or in combination with anti-CD20 antibodies. Nevertheless, the effectiveness still need to be improved, therefore one of our strategies is to find possible therapeutic targets exclusively expressed on tumour cells. FL has a frequent tendency to transform into the more aggressive DLBCL by a mechanism that still remains to be elucidated. Identifying involved signalling pathways would enable new treatment strategies to be designed for this matter. Potentially, transformation could be prevented by using treatment strategies less prone to select for aggressive sub-clones. In this study Affymetrix HG U133 Plus 2 arrays were used to analyse the gene expression profile of >50 000 transcripts in each sample of FL, transformed DLBCL and four different populations of normal tonsil B cells. All cells were purified by flow cytometry to a final purity of 96-100%. Two main data analyses were performed in this study, with the aims (i) to find genes differentially expressed in lymphoma cells compared to non malignant B cells, and (ii) to improve the knowledge of the pathways involved in transformation of FL to DLBCL, including a case study of a patient afflicted by FL that later relapsed with FL and which eventually transformed into DLBCL. In summary, this type of analysis will improve the general knowledge of the malignant transformation event and potentially lead to identification of new targets molecules enabling a more refined antibody-based therapy.

002

**PRIMARY GASTRIC LYMPHOMA: CLINICAL AND ENDOSCOPIC PRESENTATION**

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*Background.* Although the stomach is the most frequent site of intestinal lymphomas, few data are available on both clinical-endoscopic presentation of gastric lymphoma and possible differences between low-grade (LG) and high-grade (HG) lymphomas. This study assessed such aspects on consecutive primary gastric lymphoma patients. *Methods.* Clinical, histological, and endoscopic records of consecutive patients with primary LG- or HG-lymphoma diagnosed in five Italian Hospitals were retrieved. Symptoms were categorized as *alarm* or *not alarm*. The endoscopic findings were classified as *normal* (no macroscopic lesions) or *abnormal* (ulcer, erosions, nodular pattern, hypertrophic folds, polypoid mass, petechial haemorrhage pattern). *Results.* Overall, 144 patients with primary gastric lymphoma were detected, including 74 LG- and 70 HG-lymphoma. Alarm symptoms, particularly persistent vomiting and weight

loss, were more frequently present in patients with HG- than in those with LG-lymphoma (54.3% vs 28.4%;  $p=0.002$ ). LG-lymphomas presented as *normal* appearing mucosa (20.3% vs 0%;  $p=0.0004$ ) or petechial haemorrhage in the fundus (9.6% vs 0%;  $p=0.02$ ) more frequently than HG-lymphomas, being also more often confined to the antrum (47.3% vs 27.1%,  $p=0.03$ ) and associated with *H. pylori* infection (88.7% vs 52.9%,  $p<0.0001$ ). On the contrary, HG-lymphomas presented more commonly as ulcerative type (70% vs 52.7%;  $p=0.03$ ), being also more frequently diagnosed in stage >I as compared to LG-lymphomas (70% vs 21.6%,  $p<0.0001$ ). *Conclusions.* The overall prevalence of alarm symptoms is quite low and may be absent in more than 70% of patients with LG-lymphoma. A normal endoscopic finding may be depicted in patients with LG lymphoma, which is also more frequently associated with *H. pylori* infection. HG-lymphoma is detected in an advanced stage more frequently than LG-lymphoma.

003

**CYCLIN DL EXPRESSION IN B-CELL NON HODGKIN LYMPHOMA**

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*Back ground.* Disorders of the cell cycle regulatory machinery play a key role in the pathogenesis of cancer. Over expression of cyclin D1 protein has been reported in several solid tumors and certain lymphoid malignancies, but little is known about the effect of its expression on clinical behavior and patients outcome in B-cell Non Hodgkin lymphoma. *Subject and Methods.* In this study we investigated the expression of cyclin D1 in group of NHL patients and correlated the results with the clinical and laboratory data to assess the role of cyclin D1 in NHL patient. The degree of expression of cyclin D1 protein was evaluated by flowcytometry in a group of NHL patients (n=46) and in normal control group (n=10). *Results.* Cyclin D1 over expression was detected in 10 out of 46 (21.7 %) patients; they were 5/5 mantle cell lymphoma (MCL) (100%); and 5/28 large B-cell lymphoma (17.8%). All other NHL subtypes showed normal cyclin D1 expression. The clinical signs (hepatomegaly, splenomegaly, and B-symptoms, clinical staging) and laboratory data (Hemoglobin, WBCs, platelet count, and bone marrow infiltration) were not significantly different between NHL subgroup with cyclin D1 over expression and that with normal cyclin D1 expression. Serum LDH levels and lymphadenopathy were significantly higher in NHL group with cyclin D1 over expression as compared to those without. Also, Cyclin D1 over expression is associated with poor outcome of NHL patients. *Conclusions.* Cyclin D1 over expression was evident among all cases of MCL and few cases of large B-cell lymphoma. Cyclin D1 over expression might be used as adjuvant tool for diagnosis of MCL; has role in NHL biology and is bad prognostic index in NHL.

004

**ANGIOGENIC FACTORS PATTERN IS DIFFERENT IN ACUTE LYMPHOBLASTIC LEUKEMIA AND CHRONIC LYMPHOCYTIC LEUKEMIA**

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Angiogenesis is a crucial event in the survival and progression of solid tumors. The angiogenic status and the exact role of the angiogenic cytokines in lymphocytic leukemia is not fully clear. In this context, we have investigated the profile of the systemic components of angiogenic regulation in B cell acute lymphoblastic leukemia (B-ALL) and chronic lymphocytic leukemia (B-CLL) serum levels of vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF- $\alpha$ ), endostatin and matrix

metalloproteinase-9 (MMP-9) by Enzyme-Linked Immunosorbent Assay (ELISA). In B-ALL patients, sVEGF, and MMP-9 were significantly lower than control level at diagnosis ( $p < 0.001$ ,  $= 0.004$ ) and increased near to the control levels in remission ( $p > 0.05$ ). Both serum TNF- $\alpha$  and endostatin levels showed no significant difference both at diagnosis ( $\alpha > 0.05$ ) and in remission ( $p > 0.05$ ) comparing to control levels. sVEGF, sTNF- $\alpha$ , s-MMP-9 and endostatin level were not significantly correlated to peripheral white cell count or Bone marrow blast cells count, however, positively correlated to platelets count. In B-CLL patients, serum VEGF, sMMP-9 and sTNF $\alpha$  were significantly higher ( $p < 0.001$ ,  $= 0.009$ ,  $0.007$  respectively) and decreased near control levels in remission ( $p > 0.05$  for all). Serum endostatin levels showed no significant difference at diagnosis and in remission compared to control levels ( $p > 0.05$ ). Significant positive correlation between sVEGF, sTNF- $\alpha$ , sMMP-9 and peripheral white cell counts and bone Marrow lymphocytic count, and platelets count were detected. *Conclusions.* Our data suggest that the driving forces of angiogenic factors (VEGF, TNF- $\alpha$ , MMP-9) in a adult B-ALL appears different from that in B-CLL patients. Further investigation on the biology of angiogenesis in ALL is required.

#### 005

##### NON-MYELOABLATIVE STEM CELL TRANSPLANTATION IS FEASIBLE AND SAFE FOR HIGH RISK HEMATOPOIETIC MALIGNANCIES

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High mortality rate after allogeneic hematopoietic stem cell transplantation (HSCT) with myeloablative conditioning prompted us to offer HSCT with non-myeloablative conditioning (NST). Between July, 2001 and October, 2005, 26 patients [acute myeloid leukemia,  $n=1$ ; chronic myeloid leukemia,  $n=1$ ; Hodgkin's lymphoma,  $n=4$ ; aggressive non-Hodgkin's lymphoma  $n=6$ ; multiple myeloma (MM),  $n=14$ ], received peripheral NST from HLA-identical siblings. Conditioning regimen consisted of fludarabine plus 2 Gy of TBI, and GVHD prophylaxis with cyclosporine-A plus micofenolate mofetil. Eighty-eight percent were in progression of disease (PD) and 92% were undergoing their second HSCT. Non-hematologic toxicities were mild and transient. Bacterial infection occurred in 44%. Median time to granulocyte recovery was six days and only 12% achieved platelets count lower than  $20 \times 10^9/L$ . Full donor chimerism was established in 95%. Donor lymphocyte infusion was required in 6 patients, 3 because of mixed chimerism and 3 because of PD. A-GVHD grade II or higher was observed in 37%, and extensive c-GVHD in 60%. There were 12 deaths (46%), 7 transplant related mortality and 5 related to PD. OS, PFS and time to progression (TTP) were respectively 39% (95% CI, 15-63%), 33% (95% CI, 11-55%) and 64% (95% CI, 32-96%) for the whole group. MM patients had OS, PFS and TTP of 27% (CI 95%, 0-67%), 42% (95% CI, 8-76%) and 86% (95% CI, 60-100%), respectively. This approach showed feasible and safe with reduction in transplant related toxicities, even for these high risk hematopoietic malignancies.

#### 006

##### SILENCING OF E2A-PBX1 IN PRE-B LEUKEMIA CELL LINES

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RNA interference is a regulatory mechanism of post-transcriptional gene silencing that can be initiated by small interfering RNAs (siRNAs), double-stranded oligonucleotides that mediate sequence-specific mRNA degradation. Fusion transcripts of chromosomal translocations, that often characterize leukemias and lymphomas, represent potential targets for a tumor-specific RNAi approach. The chimeric protein E2A-PBX1 is created through the fusion of E2A and PBX1 genes as a result of the chromosomal translocation  $t(1;19)(q23;p13)$  in a subset of

acute lymphoblastic leukemias (ALLs). Chemically synthesized siRNAs have been used in this study to inhibit the expression of this oncogene in RCH-ACV and 697 pre-B leukemia cell lines in order to clarify its role during leukemogenesis. Effective anti-E2A-PBX1 siRNA delivery into leukemia cells was achieved by NucleofectionTM technology and siRNA cellular uptake was detected by fluorescent microscopy, using fluorescein-labeled siRNAs, and FACS analysis. Specific down-regulation of E2A-PBX1 mRNA expression demonstrated a reduction of gene expression to  $15 \pm 5\%$  (normalized levels were measured 24h after transfection using real-time Q-PCR) and the correspondent protein depletion was detected 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 invariably accompanies the  $t(1;19)$  translocation (and their transcription was detected by SYBR Green PCR). In particular, E2A-PBX1 silencing affected the EB-1 gene, which encodes for a protein that could contribute to the transformed phenotype of pre-B ALL by interfering with signals that regulate normal growth and differentiation within the B cell lineage. Furthermore, the significant decrease in the Wnt16b mRNA levels (and not of the Wnt16a isoform of Wnt16 gene), observed consequently to the fusion gene depletion, confirmed the hypothesis of Wnt16b as target of E2A-PBX1. siRNA inhibition was followed by an increase in apoptosis (2-fold increase of apoptotic cell percent, compared to the mock control 48hr after transfection) and this effect was confirmed by silencing both  $t(1;19)$ -positive cell lines and one without this translocation. The anti-E2A-PBX1 siRNA allows further analysis of the E2A-PBX1 role in leukemic gene expression functions and, eventually, may provide new therapeutic approaches.

#### 007

##### BORTEZOMIB (VELCADE) IN COMBINATION WITH CHEMOTHERAPY IN THREE PATIENTS WITH REFRACTORY HODGKIN LYMPHOMA (HL)

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Bortezomib belongs to a new class of drugs with a new mechanism of action and with a proved clinical activity against multiple myeloma (MM) and non Hodgkin lymphomas (NHL). Recently, it has been reported that bortezomib can induce apoptosis in a variety of Hodgkin lymphoma (HL) derived cells but a pilot study (Younes, Blood 2006) on refractory/relapsed classical HL has shown that bortezomib has minimal single-agent activity. Since in MM and NHL it has been shown that the activity of Bortezomib is increased when used in combination with Dexamethasone and/or chemotherapy, we tested the combination of Bortezomib (PS-341) + Adriamycin + Dexamethasone (PAD, an active regimen in MM) in 3 patients with relapsed classical HL who had received a minimum of 4 prior treatment regimens, including stem cell transplantation. Bortezomib was administered intravenously at doses of 1.3 mg/mq on day 1, 4, 8 and 11 of a 3-week treatment cycle, Adriamycin i.v. at the dose of 9 mg/m<sup>2</sup> for days 1-4 and oral dexamethasone 40 mg/die days 1-4. Therapy was relatively well tolerated with manageable toxicities. One patient had a pulmonary infection and Herpes Zoster reactivation, successfully treated with antibiotic and antiviral therapy. Trombocytopenia was not a frequent event in HL patients receiving bortezomib in combination with Dexamethasone and Adriamycin. Only one patient showed WHO grade I hematologic toxicity. Grade I-II constipation, nausea, mucositis, asthenia, myalgia, paraesthesia, were also observed. After two cycles of PAD, TC scan and PET showed a stable disease but thereafter all patients stopped therapy for disease progression after three, four and six cycles respectively. Although this regimen was generally well tolerated, no significant responses were achieved, thus confirming the minimal clinical activity of Bortezomib on HL, even when used in combination with chemotherapy. However, studies with less heavily treated patients should be performed.

008

**TONSILLAR LYMPHOMA. A CLINICOPATHOLOGIC STUDY OF 80 CASES**Daneshbod Y,\* Ramzi M,\*\* Mohammadianpanah M,\*\*  
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80 cases of with stages IE and IIE primary tonsillar lymphoma between 1980-1998 were reviewed. Complete staging was done in all cases after referral to the Radiation-Oncology unite and cases with higher stages at presentation were excluded from the study. 78 cases were non Hodgkin's lymphoma and 2 were Hodgkin's disease. The most prevalent type of non-Hodgkin's lymphoma was diffuse large B cell lymphoma followed by: follicular lymphoma, T cell rich B cell and mantle cell lymphoma. Both of the Hodgkin's disease were of mixed cellularity type. EBV infection in these cases of tonsillar lymphoma was studied using an immunohistochemical marker. The patients received radiation alone, chemotherapy alone or combination therapy. The treatment results and survival of the patients with different WHO classified lymphoma are discussed.

009

**THE PROGNOSTIC SIGNIFICANCE OF APOPTOSIS-RELATED CD95 ANTIGEN NEGATIVE EXPRESSION IN PATIENTS WITH HODJKIN'S LYMPHOMA**Datikashvili-David I, Malatsidze N, Vashakmadze M,  
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**Background.** Despite visible progress in treatment of oncohematological diseases to predict the clinical outcome and response to the chemotherapy, which leads to individualization of the treatment, still remains one of the major problems of oncology. Nowadays, apoptosis seems to play the meaning role as in pathogenesis of malignancies as in sensibility to the cytoreductive drugs via direct or CD95-dependent cytotoxicity pathways. It is proved that some apoptosis-related antigens are used as prognostic criteria of various diseases, including oncological pathologies. **Aims.** In our study we tried to find out the correlation between negative expression of CD95/Apo-1/Fas antigen on peripheral lymphocytes and clinical outcome and response to the chemotherapeutical treatment of patients with Hodgkin's Disease. **Materials and methods.** On initial stage using flow cytometer (*Becton& Dickinson*) we measured 24-hour spontaneous apoptosis of peripheral lymphocytes via specific monoclonal anti-CD95 fluorescein-isocyanat (FITC)-conjugated mouse antibodies (*Dako*) in 28 patients aged 15-75 yy. During 2001-2004yy. The patients were treated at clinic of the Department of Hematology and Transfusiology of Tbilisi State Medical Academy. Evaluation of the above mentioned apoptotic marker has been provided at the Laboratory of the Department of Immunology of Tbilisi State University. Results were statistically analyzed by means of actuarial method (Bereskin D., 1982). **Results.** Supposing poor prognostic meaning of CD95 negative expression we divided patients into two groups: I. Pts. treated with standard chemotherapeutical regimens; II. Pts. who initially underwent high dose or intensive chemotherapy. Statistical analyses showed that both overall survival and median survival differed depending on the therapeutical approach. In the I-st group OS was 20% versus 87,5% of II group pts. The same results were got analyzing median survival  $\geq 9,2$  versus 30 months. **Conclusions.** Our findings suggest that negative expression of CD95 antigen is associated with more aggressive disease and lower overall survival of HD patients and thereby predicts worse clinical outcome and response to the chemotherapy. So, we conclude that CD95 expression has prognostic consequence as independent criteria.

010

**GALECTIN-7: A ROLE IN LYMPHOID MALIGNANCIES?**Demers M,<sup>1</sup> Biron-Pain K,<sup>1</sup> Hebert J,<sup>2</sup> St-Pierre Y<sup>1</sup>*<sup>1</sup>INRS-Institut Armand-Frappier, University of Quebec, Laval, QC, Canada; <sup>2</sup>Centre de Recherche Guy Bernier, Hosp. Maisonneuve-Rosemont, Montreal, QC, CANADA*

The main thrust of this study is the possible implication of galectin-7 in hematological malignancies. Galectins constitute a family of lectins defined by shared consensus amino acid sequences and affinity for beta-galactose-containing oligosaccharides. Galectins are found in the cytoplasm, in the nucleus, or can be secreted by the cell. Galectin-7 was initially described as a marker of the differentiation levels of keratinocytes. Functionally, the intracellular form of galectin-7 is strongly associated with UVB-induced apoptosis in epidermis since sunburn/apoptotic keratinocytes express abnormally high levels of galectin-7. Nevertheless, Galectin-7 remains an enigmatic member of the galectin family and has not been extensively studied to date. Not surprisingly, its role in cancer remains largely unknown. At first glance, galectin-7 should aid in the elimination of tumour cells because its expression is induced by p53 and functions as a regulator of apoptosis, or at least negatively regulates cell growth in absence of apoptosis. In sharp contrast to the intuitively negative roles played by galectin-7 in tumour development, Lu *et al.*, (1997) has shown that galectin-7 is overexpressed in mammary tumors induced in an experimental model of chemically-induced mammary carcinomas. By combining a genomic approach and an experimental mouse model, we have found that the most prominent change that distinguishes low and high metastatic lymphoma variants was the strong upregulation of galectin-7 gene expression. To determine whether galectin-7 had a role in the dissemination of lymphoma cells in peripheral tissues, we have transduced a nonaggressive T lymphoma cell line, the 267 T lymphoma cells, with the murine galectin-7 cDNA. We found that galectin-7 accelerated the development of lymphoma as the mean survival time of mice injected with galectin-7 transfectant was significantly shorter than that of mice injected with control transfectants. We also observed that mice injected intravenously with lymphoma cells expressing galectin-7 developed large metastatic tumors in the liver and kidneys with massive infiltration of tumor cells in the parenchyma. Conversely, we found that stable transfection of lymphoma cells with a plasmid encoding antisense galectin-7 cDNA significantly inhibited the dissemination and invasion of lymphoma cells to peripheral organs, thereby increasing the survival of mice as compared to controls. Finally, to examine the clinical relevance of these findings, we have studied the expression of galectin-7 in various human lymphoid malignancies, including acute and chronic leukemias, myelodysplastic syndromes, myelomas, and lymphoproliferative syndromes. Our data have confirmed that this gene is expressed in human lymphoid malignancies. We are currently correlating our data with the morphology, immunophenotyping, and cytogenetics data collected with each specimen. Overall, our results suggest that galectin-7 may represent a new molecular target against the have uncovered the existence of a previously undescribed activity, the promotion of lymphoid malignancy, to galectin-7.

011

**PROLONGED PERSISTENCE OF BCL-2/IGH POSITIVE CD19+ CD5- CD23- CD10+/- CLONAL POPULATIONS IN LYMPHOMA-FREE SUBJECTS**De Marco F, Mantoan B, Drandi D, Pollio B, Astolfi M,  
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**Introduction.** Non-lymphoma associated Bcl-2/IgH rearrangements (NLABRs) are frequently amplified by PCR in blood of



lymphoma-free subjects (LFS), but the temporal kinetics and phenotypic nature of NLABR-positive cells are unknown. To define the natural history of NLABR-positive clones, long term monitoring of cancer-free subjects carrying these lesions has been performed. *Methods.* 125 subjects undergoing periodical blood examinations for warfarin therapy were screened for the bcl-2/IgH translocation. NLABR-positive clones were monitored using nested-PCR, real time-PCR and DNA sequencing. *Results.* Of 125 donors, 16 (12.8%) were found to be Bcl-2/IgH positive and were monitored at least every six months for a median time of 22 months (range 6-50). In half of the subjects the same NLABR detected initially was again re-amplified at follow-up thrice or more. In five the same NLABR was constantly amplified in every follow-up sample. With a median follow-up of 22 months (range 9-50), no stable disappearance of a recurrent clone has been so far recorded. All samples scoring PCR positive by nested PCR, were also quantified by real-time PCR showing a range from  $5.4 \times 10^4$  cr/10<sup>6</sup> to diploid genomes (dg) to  $2.8 \times 10^9$  cr/10<sup>6</sup> dg. The number of NLABR-positive cells appeared to be rather stable in subjects with persistent NLABR-positive clones. Subjects having mixed PCR-positive and PCR-negative results had a smaller tumor burden if compared to those constantly PCR-positive ( $p < 0.001$ ). Studies on selected populations showed that NLABR-positive cells were CD19-positive. This demonstrates that NLABRs are exclusively associated to B-cells. Moreover NLABRs were never found in the CD 23+ and CD5+ cell fraction suggesting that these antigens are not present in NLABR-positive clones. In contrast, both the CD10+ and CD 10- fraction contained NLABR-positive cells suggesting variable expression of this antigen as observed in FL. *Discussion.* This report suggests the existence of a follicular lymphoma (FL)-related clonal expansion of undetermined significance, which might be either a pre-malignant or a non-malignant counterpart of FL. Since NLABRs occurs in more than 10% of healthy subjects, this condition is expected to be highly prevalent in the general population (as observed in MGUS and CLUS) and of potential relevance for the pathogenesis of FL.

#### 012

### FLUDARABINE MONOPHOSPHATE TREATMENT IN REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA IN ELDERLY PATIENTS: RESPONSE, TOXICITY AND QUALITY OF LIFE (QOL) ANALYSIS IN 30 CASES

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*Introduction.* Fludarabine monophosphate is a purine analog with specific therapeutic activity in B-cell chronic lymphocytic leukaemia (CLL) and is an effective front-line therapy option for patients with advanced CLL. We report a retrospective analysis of the results obtained with the purine analog in elderly CLL patients resistant to clorambucil therapy. *Objective.* Response assessment, toxicity and quality of life of oral fludarabine in monotherapy given to previously treated CLL elderly patients. *Design and methods.* Response rate is evaluable in 30 patients (17 males/13 females), median age 71 years (47-87). Oral Fludarabine monophosphate (Beneflur®, Schering España) 40 mg/m<sup>2</sup> were administered orally (PO) daily on Days 1-5 each 28 days. The study design consisted in six consecutive courses. The median number of courses per patient was 4. Supportive care consisted in Trimethoprim 160 mg and sulfamethoxazole 800 mg administered orally twice daily 3 times/week and acyclovir 800 mg PO daily. Clinically and cytometric response were assessed after therapy and thereafter, every 3 months. Ten patients were in Rai low risk, and 19 intermediate and 1 high risk (21 stages A Binet, 7 Binet B and 2 Binet C). The median beta 2 microglobulin level was 2.9 mg/L (1.5-10) and 339 U/L (246-563) of Lactate dehydrogenase (LDH). Toxicity and quality of life (QoL) were determined with the WHO criteria. *Results.* 15 patients (50 %) completed 4 courses of therapy. 30 patients were evaluated for clinical response according to IWCLL and NCI criteria. Overall 66.6% out of 30 evaluable patients were responsive to treat-

ment. 60% and 6.6% of cases achieved RC and PR, respectively. The majority side effects were mild-to-moderate intensity. Hematology toxicity grade 1-2 was observed in 10 patients. Eight patients registered non hematology toxicity and it consisted in diarrhoea, anorexia and nausea and vomiting. Only one case of AHAI was reported. Overall, 3 patients died, two due to septicemia during therapy and 1 due to neurologic toxicity. Organ toxicity was represented by a fatal neurologic complication in one patient. No other organ toxicity was registered. At the baseline, all patients had a WHO performance status of 0-1. WHO performance status improved in 56% of patients. *Conclusions.* For many years, alkylating agents such a chlorambucil have been the gold standard treatment for previously untreated B-CLL patients. However, large randomized trials have confirmed the efficacy of oral fludarabine has been demonstrated for both first and second line treatment. This study demonstrated that oral fludarabine is effective and well tolerated. Moreover, the benefits of oral fludarabine extend beyond blood cell responses, and it have a positive impact on quality of life. This advantage, emphasizes the potential value of oral fludarabine in the palliative setting, where QoL is an important consideration.

#### 013

### ARRAY-CGH DETECTS CRIPTIC IMBALANCES IN PLASMA CELL DYSCRASIA (PCD)

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*Background and Aims.* Array-based comparative genomic hybridization (array-CGH) is a relatively new technology designed to rapidly screen the entire genome for unbalanced genetic aberrations. We used this technique to perform pan-genomic screening in 20 patients with plasma cell dyscrasia (PCD) in particular 16 patients with multiple myeloma (MM) and 4 with de novo plasma cell leukemia (PCL). Our aims were the following: i) to identify the most common yet undescribed genetic lesions; ii) to verify which among these lesions are linked to the natural history of PCD; iii) to establish a preliminary link with clinical outcome. *Method.* Array-CGH was performed as follows: plasma cells were purified from bone marrow samples by Myltenyi columns. Genomic DNAs, from both the tumor and healthy cells, labelled with different fluorescent dyes were cohybridized to 1 Mb resolution BAC-arrays (Spectral Genomics Inc. USA). Variations in DNA sequence copy number for each BAC clone was assessed by relative fluorescence signal intensities, providing a locus-by-locus measure of DNA copy-number changes. *Results.* Array-CGH is a feasible and robust approach and is 86% concordant with FISH for known imbalances. The median number of lesions/patient observed in our panel was 17 (4-135). Also the amount of the total genome affected by chromosomal imbalances was highly variable (median 3.9% range: 0.14%-27%). This number is superior to that reported in CLL (Drandi, ASH 2003) and in diffuse large B cell lymphoma (DLB-CL) (Chen, Blood 2006). Of 2600 BACs 934 were never affected, 864 were targeted only in one patient (pt), 401 in two pts, 296 in 3-5 pts and only 105 were targeted in six pts or more. These 105 recurrent imbalances could be attributed to 9 different abnormalities. Despite the small series we have identified five yet undescribed recurring imbalances (frequency more than 30%) that involve chromosome 19p13, 14q12, 16q12, 11q24 and 9q23. *Conclusions.* a) array-CGH allows effective pan-genomic screening; b) in PCD the genome undergoes a high degree of genetic disruption compared to other lymphoid tumors, particularly CLL; c) the overall amount of perturbed genome seem to correlate with more aggressive disease, and might be the reflection of alternative biologic features; d) a number of highly recurrent lesions have been identified. All these lesions will require further investigation to identify candidate target genes and to verify if they might be prognostically relevant.

014

#### RECOMBINANT HUMAN ERYTHROPOIETIN (RHU-EPO) EFFICIENCY IN THE PREVENTION AND TREATMENT OF ANEMIA IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES AFTER THE TRANSPLANTATION OF PERIPHERAL STEM CELLS

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**Background.** Autologous transplantation of the hemopoietic stem cells (HSCT) preceded by the highly dosed chemotherapy is a selective method of treatment during lymphoproliferative disorders. Anemia progresses nearly in all cases, and erythrocyte transfusion is a universal practice for its treatment during the posttransplant period. An inadequately low production of erythrocytes plays the key role in this process. Despite the sufficient amount of the erithroid precursors in the bone marrow, restoration of erythrocytes slows down due to the inadequate production of erythropoietin. **Aims.** The aim of this work was to determine the adequacy of endogenic erythropoietin production and the potency test of the recombinant human erythropoietin (rHu-EPO) in patients with the lymphoproliferative disorder (non-Hodgkin's lymphoma (NHL), lymphogranulomatosis (LGM) and multiple myeloma (MM), after the autologous (HSCT). **Methods.** Twenty patients with lymphoproliferative disorder were examined. Ten of them were treated with rHu-EPO - epoietin beta to prevent anemia, and other ten were included in the control group. Among the test group five patients had MM, four had LGM, and one patient had NHL. Regimens of conditioning were used: BEAM in patients with LGM and NHL, and 200 mg/m<sup>2</sup> doses of melphalan in patients with MM. 20000ME doses of rHu-EPO were inserted subcutaneously three times a week from the first day of conditioning. Clinical blood analysis, levels of serum iron (SI), serum transferrin (ST) and ferritin (SF) were measured for all patients on the first day of conditioning and on the +15th day after (HSCT). **Results.** The average level of hemoglobin and reticulocytes in the test group before treatment was 105,7±5,96 g/L and 0,4±0,19% accordingly, while these indicators in the control group were 106,6±8,28 g/L and 0,5±0,13%. The average level of thrombocytes in the test group before treatment was 249,3±26,27×10<sup>9</sup>/mm<sup>3</sup>, accordingly, while these indicators in the control group were 211,3×10<sup>9</sup>/mm<sup>3</sup>. In the test group of patients treated with rHu-EPO, the average level of s-EPO constituted 104,2±25,4 mIU/mL, mean concentration sTFR 7,3±2,12 mg/mL, indices of iron metabolism were ST-1,5±0,5 g/L, SI-23,3±3,27 micromole/l, SF-782±87,28 mg/L.

During the rHu-EPO therapy enlargement of s-EPO was registered (mean value was 128,8±18,0 mLU/mL) average concentration of sTFR (27,2±11,05 microgramm/mL) increased 3 times when compared with initial data ( $p < 0,05$ ). Indices of iron metabolism decreased a little, however they remained, within the mark for all patients (ST-2,5±0,15 g/L, SI-9,61 ±3,63 micromole/L, SF-387,5±128,3 mgr/L). However when compared with the control group, the patients treated with rHu-EPO had the average level of hemoglobin of 103,2±5,69 g/L against 95,1±4,40 g/L in the last one, the amount of reticulocytes was higher than in the control group (1,4±0,32%) and (0,3±0,10%) accordingly, which testifies to the activation of erythropoiesis. The amount of thrombocytes was higher than in the control group (157,8±35,15×10<sup>9</sup>/mm<sup>3</sup>) and (82,6±17,48×10<sup>9</sup>/mm<sup>3</sup>). The number of patients who needed the transfusion of red blood cells was higher in the control group (6 and 4 patients); average volume of the transfused red blood cells (853±81,19 and 762±114,77 mL), total volume of the transfused red blood cells (5120 and 3070 mL). The number of patients who needed the transfusion of thrombocytes was higher in the control group (8 and 6 patients); average volume of the transfused thrombocyte (1871,25±500,34 and 665±345,80 mL), total volume of the transfused thrombocyte (14730 and 2600 mL). **Conclusions.** This research showed that use of rHu-EPO at the early stages of treatment (from the first day of conditioning) is an adequate means for prevention and correction of anemia in patients treated with the use of transplantation technologies.

015

#### STUDY OF GENETIC POLYMORPHISM OF XENOBIOTIC ENZYMES IN ACUTE LEUKEMIA

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This work is a trial to study the possible association between the main genetic polymorphisms of CYP2D6, GSTM1, GSTT1 and NQO1 and altered susceptibility to leukemia. Also to correlate these genetic polymorphisms with other clinical prognostic data of the patients, their response to therapy and the possibility of relapse. This study included thirty two leukemia patients, nineteen patients with AML and thirteen patients with ALL, it also included eleven normal subjects as control group. Basic investigations for the diagnosis of AML & ALL were performed including complete blood picture, bone marrow aspirate, with cytochemistry and immunophenotyping for the detection of ALL and AML subtypes. For the detection of CYP2D6, NQO1, GSTM1 & GSTT1 genetic polymorphisms, this work has applied a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. A follow up study was also carried out to investigate the association between the outcome of these patients and the different patterns of genetic polymorphism of these four genes. Our results have demonstrated a significant increase in the frequency of CYP2D6 wild type and GSTM1 null genotype in the acute leukemia group when compared with the control group. Studying the relationship between the genetic polymorphisms of these genes and the outcome of our cases revealed that the wild genotype of CYP2D6 significantly influenced the outcome of acute leukemia in particularly the AML cases, while the GSTM1 null genotype was associated with bad prognosis among the ALL group. The GSTT1 null genotype had no impact on the outcome of acute leukemia cases. The study also revealed that patients with combined mutant CYP2D6/present GSTM1/present GSTT1 achieved the best prognosis, suggesting the synergistic impact of these genetic polymorphisms on the outcome of acute leukemia cases. This case-control study suggests a contribution of CYP2D6 and GSTM1 null variants in the development of acute leukemias. In addition GSTM1 and GSTT1 genotypes were apparently related with response, side effects and prognosis of patients with AML. **Key words.** Xenobiotic enzymes, Genetic polymorphisms

016

#### EARLY STAGE INDOLENT NON-HODGKIN'S LYMPHOMA: PROLONGED DISEASE-FREE SURVIVAL COMBINING ANTI-CD20 RITUXIMAB WITH INVOLVED FIELD RADIATION THERAPY

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**Introduction.** Among non-Hodgkin's lymphomas (NHL), the indolent subgroup includes follicular lymphoma (FL) grade 1-2 according to the REAL classification, small cell lymphocytic and MALT lymphomas.<sup>1,2</sup> They usually present with asymptomatic and slowly growing nodal or extranodal masses. Although indolent NHL usually starts as a disseminated disease, about 15% of patients present with limited disease at diagnosis.<sup>3</sup> The most common therapeutic approach for limited, stage I-II disease without risk factors is involved field radiation therapy (IFRT), allowing more than 95% local control rates.<sup>4</sup> From a recent update of Canadian datasets, the expected overall survival rates with RT alone are 79% and 62% at 5 and 10 years, respectively, while disease-free survival rates are 56% and 41%.<sup>5</sup> Over the years several groups investigated the combination of chemotherapy (CT) with radiation in order to improve overall and disease-free survival. Even if 6 randomized trials failed to show a benefit in terms of survival, other few studies reported higher disease-free survival rates if CT is combined to RT.<sup>6-10</sup> However, there is concern about the use of cytotoxic drugs in

patients with an indolent and early stage NHL. In order to deliver a systemic anti-lymphoma drug while avoiding the potential toxicity of chemotherapy, a study program was designed combining the anti-CD20 Rituximab monoclonal antibody with IFRT. Preliminary results are here reported. Patients and Methods. Since March 1999, patients with nodal or extranodal stage I-II FL, grade 1 or 2-REAL, or indolent MALT Lymphoma have been enrolled in the study program. Staging work-up included physical examination, total body CT scans, bone marrow biopsy, CBC. The feasibility of inclusion of all involved sites in a single IFRT field was assumed as a selection criteria for stage II patients. Overall, 26 patients have been treated and are evaluable: 12 had nodal FL (10 inguinal, 1 cervical, 1 axillar-cervical involvement), 10 had MALT lymphoma (4 with parotid gland, 4 with orbital and 2 with breast involvement) and 4 primary cutaneous FL. Twenty-five out of 26 had stage I disease. Treatment protocol included 4 Rituximab doses (375 mg/sqm/each dose) given at 1 week interval followed by IFRT, starting 3-4 weeks after Rituximab. All RT fields were limited to involved sites. Patients in complete remission (CR) after immunotherapy received 30.60 Gy in 17 fractions, all other patients received 36 Gy. Standard RT techniques were usually employed, with single or opposite shaped fields; in selected cases (parotid gland and ocular localizations), a CT-based 3D-conformal radiation therapy approach was chosen, in order to obtain the maximum sparing of critical normal structures. Treatment of primary cutaneous FL was delivered with a combination of 6 and 9 MeV electrons and shaped fields, all other treatments with 5 or 6 MV X photons. Restaging work-up included physical examination, total body CT scans, CBC. All 4 patients with orbital MALT lymphomas were also studied with MRI before and after treatment. MRI-CT image co-registration was useful also in contouring RT target volumes with more accuracy. Response criteria for lymphoma, adapted from Cheson et al. were employed to evaluate response.<sup>11</sup> **Results.** Overall, 4 patients had no more signs of disease following the surgical biopsy; at the end of the 4 Rituximab doses, 4 more patients (3 with nodal involvement and 1 with parotid gland involvement) reached CR, 13 patients went into partial response (PR), while no evidence of response (NR) was documented in 3 patients. Ultimately, all 26 patients were in CR following IFRT. Treatment was very well tolerated. In 4 patients mild Rituximab-related side effects were observed (fever with chills); no hematological toxicity was recorded. Mild radiation-induced xerostomia (grade 1) was recorded at 2 years from RT in a patient treated for parotid gland MALT lymphoma. No severe late toxicities have been observed. So far, 13 patients relapsed outside treatment field, 1 with changed histological pattern (diffuse large B-cell lymphoma) and 2 with same histological features (FL). Time to treatment failure was 54, 50 and 66 mos. respectively. At present, at a median follow-up of 32 mos. (range 6-88 mos.), 23 patients (88%) are alive in continuous, unmaintained CR. **Conclusions.** Administration of four Rituximab doses followed by IFRT appears to be a safe, tolerable and active combined modality regimen for patients with grade 1-2 FL or low-grade MALT lymphoma presenting with limited stage disease. The CR rate of 100% is similar to the one previously reported in RT-only series. Focusing on the impact of anti-CD20 on relapse rate and disease-free survival, these preliminary data suggest a benefit in favour of the combined modality treatment in terms of reduced relapse rate (3/26, 11%) and prolonged time to treatment failure (50-66 months), compared to studies employing RT as single agent. Thus, combining Rituximab with RT might result in improved event-free survival, without the need of chemotherapy administration. Furthermore, the addition of Rituximab might allow to reduce RT doses and treatment volumes, minimizing acute and late effects.

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## 017

### IN VITRO ACTIVITY OF BROSTALICIN ON HEMATOLOGICAL MALIGNANCIES

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Brostallicin is a DNA minor groove interacting agent, currently in Phase II trial. The drug has anti-proliferative and strong pro-apoptotic activity in experimental tumors. The mechanism of DNA alkylation is novel: brostallicin binds covalently to DNA only in the presence of glutathione/glutathione S-transferase (GSH/GST), as a consequence, unlike other cytotoxics, it has enhanced activity in tumors with high GSH/GST levels. Phase I/II clinical data from patients with solid tumors show neutropenia as dose-limiting toxicity, while erythropoiesis and platelets are less affected. So, hematologic tumors may represent an important target for brostallicin. In order to study the activity profile of brostallicin in this tumor type and try to identify the most responsive subgroups, we have tested its effect on proliferation and viability (alamar blue vital dye added to the cells 48 h after treatment) of 19 human cell lines derived from different subtypes of hematologic tumors. Cell lines tested included: 4 acute lymphoblastic leukemias (ALL), 7 acute myeloid leukemias (AML), 1 chronic lymphocytic leukemia (CLL), 6 B-non Hodgkins lymphoma (B-NHL) and 1 chronic myelogenous leukemia (CML). All cells were characterized for gene expression of GST isoenzymes and multidrug-resistance related markers (MDR-1, BCRP, MRPs, LRP). Brostallicin was tested in parallel with doxorubicin (DX). In dose-response experiments, brostallicin was highly cytotoxic for 17 out of 19 cell lines, with an IC50 varying from 16 to 488 nM (median 51 nM). Resistance to brostallicin was found in 1/6 B-NHL and 1/7 AML line (IC50



1518 and 1457 nM, respectively). This compared favourably with the activity of DX (median IC<sub>50</sub> 141 nM), being the cytotoxic effect of brostallicin comparable or better than DX in 78% of the cell lines. Cytotoxicity was due to induction of apoptosis as shown by annexin V binding and detection of cleaved caspase 3 following treatment with Brostallicin. All cell lines tested expressed the GST-P1 protein and no correlation was found between sensitivity of cells to brostallicin or DX and expression of this GST isoform. We next studied the capacity of brostallicin to inhibit the colony formation of leukemic cells in methylcellulose at doses and times of exposure reached in patients at the selected Phase II dose (AUC<sub>0-8</sub> 881±208 ng h/mL). Exposure of leukemic cell lines to brostallicin for 1 h led to a 40-69% colony growth inhibition (mean 50%) at the dose of 100 nM (78 ng/mL), thus further supporting the effect of this drug on these cells. Studies are in progress to investigate the mechanism of action of brostallicin in terms of cell cycle block and induction of apoptosis. The results obtained so far suggest that the drug induces a block in G<sub>2</sub> in some cell lines. We conclude that the activity of brostallicin on hematologic neoplasms, including acute leukemias and B-NHL, warrants further investigation. Studies on freshly isolated leukemic samples are ongoing.

### 018

#### HBV REACTIVATION IN A HBSAG NEGATIVE PATIENT WITH B-CELL CHRONIC LYMPHOCTIC LEUKAEMIA UNDER FLUDARABINE, CYCLOFOSFAMIDE AND RITUXIMAB

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HBV reactivation in HbsAg negative patient under immunosuppressive therapy is a phenomenon, recently described in few cases, that leads to exitus in almost all patients because of the lack of prophylaxis with lamivudine. Here we show the case of a reactivation in a patient without surface antigen but exclusively with antibodies anti core. On April 1999 a 70 old year female was diagnosed as B-CLL stage II according Rai classification. Her liver tests were normal; HbsAg, HbsAb and HCV were negative, only HbcAb titer was positive. On June 1999 she was treated because of abdominal bulk lymphadenopathies according VCP protocol obtaining a good partial response. Successively she started Interferon alpha 3000000 UIx3/week as maintenance therapy from November 1999 until June 2000, when her disease progressed because of bulk lymphadenopathies. Then she received fludarabine and cyclofosfamide for four cycles every 21 days achieving a good partial response (July 2003) again, that maintained under IFN therapy until August 2005. On October 2005 she was treated according FCR protocol (Fludarabine, Cyclofosfamide, Rituximab); at this time her liver tests were always normal, and her viral markers were unmodified. After five courses of chemotherapy in occasion of the first red blood transfusion we found HbsAg positive, HbsAb negative, HbcAb positive, HbeAg positive, while her liver tests were absolutely normal (AST 12 U/L, ALT 20 U/L, Bilirubine 0,9 mg/dL, Albumine 3,8 g/dL). HBV DNA copies were 890000/mL Therefore on March 2006 chemotherapy stopped and lamivudine therapy started. On July 2006 at last control her liver tests were always normal, while her HBV DNA copies decreased to 270000/mL. In literature few cases of HBV reactivation under chemotherapy in patients without surface antigen but exclusively with HbsAb and/or HbcAb are reported. Generally this unusual reactivation causes fatal death for severe and quick hepatic failure. Here we have shown an HBV reactivation that causes only a chronic infection. At the start of chemotherapy our patient showed only HbcAb. This pattern is typical of a carrier for a mutant HBV who replicates to low level. At reactivation her serology tests were HbsAg, HbeAg and always HbcAb positive. Unusually her liver tests were never abnormal. At the moment we can consider the patient as chronic HBV carrier with high replication but without signs of cytolysis. Actually at the best of our knowledge this type of reactivation is the combined result of an occult long-lasting infection and the immunosuppressive chemotherapy effects.

### 019

#### RESPONSE-ADAPTED ABVD CHEMOTHERAPY AND INVOLVED FIELD RADIATION FOR INTERMEDIATE STAGE HODGKIN DISEASE: A GISL/NHLSG TRIAL

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Most of the patients with advanced stage Hodgkin lymphoma can be cured with a standard course of six cycle of ABVD chemotherapy plus involved field radiotherapy (IF). Patients with less advanced stage or with a more responsive disease could possibly achieve a cure with a shorter course of chemotherapy. In 1992, in the pre-PET era, the GISL addressed the issue of the proper number of chemotherapy cycles planning a response-oriented, ABVD-based study for intermediate stage Hodgkin's lymphoma patients. *Patients and Methods.* From January 1992 to December 2002, 218 patients younger than 70 were enrolled by 14 GISL/NHLSG institutions. Eligible patients had histologically confirmed and clinically staged Hodgkin's disease and were previously untreated. Patients with unfavourable disease included those with Ann Arbor stage I-III plus at least one among the following: B-symptoms, bulky tumor or extranodal localization. Bulky lymphadenopathy was defined as a mediastinal-mass to intrathoracic-cavity ratio of one third or greater on upright chest radiograph or a peripheral lymph node mass greater than 10 cm in longest diameter. The required clinical staging evaluation included: history and physical examination; CBC count with differential, erythrocyte sedimentation rate, and routine renal and hepatic chemistries; chest radiograph, cervical, thoracic and abdominal computed tomography (CT) scan with contrast. Treatment Plan. Treatment included a first step of three ABVD cycles followed by an early restaging. Those patients in CR or CRu after 3 cycles were planned to receive one additional ABVD cycle while patients in PR were planned to receive 3 additional ABVD cycles. IF radiotherapy was recommended on sites of bulky disease and on residual masses at the end of ABVD courses. *Results.* The median age was 30 years (15-68) with an M/F ratio of 0.7. Nodular sclerosis accounted for 75% of the diagnoses. Seventy-eight percent of cases were in stage II, 7% stage I and 15% stage III; B-symptoms, bulky tumor and ESR>30 were recorded, respectively in 15%, 20%, 25%, and 80% of cases. Of the 206 evaluable cases, 34% of patients were in CR at early restaging, 11 in CRu and 47% in PR; four patients did not respond, and four were early drop-outs. Overall 57% of patients received 4 ABVD cycles, 47% six cycles, and 85% received the planned IF-RT. The CR-CRu rate was 72% at the end of chemotherapy and increased to 93% after the radiotherapy. First events included: 18 relapses, 14 less-than-CRs or progressive diseases and two deaths in CR. Of the 34 patients with recurrent or progressive disease, 12 subsequently died, the others are in second or third remission. With a median follow-up of 60 months (24-162), five-year OS and EFS (median-SE) are 96.7%-2.7% and 83.8% - 3.6%, respectively. Early responders at the third cycle, who received only four ABVD cycles had an excellent outcome with similar EFS (90% vs 84%) but better OS (100% vs 91%  $p=0.003$ ) compared to the late responders. *Conclusions.* A response oriented chemotherapy program is feasible and safe in intermediate stage Hodgkin's lymphoma patients. Overall, with a flexible number of ABVD the OS and EFS of present series matches nicely with that expected for this setting of patients. Early clinical restaging identify cases who have an excellent outcome even with a shortened ABVD course.

020

**RESPONSE-ORIENTED ABVD CHEMOTHERAPY AND INVOLVED FIELD RADIATION FOR INTERMEDIATE STAGE HODGKIN DISEASE. A GISL/NHLSG TRIAL**

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021

**PRE-EMPTIVE TREATMENT WITH CIDOFOVIR FOR CYTOMEGALOVIRUS ANTIGENEMIA IN CLL PATIENTS ON THERAPY WITH ALEMTUZUMAB AND IN AUTOLOGOUS BONE MARROW RECIPIENTS.**

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Cytomegalovirus (CMV) is an important cause of morbidity and mortality in patients who have undergone severe immunosuppressive therapy. Ganciclovir continues to be the first choice for pre-emptive therapy, but it needs multiple intravenous daily administration for three weeks and may cause myelosuppression. Cidofovir is a non myelotoxic nucleotide analogue effective against CMV; its favourable pharmacokinetic profile allows a once-a-week dosing. We reviewed a database on 110 consecutive Autologous Stem Cell Transplant (ASCT) and that of 15 Chronic Lymphocytic Leukemia (CLL) patients treated with alemtuzumab. All patients were virologically monitored by quantification of pp65 antigenemia in peripheral blood. Cytomegalovirus infections were identified respectively, in 13 of 110 (12%) ASCT group and in 10 of 15 (66%) CLL group. Nine out of 23 CMV reactivation showed manifestation of the infection. All patients were treated on outpatient basis. Patients with a positive pp65 assay were treated with cidofovir 5 mg/kg once-a-week for two weeks followed by one or two doses every two weeks. Twenty-three patients (13 autologous, 10 alemtuzumab) had 23 episodes of CMV-pp65 detection treated with cidofovir. The first positive antigenemia occurred after a median of 36 days from starting treatment (range 5-20) and the median antigenemia level at first appearance was 2 (range 1-89). The treatment produced regression of symptoms in all cases and clearance of the virus in 21 (11 post-transplant 84%; 10 post alemtuzumab 100%), stained by CMV antigenemia. Median duration of therapy was 21 days (range 14-30 days) and the time to the first undetectable antigenemia was seven days (range 7-28). We did not observe any further CMV reactivations, also in six of the ten patients who restarted treatment with alemtuzumab after the end of pre-emptive therapy. We did not observe any of the side effects potentially related to cidofovir administration: notably, none of the patients experienced renal toxicity, proteinuria, nausea or vomiting, ophthalmological or neurological toxicity. In our experience, pre-emptive therapy of CMV infection with cidofovir is safe and effective. In our opinion it could be considered an interesting alternative to ganciclovir for pre-emptive therapy, particularly advantageous for treatment of CLL and ASCT ambulatory patients at low risk of developing CMV disease.

022

**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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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. We have investigated, in a small cohort of young untreated CLL patients, the feasibility and effectiveness of a combination therapy using alemtuzumab consolidation to improve the quality of response to FC-R induction. In our institution we treated 12 patients (4 F and 8 M; median age: 45 years, r.: 35-52 years; Rai stage III-IV) with 6 cycles of FC-R (fludarabine at a dose of 25 mg/m<sup>2</sup> i.v. on days 1-3, cyclophosphamide at a dose of 250 mg/m<sup>2</sup> i.v. on days 1-3, and rituximab at a dose of 375 mg/m<sup>2</sup> 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. 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 +10) all patients are alive and in molecular CR. FC-R is highly active as initial therapy also in young CLL patients. However, a consolidation therapy with alemtuzumab seems to be required for achieving a stable molecular CR. Moreover our preliminary results show acceptable toxicity profile of this therapeutic approach.

### 023

#### OUTCOME OF PATIENTS PROGRESSING OR RELAPSING AFTER PRIMARY TREATMENT WITH TWO CYCLES OF CHEMOTHERAPY AND RADIOTHERAPY FOR EARLY STAGE (FAVORABLE) HODGKIN'S DISEASE

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**Purpose.** To evaluate treatment outcome of patients (pts) with early stage (favorable) Hodgkin's disease (HD) with disease progression or relapse after primary treatment with two cycles of polychemotherapy followed by radiotherapy. **Patients and Methods.** From 1994 to 2002 pts with early stage HD were enrolled in two trial generations (HD7/HD10) of the German Hodgkin Study Group (GHSG). HD7 randomized CS IA-IIB patients with no risk factors (RF) to extended field radiotherapy (EFRT) alone or to 2 cycles of ABVD and EFRT. The HD10 trial randomized CS IA-IIB pts to 2 or 4 cycles of ABVD and 20 or 30 Gy involved field RT (IFRT). Treatment outcome and prognostic factors (PF) in pts with progressive or relapsed disease after 2 x ABVD + EF RT (HD7 Arm B) or 2 x ABVD + IF RT (HD10 Arm C + D) were retrospectively analysed and compared to pts registered in our database with treatment failure after RT alone or after 4 or 8 cycles polychemotherapy as front-line therapy. **Results.** A total of 1831 pts with early stage HD were enclosed in HD 7 and HD 10. 915 pts were treated with two cycles of ABVD followed by EFRT in 316 patients (HD7 Arm B) or IFRT in 599 pts (HD 10 Arm C+D). 35 patients had progressive (17%) or relapsed HD (83%). Pts characteristics at relapse: median age: 40 yrs (range 19-72 yrs); histology: MC 39%, NS 37%, LCRHD 9%, LPHD 9%, LD 3%, not classified 3%; stage: CS I 26%, CS II 43 %, III 20%, CS IV 11 %; B symptoms 8%; anemia (m < 12.0 g/dL; f < 10.5 g/dL) 23%; outfield relapse 40%. At progress/relapse 29% were treated with BEACOPP escalated, 29% with HDCT/ASCT, 20% with COPP-ABVD-like regimens, 14% with BEACOPP baseline, and 8% with salvage RT. At 58 months median follow-up FF2F and OS were 49% and 62%,

respectively. According to our recently developed prognostic score for relapsed HD (PF: duration of first remission, stage at relapse and anemia at relapse) patients with 2 or more RF had a FF2F and OS of 15 and 18% compared to 67% and 82% for pts with 0 or 1 RF. FF2F and OS at 58 months were comparable for pts treated with HDCT/ASCT (FF2F: 70%, OS 81%) or BEACOPP escalated (FF2F 64%, OS 78%). In contrast, pts treated with COPP-ABVD like regimens (FF2F 30%, OS 43%) or BEACOPP baseline (FF2F 19%, OS 22%) did worse. Compared to pts receiving RT as front line therapy alone pts treated with 2 x ABVD had a poorer outcome (RT alone: FF2F 79%, OS 82%). The outcome of pts pretreated with 4 (intermediate stages) or 8 cycles (advanced stages) of polychemotherapy at first diagnosis did not differ significantly and was inferior to pts pretreated with 2 x ABVD + RT. **Conclusions.** Disease progression or relapse after primary treatment with 2 x ABVD followed by radiotherapy is a rare event. The prognosis of pts treated with 2 x ABVD is impaired compared to patients treated with front-line radiotherapy alone. Modern treatment regimens like BEACOPP escalated or HDCT/ASCT should be used to salvage patients relapsing after a brief chemotherapy course and RT for early stage (favorable) HD.

### 024

#### PERIPHERAL T-CELL LYMPHOMA HOW UNUSUAL CAN IT BE AT PRESENTATION?

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**Introduction.** About 50% of cutaneous T-cell lymphomas fall between the plaques and patches of mycosis fungoides or erythroderma of Sezary syndrome. However, there is a large pleomorphism on presentation, inside or outside these categories. The authors present a case of peripheral T-cell lymphoma (PTCL) non otherwise specified (nos), with a singular cutaneous presentation and evolution, very well documented by several photographs. **Clinical case.** The patient is a 68 years old Caucasian male, with previous history of psoriasis (over 40 years), under topical medication and systemic corticotherapy, diabetes mellitus type II, ischemic heart disease (3 vessel compromise) with two acute coronary events in 2006, and resected prostatic adenocarcinoma 5 years ago. A few days after a facial trauma without injury, appeared a papule in the right cheek (zygomatic region), gradually becoming a nodule and then a tumour, hard and reddish, slightly oval, adherent to subcutaneous plans, irregular in surface and contour, with roughly 12cm of larger diameter (between pre-temporal region and antero-lateral region of the neck, inferior to the chin). Treatment either with antibiotics or either attempted drainage was unsuccessful. In an ultrasonography as in a CT scan of the face, it was heterogeneous and seemed to involve subcutaneous adjacent tissue. Another tumour appeared slightly later, on the pavilion of the left ear, with the same general characteristics, but round and very prominent with 4 to 5 cm larger diameter. One month after, erythematous subcutaneous nodules started to appear all over the body, especially in the head, shoulder and neck. A body CT-scan revealed exuberant plurifocal cutaneous and subcutaneous alterations that spared deeper plans, and also excluded profound lymphadenopathies or organ infiltration; also there was no bone marrow infiltration. Biopsies of several nodular lesions revealed PTCL (nos), with intense infiltration by medium sized lymphoid cells with irregular nuclei, some with scant chromatin and visible nucleoli; CD2 and CD43 diffusely positive, in less number CD3 and CD45 Ro positive, rarely CD 56 positive; B markers, CD5, CD4, CD8, CD30, TdT, TiA negative; proliferative index (Ki67) about 70% It was initiated low dose local radiotherapy with regression of facial lesions, but progressive appearance of more groups of subcutaneous and cutaneous erythematous nodules on 4 limbs and anterior thorax. After ending radiotherapy, chemotherapy was begun with CVP (cyclofosfamide 600 mg/m<sup>2</sup>, reducing cardiac toxicity). **Commentary.** PTCL represents 7% of all NHL. PTCL (nos) accounts for about 50% of all peripheral T-cell

lymphomas but due to the pleomorphism of its various subtypes only 5% are true primary cutaneous forms. Besides its rarity, its diagnosis is not easy because even CT-scans can misinterpret infiltration or swelling as inflammation or infection. Currently, the hypothesis that auto-immune diseases and psoriasis are actually involved in lymphomagenesis is not to discard. The main feature of this lymphoma, in this case, is its aggressiveness, and its poor prognostic: high IPI, CD 30<sup>+</sup> and more than 10% skin involved. There are several treatment approaches, all including radiotherapy with or without chemotherapy and retinoid analogues, with no consensus on best therapy and low rates of responses and frequent relapses, new therapeutic approaches are urgently needed to treat cutaneous PTCL.

## 025

### DIFFUSE LARGE B-CELL LYMPHOMA: MODIFIED NHL-BFM-90 PROGRAM IN ADULT PATIENTS

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**Key words.** Diffuse Large B-Cell Lymphoma, modified NHL-BFM-90 protocol, survival. **Background.** Diffuse large B-cell lymphomas (DLBCL) are a heterogeneous group of lymphoid tissue malignancies, varying in cellular content, phenotype, cytogenetics, site of presentation and outcome. **Aims.** to evaluate the efficacy of intensified induction chemotherapy regimen by NHL-BFM-90 protocol in adults with nodal presentation of Diffuse Large B-Cell Lymphoma (DLBCL), stage III-IV and stage II with bulky disease (Ann-Arbor classification). **Patients and Methods.** twenty one patients with nodal presentation of DLBCL (range, 18-66 years) participated in the study performed in Russian Hematological Research Center between January 2002 and March 2006. The diagnosis was based on World Health Organisation (WHO) classification. The stage II with bulky disease, III, IV was diagnosed in 7, 6, 8 patients respectively. All the patients had at least 1 unfavourable prognostic factor by International Prognostic Index (IPI). Extranodal involved arears (liver, bone marrow, pleura, uterus, stomach, orbita, neuroleukemia, lungs, peripheral blood) were registered in 9 (42,8%) patients at diagnosis. Bone marrow was involved in 6 patients (28,5%). Serum lactate dehydrogenase level was increased in 16 patients (76,1%). All the patients were treated with 4-6 induction courses by modified NHL-BFM-90 protocol (prephase: prednisone 30 mg/m<sup>2</sup> per os in 1-5 days, cyclophosphamide 200 mg/m<sup>2</sup> i/v in 1-5 days. Course Aa: ifosfamide 800 mg/m<sup>2</sup> i/v 1-5 days, methotrexate 1,5 g/m<sup>2</sup> i/v during 12 hours in 1 day, vincristine 2 mg i/v in 1 day, doxorubicin 25 mg/m<sup>2</sup> in 1-2 days, cytarabine 150 mg/m<sup>2</sup> i/v twice a day in 4-5 days, etoposide 100 mg/m<sup>2</sup> i/v in 4-5 days, dexamethasone 10 mg/m<sup>2</sup> per os in 1-5 days. Course Bb: cyclophosphamide 200 mg/m<sup>2</sup> i/v in 1-5 days, methotrexate 1,5 g/m<sup>2</sup> i/v during 12 hours in 1 day, vincristine 2 mg i/v in 1 day, doxorubicin 25 mg/m<sup>2</sup> in 4-5 days, dexamethasone 10 mg/m<sup>2</sup> per os in 1-5 days. If a complete remission (CR) was not achieved after 2 courses (Aa-Bb) 4 patients were treated with course C: cytarabine 2 g/m<sup>2</sup> i/v twice a day during 3 hours in 1-2 days, vincristine 2 mg i/v in 1 day, etoposide 150 mg/m<sup>2</sup> i/v during 1 hour in 3-5 days, dexamethasone 20 mg/m<sup>2</sup> per os in 1-5 days). **Results.** sixteen patients (76,1%) achieved a CR, all patients are alive in a CR (range, 4-50 months). Five patients were refracted to our treatment. Four of them had a bone marrow involvement. One of them died of disease progression, one patient was died of unknown cause, 3 are alive: 1 is alive after a sequential high-dose therapy, 2 is alive after a salvage therapy. Two and half year overall survival was 74%, two year event-free survival was 75%. So, the efficacy of modified NHL-BFM-90 protocol in adult patients with nodal presentation of DLBCL with features of unfavourable prognosis is high. In a current study we present an intermediate data about treatment regimen, which be specified during continued trial. **Conclusions.** An efficacy of modified NHL-BFM-90 protocol in unfavourable prognosis patients with stage III-IV and stage II with bulky disease (without bone marrow involvement) of DLBCL nodal presentation is high.

## 026

### FLUDARABINE ALONE OR IN COMBINATION WITH CYCLOPHOSPHAMIDE AND RITUXIMAB INDUCES APOPTOSIS *IN VITRO* IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Reduced apoptosis has a major pathogenetic role in chronic lymphocytic leukemia. In this paper a report on the additive apoptotic action of certain chemotherapeutic agents (fludarabine, cyclophosphamide, rituximab) and aspirin on the apoptosis of chronic lymphocytic leukaemia cells is given. Peripheral blood samples from CLL patients and healthy individuals were analyzed by flow cytometry using the annexin V technique, which is very sensitive for the detection of early stages of programmed cell death. 10 mmol/L of aspirin served as a positive control to the measurements. 10 µg/mL of fludarabine, 1 µg/mL of cyclophosphamide and 10 µg/mL of rituximab were tested. The highest rate of apoptosis was attained by fludarabine in CLL lymphocytes, in normal lymphocytes it acts only after a long incubation period. Significant increment in apoptotic cells<sup>TM</sup> fraction using cyclophosphamide and rituximab was not ascertained, however used together with fludarabine these drugs had an additive apoptotic effect. The combinations of fludarabine+cyclophosphamide and fludarabine+cyclophosphamide+rituximab increased the apoptosis rate of B-CLL cells. Significant correlation was showed between the ratio of apoptotic cells *in vitro* and *in vivo* effect of fludarabine alone and in the above-mentioned combinations.

## 027

### ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT) FOR RELAPSED OR REFRACTORY LYMPHOMAS. ANALYSIS OF A 4 CENTRE ACTIVITY

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**Background.** Allogeneic stem cell transplantation is a potentially curative treatment for patients with Hodgkin and non Hodgkin's Lymphomas in whom conventional therapy or autologous SCT (autoSCT) have failed. In these patients, conventional alloSCT is associated with a high transplant related mortality (TRM), particularly in the case of a previous autoSCT. Consequently, reduced intensity conditioning regimens (RIT) are usually indicated for most lymphoma patients even though the rate of disease recurrence is still high and the most appropriate conditioning regimen remains to be defined. **Aim.** A retrospective analysis was performed in order to identify prognostic factors in terms of survival, relapse and non relapse mortality as well as acute and chronic GVHD for lymphoma patients undergoing alloSCT. **Methods.** Seventy-seven patients with relapsed or refractory lymphoma, who underwent alloSCT at our institutions (Bergamo 36, Bolzano 11, Verona 20, Vicenza 10) were evaluated. The median age at transplant was 41 years (range 17-62) and the median follow-up 26 months (range 4-122). According to histotype, we divided the patients in 4 groups: indolent lymphomas (follicular and lymphocytic lymphoma or B-CLL, 20/77=26%), aggressive lymphomas (DLBCL, anaplastic, lymphoblastic, mantle cell lymphoma, NK lymphoma, 26/77=34%), Hodgkin's Lymphoma (18/77=23%) and T-Lymphomas (13/77=17%). All patients had received more than 2 lines therapy and 66% of them underwent an autoSCT. AlloSCT was performed in 2ndCR or nCR in 29/77 patients (37%); 18/77 patients (23%) were in chemo sensitive PR and 30/77 (39% 40%) had advanced disease. Twenty seven patients (35%) received a matched unrelated transplant. A myeloablative conditioning with TBI was administered in 14 patients (18%), without TBI in 16 (20%), whereas the vast majority received a reduced intensity conditioning (RIT) (47/77= 61% 62%). ATG or Alemtuzumab were part of conditioning regimen in 30



patients, who received unrelated alloSCT. *Results.* All patients engrafted. With a median follow-up of 26 months, the Overall Survival is 65% at 24 months for the whole group. Forty-three patients (56%) are in continuing complete remission. Relapse rate is 18% (14/77). After transplant, 20 of 48 patients with evidence of disease at the time of alloSCT reached CR; 9 of these patients (45%) relapsed within the first year. Relapse rate of patients in CR at transplant was 17% (5/29). Non relapse mortality at day + 100 was 14% (11/77): 8/47 in the RIT group, 3/30 in the group treated with conventional regimen. Thirteen patients died due to progression. Acute GVHD (clinical grading  $\geq$ II) was observed in 20/77 (25%) patients: in 3/20 cases the GVHD was of grade III-IV. Extensive chronic GVHD was diagnosed in 20 cases. 42 patients did not experience any GVHD. The univariate analysis showed that the overall survival significantly correlates with age at transplantation ( $\leq$ 45 years vs >45 years) ( $p=0.005$ ), chronic GVHD ( $p=0.002$ ) and disease status at transplant (CR or near CR versus no CR) ( $p=0.012$ ). OS was not significantly different among the clinical-histological groups (53% for aggressive lymphomas, 62% for Hodgkin's Lymphomas, 75% for T-NHL and indolent lymphomas, respectively). Of the 12 patients who received DLI for disease recurrence, 8 suffered rapid disease progression, 4 developed GVHD and only 3 remain alive although with active disease. *Conclusions.* AlloSCT may represent a salvage therapeutic option for high risk lymphoma patients particularly those suffering disease recurrence after autoSCT. The achievement of clinical remission before transplant and the age of patients are of crucial importance for the outcome. However more studies on larger number of patients are needed to optimize the conditioning regimen choice, the immunosuppressive therapy after transplant and to find the role of DLI, in order to counteract the high risk of relapse in the first year after HSCT.

## 028

### DISSEMINATED CITOMEGALOVIRUS INFECTION IN A PATIENT WITH NON-HODGKIN'S LYMPHOMA, PERIPHERAL T CELL, UNSPECIFIED (VARIANT-LENNERT'S)

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CMV infection is a major cause of mortality after allogeneic bone marrow transplantation (BMT). Among autologous BMT recipients, the frequency of CMV infection has been reported to be comparable to that in allogeneic BMT recipients (approximately 40-50%). However, the frequency of serious CMV disease has been reported to be considerably lower, generally ranging from 1% to 9%. Nevertheless, viral infection and subsequent disease are rarely reported in haematological diseases unrelated to these conditions. We report here the case of CMV pneumonia and disseminated disease in a patient affected by unusual form of non-Hodgkin's lymphoma (NHL): NHL peripheral T cell, unspecified, variant-Lennert's. A 41-year-old woman had been diagnosed with peripheral T-cell lymphoma unspecified (variant-Lennert's) based on biopsy findings of the cervical lymph node. Staging procedures disclosed that the disease was at clinical stage IIIa. Serological status for CMV had not been performed. She was treated with three courses of standard megaCHOP chemotherapy (Cyclophosphamide 2000 mg/m<sup>2</sup>, Doxorubicin 90 mg/m<sup>2</sup>, Vincristine 1,4 mg/m<sup>2</sup>, and prednisone 60 mg/m<sup>2</sup> by five days) alternated with three courses of ESHAP chemotherapy (Etoposide 40 mg/m<sup>2</sup> days 1-4, Cytarabine 2000 mg/m<sup>2</sup> day 5, Cisplatin 25 mg/m<sup>2</sup> days 1-4, and Prednisone 200 mg total dose, days 1-5) After six courses of the chemotherapy, size of lymph nodes was similar or bigger, and high-grade fever and constitutional symptoms appeared. Cervical lymph node biopsy was performed again which showed the same diagnostic (Lennert's lymphoma). EPOCH rescue polychemotherapy was started (cyclophosphamide, etoposide, doxorubicin and prednisone). There was not hystocompatible related donor. After 10 days post second course of EPOCH the patient was admitted to the hematology department because fever and neu-

trophenia. On admission, the blood cell count showed: hemoglobin, 11.5 g/dL; WBC, 600/mm<sup>3</sup>, with granulocytes 45%; platelets 44000/mm<sup>3</sup>. Empirical antibiotic therapy with imipenem and teicoplanin was initiated besides G-CSF (300  $\mu$ s.c/day), and 48 hours later amikacin was added. Chest X-ray was normal. Four days after the admission, fever disappeared and blood count recovered normal values. At day +15 post EPOCH, a deterioration of the overall clinical occurred with dyspnea; severe hypoxia (pO<sub>2</sub> 32 mmHg) and a chest X-ray showing bilateral interstitial opacities more evident in the right lower lobe. On the basis of this finding, ventilatory support, antiviral prophylactic therapy with aciclovir and antibiotic therapy with meropenem, teicoplanin and cotrimoxazol were started. Two days later, renal function deteriorated rapidly, with persistence of respiratory failure despite ventilatory support and the patient died. Autopsy was performed, which showed infiltration of retroperitoneal lymph nodes by medium to large-sized lymphocytes that were CD4+. The presence of cytomegalovirus (CMV) was demonstrated by immunohistochemical studies at lung, renal, pancreatic and parathyroid tissues. CMV is an important cause of morbidity and death among immunocompromised patients, and it causes a wide variety of disease manifestations, including fever, cytopenia, esophagitis, enterocolitis, hepatitis, adrenalitis, pneumonia, retinitis, encephalitis, and disseminated disease. The frequency of serious CMV diseases is especially high among patients with cell-mediated immunodeficiencies, such as bone marrow and organ transplant recipients and persons with AIDS. However, CMV infection and subsequent disease are rarely reported in hematological diseases unrelated to these conditions. The most significant risk factor for CMV pneumonia is the CMV serostatus prior to transplant, being significantly higher among patients who are seropositive than among patients who are seronegative. Other risk factors are the use of unrelated or mismatched donors, acute and chronic GVHD, CMV antigenemia, delayed lymphocyte engraftment, low CD4 counts, CMV-specific T-cell immunity and the viral load. Aggressive anti-T-cell therapy, such fludarabine or Campath, introduced also in lymphoma and leukemia patients, increases the risk for CMV disease. All these data indicate that T cells play a crucial role in controlling CMV infection and disease. Patients with T-cell lymphomas have profound immunodeficiency with diminished T-cell function and disappearance of the CD8 cytotoxic population. Peripheral T-cell lymphoma (PTCL) are non-lymphoblastic NHL of T-cell phenotype other than mycosis fungoides that represent 3-10% of all NHL. The most common entity (43% of all PTCL), is CD30+ anaplastic large cell lymphoma (ALCL), divided into classical ALCL, small cell variant of ALCL and primary cutaneous ALCL. Lennert's lymphoma represents the 30% of all PTCL and is an unusual form of nodal lymphoma morphologically defined by the presence of large numbers of epithelioid cells interspersed with medium- to large-sized lymphomas cells. Subsequent immunohistochemical studies revealed that these lymphomas possess CD3+, CD4+ and CD8- helper/inducer T-cell phenotype, although some of them could be either CD4+ or CD8+. However, Lennert's lymphoma was not recognized as a distinct clinicopathological entity in Revised European-American lymphoma (REAL) and World Health Organization (WHO) classification probably because of its ambiguous diagnostic criteria, and was categorized in *peripheral T/natural killer (NK)-cell lymphoma of unspecified type*. Originally, Lennert's lymphoma was recognized as an indolent type of peripheral T-cell lymphomas. However, a recent study analysing 108 patients with Lennert's lymphoma reported that the median overall survival is only 16 months. Especially, the prognosis of advanced disease (stage III/IV) was significantly worse 11. In our case, PTCL by itself, could cause diminished T-cell function that cause an altered CMV-specific immunity and an aggressive CMV disease with fatal outcome. Thus, clinical risk factors for CMV disease will need to be further defined in controlled trials so that prophylactic and pre-emptive strategies can be targeted rationally. These risk will need to be continually reassessed, because the acquired and cumulative immunodeficiencies of patients with hematologic malignancies are in a state of flux and they reflect changing chemotherapeutic and supportive-care regimens.



## 029

**FOLLICULAR LYMPHOMA PRESENTING AS PULMONARY LYMPHANGITIC AFFECTATION RESOLVED WITH FCM-R QUEMOTHERAPY**

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A 63-year-old female patient developed progressive enlargement of general lymph nodes. She was diagnosed as follicular non-Hodgkin lymphoma by lymph node biopsy. A body computed tomography (CT) showed multiple lymph nodes enlargement in all territories, and in the pulmonary area, CT-scan revealed thickening of the peribronchiolvascular interstitial, interlobar septa and interlobular fissures. She denied exposure to tuberculosis. On physical examination, the lungs were clear on auscultation and the patient did not complain of cough dyspnea, fever or chest pain. A pulmonary lymphangitic affection secondary to follicular lymphoma was suggested. The patient underwent chemotherapy with FCM-R treatment (fludarabine 25 mg/m<sup>2</sup>/day over 3 days, cyclophosphamide 200 mg/m<sup>2</sup>/day over 3 days, mitoxantrone 6 mg/m<sup>2</sup> 1 day, and Rituximab 375 mg/m<sup>2</sup> 1 day) 6 cycles every 21 days. After 3 cycles a complete remission was achieved with complete resolution of the thoracic-CT findings. Lymphangitic carcinomatosis, intrapulmonary spread of metastatic neoplasm via lymphatics, and adjacent connective tissue is frequently seen with lung, breasts, stomach, pancreas and prostate cancers, but a novo presentation of follicular lymphoma with extensive lymphatic spread is rare. The most often striking presenting features are progressive, unremitting dyspnea, non productive cough and tachypnea. Radiographs usually show a coarse reticulonodular pattern, which is more obvious in the lower zones, but in about half the cases, may be completely normal. CT scans show nodular thickening of the bronchovascular bundles, thickened interstitial lines, polygonal lines, and beaded interlobular septae. Bronchoscopy with transbronchial biopsy is the diagnostic procedure of choice. We have reported an atypical case of follicular lymphoma presenting as pulmonary lymphangitic affection, with atypical presentation in terms of symptoms but with typical presentation of radiographic examination of an interstitial pulmonary, that had resolved completely with FCM-R chemotherapy.

## 030

**FAVORABLE INFLUENCE OF RITUXIMAB WITH HIGH DOSE SEQUENTIAL CHEMOTHERAPY (R-HDS) PROGRAMS AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN SALVAGE TREATMENT FOR PATIENTS WITH REFRACTORY OR RELAPSED B-CELL NON-HODGKIN'S LYMPHOMA**

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The high-dose sequential (HDS) chemotherapy regimen followed by autologous stem cell transplantation (ASCT) proved to be an effective salvage therapy for patients with refractory or recurrent aggressive non-Hodgkin's lymphoma (NHL) (Cortelazzo *et al.*, Br J Haematol, 2001). The addition of rituximab to HDS (R-HDS) could enhance the sensitivity to rescue treatment, increasing the number of transplant eligible patients and improving the outcome of ASCT. To assess the role of R-HDS salvage therapy we evaluate retrospectively the clinical outcome of two consecutive cohorts of patients with indolent (n=55) or aggressive (low grade transformed=50; DLBCL=89) NHL treated with HDS chemotherapy with or without rituximab. At enrolment 135 patients (70%) were at high risk being primary refractory (primary resistant, n=33; partial responders, n=51), early relapsed (<12 months) after first line treatment (n=21) or relapsed more than twice (n=30). Eighty-six patients (44%), median age 49 years (range 18-66 years) were treated with HDS alone from 10/92 to 5/99 (group1), whereas 108

(56%), median age 53 years (range, 23-68 years) were given R-HDS from 6/99 to 11/05 (group 2). Both groups were comparable regarding adverse prognostic features such as age, histology, B symptoms, bulky disease, bone marrow infiltration, median number of previous chemotherapies, risk factors according to IPI and disease status at enrolment except for a higher prevalence of partial responders in group 1 (34% v. 20%;  $p=0.04$ ). Further, nearly one third of patients in both groups received involved field radiotherapy (IFRT) after front-line treatment. The salvage program consisted of a debulking phase of two or three cycles of either doxorubicin- or cisplatin-containing chemotherapy followed by the original HDS chemotherapy regimen (n=91): high dose (HD)-cyclophosphamide (CTX) 7 gr/sqm, HD-methotrexate 8 gr/sqm, HD-etoposide 2 gr/sqm or a modified version (n=103) in which HD-methotrexate was replaced by HD-Ara-C (2 g/sqm every 12 hours for 6 days). Rituximab (375 mg /sqm) was given twice after HD-CTX and HD-Ara-C in 108 patients (56%). After HDS chemotherapy program, a BEAM (carmustine BCNU, 300 mg/sqm; etoposide, 200 mg/sqm; Ara-C, 4000 mg/sqm; L-PAM 140 mg/sqm) or HD-mitoxantrone plus melphalan (60 and 180 mg/sqm, respectively) conditioning regimen with autologous PBSC transplantation was planned. Thirty-five patients (18%) received IFRT at the end of rescue program. After salvage chemotherapy, prior to conditioning regimen, the overall response rate (ORR) was 84% in group 1 and 94% in group 2 with 29 and 81 patients achieving complete remission (CR, 34% v 75%;  $p=0.0001$ ). After HDS 76 patients (88%) in group 1 and 97 (90%) in group 2 underwent autologous transplantation with a median number of 7.2 and 6.2  $\times 10^6$  cells CD34+/kg (range 3-18 and 2-27  $\times 10^6$  cells CD34+/kg) transplanted. At the completion of treatment, 52 patients in group 1 and 94 in group 2 achieved CR (60% v 87%;  $p=0.0001$ ). Three patients (1.5%), two in group 1 and one in group 2, died during treatment. Eventually, 55 patients (64%) in HDS group relapsed, while one patient developed a bladder carcinoma. In R-HDS group 27 patients (25%) relapsed, whereas one patient died of secondary MDS and one developed a thyroid carcinoma. With a median follow-up of 36 months (range 3-154) in group 1 and 21 months (range 1-81 months) in group 2, the 5-year estimated overall survival (OS), event-free survival (EFS) and disease-free survival (DFS) are 47%, 33%, 49% and 75%, 60%, 70%, respectively ( $p=0.0001$ ). In the Cox multivariate analysis low-grade histology ( $p=0.05$ ), HDS supplemented by Rituximab ( $p=0.04$ ) and the achievement of CR prior to auto-transplant ( $p=0.0001$ ) emerged as favourable independent prognostic factors, while bulky disease ( $p=0.01$ ) and multiple relapse ( $p=0.02$ ) were adverse prognostic factors for EFS. In conclusion, the present retrospective study shows that the addition of rituximab to HDS significantly improves the CR rate increasing the number of transplant eligible subjects and prolongs the survival of these high risk patients.

## 031

**MAINTENANCE THERAPY WITH RITUXIMAB IN GASTRIC MARGINAL ZONE NON-HODGKIN'S LYMPHOMA**Pitini V,<sup>1</sup> Altavilla G,<sup>1</sup> Buda C,<sup>1</sup> Arrigo C,<sup>1</sup> Cavallari V,<sup>2</sup> Naro C<sup>1</sup>  
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Five to 10% of primary gastric MALT Lymphomas do not show any sign of *Helicobacter pylori* (HP) infection and almost 30% of the HP-infected patients (pts.) are resistant to antibiotic therapy or experience tumor relapse after treatment. Recently, Rituximab has been reported to induce an overall response rate of around 75% with better results as first line therapy. Aim of the present study is to investigate the relevance of maintenance therapy with Rituximab in this setting. *Patients and Methods.* The study population included 9 pts. (6 men, 3 women with a median age of 61 years) with histologically proven gastric MALT lymphoma not eligible for first-line anti-HP therapy. Stage of disease at diagnosis was II in 7 pts. and IV in 2 pts. (Lugano staging system). Gastric mapping protocol was used in all pts. to obtain multiple biopsies that allowed us to monitor histology, molecular status (PCR for the IgH gene, RT-PCR for

t(11;18) and FISH analysis t(11;18), t(1;14), t(14;18), t(3;14). *Treatment.* Two cycles of Rituximab (375 mg/m<sup>2</sup>/week x 4 weeks) spaced 6 months between them followed by a single 375 mg/m<sup>2</sup> infusion every 2 months for 4 times. *Results.* After two cycles of Rituximab histologic complete (CR) and partial (PR) responses (criteria of Wotherspoon) were observed in 7 pts. (4CR+3PR). The pts. with stage IV disease at study entry obtained a stable disease. Molecular studies showed the persistence of the malignant clone in 5 pts. with histologic response (2CR+3PR). At the end of treatment, two pts. with PR achieved CR, molecular studies showed residual disease in 3 pts.; with a median follow-up of 30 months, only two pts. relapsed at 12 and 13 months respectively. *Conclusions.* In patients who have objective response with single-agent Rituximab therapy maintenance treatment improve molecular and clinical response.

### 032

#### DISSEMINATED BURKITT'S LYMPHOMA IN A PATIENT WITH CHRONICALLY RELAPSING CROHN'S DISEASE

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Several chronic inflammatory conditions are associated with an increased risk of developing malignant lymphomas. Although such risk is not documented in the whole population of patients with Crohn's disease (CD), the subset of those previously treated with immunomodulators and suffering from concomitant Epstein-Barr virus (EBV) infection seems to be associated with Burkitt's lymphoma, a mature B-cell neoplasm that in the non-endemic form often presents with small-bowel involvement. We report the case of a 48-year-old woman with relapsing ileal CD without immunosuppressive therapy or EBV infection, who developed Burkitt's lymphoma after a 24-year disease course. She had been initially treated with mesalazine, ciprofloxacin and metronidazole, but more recently underwent the surgical resection of a histologically proven inflammatory mass involving the ascending colon, ileum, right ureter and ovary. In December 2005, because of abdominal pain, rectal bleeding and severe anemia, she underwent radiological and endoscopic examinations, that showed multiple tumoral masses involving the stomach, ileum, uterus, and pelvic lymph-nodes. Gastric and bone marrow biopsy revealed the typical histological appearance of Burkitt's lymphoma (CD20+, CD10+, Bcl-6+ phenotype) with 100% proliferative rate. In addition, c-myc gene rearrangement was detected in the neoplastic population. EBV genome was not found in the neoplastic cells by means of PCR. Bone marrow trephine showed an extensive infiltration of lymphoma cells. Patient was treated using a third generation chemotherapy protocol including Rituximab, achieving only a partial response, with residual disease in the uterus. After surgical excision of the remaining pelvic mass, she was started on high-dose sequential chemotherapy as salvage regimen. She will soon undergo autologous peripheral blood stem cells transplantation. This is a case of Crohn's disease developing Burkitt's lymphoma without immunosuppressive treatment or EBV infection that suggests other possible links between the two disease entities.

### 033

#### COMPARISON OF DENDRITIC CELLS GENERATION METHODS FROM PERIPHERAL BLOOD MONOCYTES FOR IMMUNOTHERAPY OF B-CLL PATIENTS

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B-CLL, which is the most common B-cell malignancy, still remains incurable disease. Therefore, novel therapeutic strategies are very much needed. One of the most promising is immunotherapy with dendritic cells (DC). DC are professional antigen-presenting cells with a potential for inducing antitumour responses. Their frequency in peripheral blood (approx-

mately 0,1% of leukocytes) is too low for immunotherapy. So development of simple and reproducible methods for *ex vivo* production of large numbers DC is prerequisite for their widespread clinical application. It was reported previously, that B-CLL lymphocytes might impair DCs function and maturation *in vivo*, as well as in *in vitro*. Here we report the comparison of DCs generation methods in B-CLL patients. *Material and Methods.* Peripheral blood monocytes were obtained either from adherent fraction of PBMC leukapheresis product or from 150 mL peripheral blood (PB) taken by phlebotomy. After mononuclear cells isolation from PB, monocytes were enriched by immunomagnetic depletion of CD19+ B cells or by positive selection of CD14+ cells using the VarioMACS magnetic sorter. Then, precursor cells were cultured in RPMI-1640 supplemented with GM-CSF (100 ng/mL) and IL-4 (50/mL). TNF (50 mg/mL) was used to induce the final maturation of DCs. Pulsing with autologous tumour lysates was performed before maturation of DCs. DC were evaluated in FACS analysis for phenotype expression and phagocytosis function. Autologous T cell responses to DCs pulsed with tumour lysates were measured in tritiated thymidine incorporation assay. *Results.* We found no differences in the median number of DC obtained with three used approaches: 4.8x10<sup>6</sup> (leukapheresis) vs 5.0x10<sup>6</sup> (PB, CD19+ depletion), vs 5.8x10<sup>6</sup> (PB, CD14+selection). Mean viability of DCs was 93% (leukapheresis) vs 97% (PB). Mean DC purity (CD83+, CD80+, HLADR+) exceeded 90%, irrespective of the used method. The number of lymphocytes contaminating DC vaccines generated from adherent fraction of PBMC from leukapheresis product as well as from PBMC after partial CD19+ depletion was significantly higher than in vaccines gained from CD14+ positive selected cells (107.5x10<sup>6</sup> vs 42.5x10<sup>6</sup> vs 6,3x10<sup>6</sup>, res.). However no significant differences in the function concerning FITC-dextran engulfment as well as the ability of autologous DC to stimulate proliferation of T cells was found between monocytes derived DCs from B-CLL patients and healthy donors. *Conclusions.* All presented methods of autologous DC generation resulted in development of comparable amount of fully mature and functional DC. The removal of CD19+ lymphocytes allowed to receive much higher number of DCs, though the function of DCs generated in the presence of leukemic cells was not impaired, as compared to the healthy donors.

### 034

#### POTENTIAL ROLE OF MFV-LIKE VIRUSES (MFLV'S) IN LYMPHOMAGENESIS

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Micro-Foci inducing Virus (MFV) was initially isolated from a space-time association (cancer-cluster) of neuroblastoma cases and subsequently from sporadic cases of Burkitt's Lymphoma displaying typical (t(8;14)) or variant (t(2;8) or t(8;22)) translocations (MFV-like Viruses or MFLV's). Experimental models in rodents (mice/rats) show that MFV is capable of inducing remarkable tumorigenesis in newborns of infected parents, although young adults in general and the infected parents themselves appear to be immune to these neoplasms. Initial isolation, cloning and sequencing demonstrate that MFV's/MFLV's contain a dsRNA genome and belongs to the Reoviridae Family, although with variable degrees of sequence homologies. Alteration of dsRNA molecules (RNAi) was recently associated with human cancers, especially with human leukaemia and lymphoma. Genes encoding microRNA's (miRNA's, believed to be involved in development, differentiation and even malignant growth), are frequently deregulated in their expression, as well as deleted, mutated or amplified in different hematologic neoplasms. In view of our discovery of MFLV's in human paediatric lymphomas and the very large amounts of dsRNA produced in the initial acute MFLV infection (> 1000 times the amounts in mock-infected controls), it is proposed that a previous infection with MFV/MFLV causes dramatic changes in expression of RNAi/miRNA molecules, thus leading to cellular transformation and lymphomagenesis.



## 035

**FOLLOW-UP RESULTS OF IBRITUMOMAB TIUXETAN RADIOIMMUNOTHERAPY IN RELAPSED OR REFRACTORY FOLLICULAR NHL**

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Radioimmunotherapy (RIT) with Ibritumomab Tiuxetan have demonstrated efficacy in the treatment of relapsed or refractory follicular lymphoma. The therapy is well tolerated with scarce and manageable adverse events. The advantage of safe administration in outpatient regime, and the collaboration of a multidisciplinary team offer an interesting alternative to conventional therapy in relapsed NHL. We present our experience in therapy of relapsed or refractory follicular NHL treated with RIT with the same protocol in the same centre. *Patients and Methods.* 14 follicular NHL have been included in a clinical coordinate protocol of RIT during the period September 2005- July 2006. All patients were adults with histological confirmed relapsed or refractory follicular or transformed CD20+ NHL with less of 20% of B-lymphocytes in bone marrow and more than  $100 \times 10^9/L$  platelet counts. Patients received an IV infusion of Rituximab 250 mg/m<sup>2</sup> and 7 days later a second IV infusion of Rituximab 250 mg/m<sup>2</sup> followed by <sup>90</sup>Y-ibritumomab tiuxetan 0.3 mCi/kg (if platelets  $100-124 \times 10^9/L$  or 0.4 mCi/kg (if platelets  $\geq 125 \times 10^9/L$ ). *Results.* 50% females, mean age: 57.6y (37-77), in 6 patients RIT was administered as second line of therapy and in 8 as third or more. The dose was 0.4 mCi/kg in 8 patients and 0.3 mCi/kg in 6. No immediately adverse events were appeared, the haematological effects were neutropenia grade 2-3 (nadir: +4-+7 weeks; mean:  $1.1 \times 10^9/L$  range: 0.4-2.2), 4 patients need support with G-CSF, thrombocytopenia grade 3-4 (nadir: +4-+8 weeks (mean:  $70 \times 10^9/L$ ; range: 7-162) 2 patients need platelet transfusion support, only 2 patients showed mild haemoglobin decrease. The 9 valuable patients showed overall response at 12 weeks after therapy (8 CR, 1 PR). At present not relapse has been evident. *Conclusions.* RIT is effective in follicular relapsed-refractory NHL. Toxicities were primarily haematologic and reversible. It is necessary longest time of follow-up to determine long-term durable remissions and later adverse events.

## 036

**CHOP EVERY 14 DAYS IN NAIVE PATIENTS WITH DIFFUSE B-LARGE CELL LYMPHOMA**

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*Introduction.* Some studies have shown that patients with aggressive lymphoma may benefit from dose intensified schedules as CHOP-14. The addition of rituximab (R) improves response rate and survival. The support with G-CSF in dose intensification regimes may provide a good complementation of courses and advantage compared with schedules standard-dose as R-CHOP. *Purpose.* To evaluate the efficacy of R-CHOP-14 in naive patients with diffuse B- large cell lymphoma (DBLC) (REAL classification). *Design:* observational, prospective and multicentric in a consecutive and previously untreated patients diagnosed of DBLC CD20+. *Exclusion criteria:* HIV positive, other malignancies and CNS involvement. *Patients and methods:* Since June 2003 to June 2006, 56 patients were included in

an R-CHOP regimen administered every 14 days (8 courses). At baseline assessment: clinical and physical exam, blood counts, serum and urine biochemistry, albumin, 2-microglobuline and LDH level, body scan, bone marrow biopsy. Patients were classified according ECOG, clinical stage and IPI. All patients have received prophylaxis with G-CSF. Re-staging studies were performed every 4 cycles. Responses were classified as complete remission (CR), partial remission (PR), and non response (NR). *Statistical analysis:* Overall survival (OS), relapsed free survival (RFS). Survival analysis was performed using Kaplan-Meier and Cox regression. *Results:* Mean age 50.13 years (20-78), 19M/37F. ECOG 0(24), 1(18), 2(3), 3(11). B symptoms 30 patients (53.5%). IPI score 0-1(21), 2(12), 3(8), 4 (15), stage I(4), II(9), III(13), IV(30). Extranodal location 33 (59%): bone marrow 13, lung 8, liver 6, CNS 4. After 4 cycle: 50 evaluable patients; response: 50 (91%), 15 CR (27%), 35 PR (64%), 5 NR. After 8 cycle: 40 valuable patients, 38 CR (95%), 2 PR. 5 patients have relapsed and 12 died (progression 6, infection 6; > 70 years). Adverse events 187 episodes: neutropenia III-IV 107 (72%), thrombocytopenia III-IV 21(16%), infection 22 (11.7%): pneumonia 8(4.3%), neutropenia febrile 5(2.7%), gastrointestinal 5 (2.7%). Mean OS was 34.3 months and mean RFS 38.9 months. *Conclusions:* A high response rate to R-CHOP 14 in adults naive DBLC patients was observed in this study with acceptable toxicity. No differences in response were observed according to age groups but higher myelotoxicity and adverse events was present in older than seventy

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## 037

**MAINTENANCE TREATMENT WITH RITUXIMAB IN FOLLICULAR NHL**

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*Introduction.* Preceding studies have shown that Rituximab (R) prolongs relapsed free time (TFR) and response duration (RD) in follicular NHL (FNHL) when given either together with chemotherapy or alone as maintenance after therapy. The best schedule of maintenance with R still remains unknown. *Aim of study:* to evaluate the relapsed free time in FNHL patients using R as maintenance after being treated with an induction schedule that included R. *Design:* observational, prospective trial in previously patients with FL treated with chemotherapy + R in complete remission (CR). *Patients and methods.* Since January 2002 to December 2005, 23 previously treated FL grade I or II patients with one R-chemotherapy line were included. At diagnosis baseline assessment: age, gender, clinical and physical exam, blood counts, serum and urine biochemistry, albumin, 2-microglobuline and LDH level, body scan, bone marrow biopsy. Patients were classified according to ECOG, clinical stage and FLIPI. Patients in complete remission (CR) after chemotherapy received R 375 mg/m<sup>2</sup> x4 weekly every 6 months for 2 years. Re-staging studies were performed at every cycle: CR, partial remission (PR) and relapse (R). Toxicity events have been noted. *Statistical analysis.* Overall survival (OS), relapsed free survival (RFS). Survival analysis was performed using Kaplan-Meier and Cox regression. *Results.* Mean age 53.1 (35-75), 12 F/11M; ECOG 0(14), 1(8), 2(1); B symptoms 10; FLIPI score 0(2 patients), 1(10), 2(7), 3(4); stage I (2 patients), II(4), III(9), IV(8); grade I(10), II(13). *Therapy schedules:* R-CHOP (69.5%), R-CHOP+RTP (8.7%), R-CMF (17.3%), R-CF (4.3%). At present 21.7% of patients have completed maintenance for two years, 30.4% have received 3 courses, 60.8% two and 1 100%. None of them have relapsed. Adverse events: two patients were excluded because of grade 3-4 neutropenia and two referred erythema while infusion of R. OS: mean 28.2 months (10-61); RFS: mean 22 months (4-53). *Conclusions.* R maintenance seems to be effective in FL. The tolerance has been good in most of the patients and only two cases presented severe adverse events, neutropenia, in those patients who had received QTP with fludarabine+R. It is necessary a longer follow-up to consider the magnitude of the effect obtained with R maintenance.

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038

**REACTIVITY OF AN ANTI NS3-HCV-INDUCED AUTOANTIBODY IN HCV-POSITIVE GASTRIC TISSUE BY 2D GEL IMMUNOBLOT**

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**Background and Aims.** In a previous study we demonstrated an IgM cross reactivity against Fc region of human IgG and HCV-NS3 antigen in HCV-induced type II mixed cryoglobulinemia (MC). We identified NS3 and Fc epitopes recognized by these IgM by epitope excision approach. HLA determination confirms their ability to present these epitopes. The same cross reactivity has been observed with a monoclonal antibody, (mAb B3-18), obtained after immunization of a mouse with one of the identified NS3 epitopes. Previous observations indicate that HCV may localize in several tissues besides the liver (such as gastric mucosa) possibly inducing some of the extrahepatic manifestations of chronic HCV infection. The aim of this study is to characterize the mAb B3-18 reactivity by an immuno-proteomic approach. Such novel information may be of interest to identify new potential antigens implicated in HCV pathogenesis useful to design new molecules for diagnostic or therapeutic approaches. **Methods.** Proteins were extracted from 12 gastric biopsies of patients with gastric disturb and HCV-infection (one of them with a concomitant malt lymphoma of the stomach) and from 3 patients with gastric disturb but without HCV infection as control. Proteins were analysed by 2D gel electrophoresis and mAb B3-18 immunoblot. Proteins recognized by mAb B3-18 were identified by MALDI-TOF mass spectrometry. Differential protein expression levels between patients and controls were detected by 2D-DIGE. Samples were also tested by immunohistochemistry with the mAb B3-18, by VDJ-BCR and VDJ-TCR gensecan analysis and by KIR/ligands haplotypes. **Results.** MAb B3-18 is functional both in 2D gel electrophoresis and in immunohistochemistry. Two specific proteins were identified by B3-18 immunoblot in HCV-infected patients: 18 kDa antrum mucosa protein (AMP 18) and TFIZ1. Both proteins function as growth factors at least partly responsible for maintaining a functional gastric epithelium by seeking evidence of epithelial cell mitogenic activity. They are expressed and secreted in normal gastric mucosa but down-regulated in gastric cancer. These proteins, as well as some others, also showed an up-regulation in gastric mucosa from HCV-infected patients. MAb B3-18 reactivity in immunohistochemistry was restricted to gastric tissue surrounding the NHL lesion. An oligo/monoclonal B cell pattern was found in 3 patients. The characterization of KIR/ligand is in course. **Conclusions.** The use of a monoclonal antibody miming IgM autoantibodies from HCV+ patients with type II MC and a concomitant immunocytoma, is useful to identify autoantigens that could sustain HCV-related B cell proliferations and HCV infection-associated antigens.

039

**A MULTICENTER PHASE II TRIAL OF AN OUTPATIENT-BASED STRATIFIED SALVAGE APPROACH FOR ADVANCED LYMPHOMA**Spencer A,<sup>1</sup> Grigg A,<sup>2</sup> Catalano J,<sup>3</sup> Leahy M,<sup>4</sup> Underhill C,<sup>5</sup> Arthur C,<sup>6</sup> D'Rozario J,<sup>7</sup> Lowenthal R,<sup>8</sup> Taylor K<sup>9</sup>

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We have previously demonstrated significant anti-lymphoma activity using vinorelbine and gemcitabine with an overall response rate (ORR) in relapsed or refractory cases of 55%. Based on these data a larger multicentre study incorporating vinorelbine and gemcitabine with or without ifosfamide was conducted. Patients were stratified into Group 1 (G1 - first relapse, durable first remission - follicular NHL (FL) >12 months, all other NHL sub-types and Hodgkin's lymphoma (HL) >6 months); Group 2 (G2 - primary refractory, > first relapse, or non-durable first remission); or Group 3 (G3 - relapse following stem cell transplant therapy). Two regimens were evaluated. VGF (vinorelbine 25 mg/sqm days 1 and 8, gemcitabine 1000 mg/sqm days 1 and 8, pegfilgrastim 6 mg SC day 9); F-GIV (same as VGF but with ifosfamide 3000 mg/sqm day 1). G1 and G3 received VGF and G2 F-GIV. Following 2 cycles patients were re-staged. Patients demonstrating disease progression were removed from trial. Responsive patients (>50% reduction in all previous sites of disease and gallium or FDG-PET negativity) received 2 further cycles of the same therapy, the remainder *escalated* to F-GIV (G1 and G3) or IVAC (G2) (inpatient ifosfamide, VP-16 and Ara-C). Ninety patients were accrued between December 2002 and December 2004 at 10 sites (G1 = 26, G2 = 52 - 27 primary refractory, G3 = 12. Median age of 58 years (range, 17-78). Diagnoses were DLBCL = 40, FL = 19, HL = 17, PTCL = 6, others = 8. LDH was elevated in 69%, 79% and 92% of G1, G2 and G3 patients respectively. G1 and G2 have received 79 and 140 cycles of VGF and F-GIV, respectively, with cycle grades 3/4 neutropenia or thrombocytopenia occurring in 24% and 18% (VGF) and 68% and 55% (F-GIV) of patients. G3 patients have received 28 and 8 cycles of VGF and F-GIV, respectively, with no unexpected toxicities. ORR after 2 to 4 cycles of treatment was 53% (n=47). Escalation from VGF to F-GIV or from F-GIV to IVAC produced improved disease responses in 5 of 12 and 4 of 10 cases, respectively. 29 patients proceeded to stem cell transplantation. With a median follow-up for surviving patients of 30 months the estimated 3 year OS is 42% (transplant 73% vs no transplant 27%,  $p < 0.05$ ) with group specific OS of G1 55%, G2 34% and G3 47% (G1 vs G2,  $p = 0.06$ ). Disease-specific OS at 3 years were DLBCL 32%, FL 54% and HL 64% (DLBCL vs HL,  $p < 0.05$ ). We conclude that both VGF and F-GIV can be safely administered on an outpatient basis and show significant activity against a variety of advanced lymphoma types. The impact of initial or delayed therapeutic escalation beyond the standard VGF regimen appears to be modest.

040

**THE MILAN INT-REGIMEN FOR ADVANCED STAGE BURKITT'S LYMPHOMA IN CHILDREN AND ADOLESCENTS, CONSOLIDATED RESULTS**

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*Background.* We previously showed the outcome of advanced stage Burkitt's Lymphoma (BL) achieved by two consecutive single-institute trials since 1987, basing on moderately-intensive chemotherapy regimens (JCO 2002;20:2783). *Purpose.* We report on the up-dated results of the current 2-month regimen (regimen II) prospectively adopted at our Unit since 1992. *Methods.* Forty-one children with Murphy stage III-IV BL, median age 9 years (2.1-17 years) were enrolled. 80% had LDH level > 500 IU/l (67% > 1000). There were 7 girls and 34 boys. 14 had Murphy stage IV (6 had CNS involvement, 6 had bone marrow infiltration, 2 had both); 27 had stage III. Chemotherapy regimen as follows: after a 5-week cytoreductive chemotherapy consisting of VCR (1.4 mg/m<sup>2</sup> day 0 and 35), CPM (500 mg/m<sup>2</sup>

day 0 and 1), VP16 (500 mg/m<sup>2</sup> day 14), DOXO (50 mg/m<sup>2</sup> day 24), HDMTX (150 mg/Kg day 7, 250 mg/Kg day 21) and weekly intrathecal MTX or ara-C, on day 42, HDara-C (7 g/m<sup>2</sup>) plus CDDP (80 mg/m<sup>2</sup>) were given as a 4-day continuous infusion consolidation. *Results.* EFS, DFS and S at 5 years were 87.4%±5%, 89.6%±5%, and 90.1%±4% respectively, for 41 evaluable patients (median follow-up 6.5 years, range 0.5-12.1). CNS and/or BM invasion, within the limits of our small series, were overcome as negative prognostic factors (88%±6% DFS for children without BM a/o CNS, 93%±7% for those with BM a/o CNS). Four pts relapsed (at 3, 5, 6 and 27 month, respectively), of whom 3 died of disease. Actually, the late failure occurred in child who was afterwards diagnosed as having Duncan syndrome, and developed subsequent different B-cell lymphomas. One treatment-related death occurred (acute cardiomyopathy following HDara-C). Infections after the HDara-C/CDDP-related myelosuppression were always manageable. *Conclusions.* The previously reported results on our 45-day intensive chemotherapy program scheduled for disseminated BL confirmed to be of value in the setting of the current strategies for B-cell NHL. Again, survival is satisfactory for CNS+ patients too. This regimen provided patients with an high chance to survive event-free and late sequelae-free.