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# PUBLICATIONS

## PATHOLOGY AND BIOLOGY

### 288
### GENETIC INTEGRITY AND EXPRESSION OF E3 UBIQUITIN LIGASES INACTIVATING P53 AND CLINICAL COURSE OF CLL PATIENTS WITH WILD-TYPE TP53

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**Introduction:** Genetic abnormalities in the TP53 tumour suppressor pathway are major predictors of poor outcome in CLL patients. Nevertheless, a fraction of TP53 wild-type patients still experience aggressive disease. Since polyubiquitination-mediated degradation of p53 is the principal mechanism controlling the cellular amount of this protein, we hypothesized that genetic amplifications and/or overexpression of p53-specific E3 ubiquitin ligases might be associated with clinical features of the disease.

**Methods:** The study included a retrospective cohort of 117 TP53 wild-type CLL patients with available clinical information. The median follow-up was 12.3 months (range 1.1–79). Copy number analysis of MDM2, COP1, HUWE1 and PIRH2 was performed with TaqMan Copy Number Assays and CopyCaller software. The relative expression levels of MDM2, COP1, HUWE1 and PIRH2 were determined by real-time PCR with SYBR Green Dye.

**Results:** Copy number analysis revealed amplifications only for the MDM2 locus. In line with chromosomal localization of MDM2, MDM2 gains are more frequent in patients with chr.12 trisomy (9/14 patients with trisomy exhibited more than three MDM2 copies, $p < 0.001$, Fisher exact test). Nevertheless, MDM2 copy gains can occur independently of chromosome 12 trisomy, since MDM2 copy gains were present in five chr.12 diploid patients. CLL patients with MDM2 gains had significantly higher levels of MDM2 transcript in comparison with those without MDM2 amplifications ($p < 0.001$). MDM2, PIRH2 and HUWE1 expression was significantly higher in patients with advanced disease (Rai 3–4; $p = 0.02$, $p = 0.01$ and $p = 0.008$, respectively). MDM2 copy gains and increased expression of MDM2 (above median) were associated with elevated LDH levels ($p = 0.03$ and $p = 0.001$, respectively). Higher MDM2, PIRH2 and HUWE1 expression was also observed in those patients who relapsed after completion of first-line treatment during the follow-up time ($p = 0.02$, $p = 0.02$ and $p = 0.017$, respectively). Patients with PIRH2 expression above median had a lower probability of treatment-free survival (log-rank test, $p = 0.04$). Neither of the assessed parameters in the studied group was associated with lymphocytosis doubling time, freedom from progression or overall survival.

**Conclusions:** In conclusion, in patients without TP53 abnormalities, expression level and genomic integrity of E3 ubiquitin ligases MDM2, PIRH2 and HUWE1 exhibit significant associations with stage of the disease at diagnosis. Neither of these factors exhibited a significant role in determining overall and relapse-free survival, albeit given the relatively short follow-up time (median 12.3 months); further observations are needed.

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### DISSECTION OF GENOMIC ALTERATIONS IN A SERIES OF 34 MYC-REARRANGED LYMPHOMAS BY SNP ARRAY AND SEQUENCING TP53, P14ARF, ID3, AND CCND3 GENES

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**Introduction:** The deregulation of the oncogene *MYC* through chromosomal translocation is the genetic hallmark of Burkitt lymphoma (BL). It is also encountered in various lymphoproliferations, especially in DLBCLs and in lymphomas intermediate between BL and DLBCL (BL/DLBCL). It is usually associated with aggressiveness.

The *MYC* deregulation contributes to BL development but is not sufficient. Several cooperating genomic and epigenetic changes have been identified: inactivation of the p14arf/MDM2/TP53 pathway, overexpression of the microRNA cluster miR-17-92 and, more recently, constitutive activation of the ID3/TCF3/CCND3/ pathway.

**Methods:** We dissected the pattern of genomic aberrations in a well-characterized series of 34 lymphomas with *MYC* rearrangement: 26 BL (13 adults/13 children), 5 BL/DLBCL (4 adults/1 child) and 3 DLBCL (2 adults/1 child). We looked for genome-wide copy number variations (CNV) and copy-neutral loss of heterozygosity (CN-LOH), and we sequenced the whole coding sequence of *TP53* and *P14ARF* and hot spot mutations of *ID3* (exon 1) and *CCND3* (exon 6).

**Results:** The mean number of CNV and of CN-LOH varied according to the histological subtype. In BL, they were 2.0 [0–9] and 3.9 [1–10] respectively. DLBCL as well as BL/DLBCL had a higher amount of CNV: 6.7 [0–16] and 9 [4–20], respectively, but they differed in the number of CN-LOH: 6.7 [1–13] for DLBCL while 2 [1–5] for DLBCL/BL.

We detected 13q gains/amplifications in seven cases associated with a complex molecular karyotyping, mainly in children (four BL and one DLBCL) rather than in adults (two DLBCL). The minimal amplified region has been restricted to 3.72 Mb at 13q31 including the miR-17-92 cluster.

The p14arf/MDM2/TP53 pathway was mutated in 73% of BL. *TP53* (58%) and *P14ARF* (20%) were mutated in a mutually exclusive pattern in BL except for one patient. *P14ARF* mutations were only detected in BL, while *TP53* mutations were also detected in three-fourths BL/DLBCL and in one-third DLBCL.

The *ID3* mutations were nearly exclusively detected in BL (60%). Only one case out of the four BL/DLBCL showed an *ID3* mutation. *ID3* is more frequently mutated in adult BL (83%) than in children BL (38%). *ID3* mutations seemed to be associated with a poorer clinical outcome: 50% alive when mutated versus 73% without. The *ID3* mutations were more frequent in the absence of *P14ARF* mutations than in its presence, 74% vs 20%, respectively.

The *CCND3* mutations were only detected in BL (32%), three-fourths in children. Mutations of *CCND3* and/or *ID3* mutations were detected in 68% of BL: they were always associated with adult BL but not with children (three-fifths BL without ID3 mutations presented a *CCND3* mutation).

**Discussion**: Our series confirm that the screening of *ID3* and *CCND3* mutations may be useful for the distinction between BL and DLBCL and also BL/DLBCL. *CCND3* had an added value in childhood BL. The suggested adverse prognosis associated with *ID3* mutations should be confirmed on a larger series. However, it may be partially explained by the fact that *ID3* is more frequently mutated in adult BL.

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**CHROMOSOME ABNORMALITIES IN MANTLE CELL LEUKEMIA: A STUDY OF 96 CASES**

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**Introduction:** Mantle cell lymphoma typically presents as a nodal disease; however, occasional cases present in a leukemic phase with marked peripheral lymphocytosis. A variant called 'indolent mantle cell leukemia' was described in the literature characterized by mild–moderate lymphocytosis, isolated translocation of t(11;14)(q13; q32), and slow clinical progression. Sporadic publications also noted aggressive behavior in other cases of mantle cell leukemia. To better understand the nature of the disease, we analyzed a large cohort of mantle cell leukemia to determine chromosome abnormalities and their related clinical features.

**Methods:** From our database over a 2-year period, we identified 96 consecutive cases of mantle cell leukemia with t(11;14) and absolute peripheral lymphocytosis (>5000/μL). Karyotyping and/or fluorescence *in-situ* hybridization was performed on all cases. The frequencies of chromosome abnormalities in addition to t(11;14) were evaluated and analyzed with clinical data including age, gender, and complete blood count.

**Results:** There were 63 males and 33 females (male : female = 1.9:1) with a median age of 68 years (ranging from 47 to 90). Additional cytogenetic abnormalities were detected in 62 (65%) patients, including 13q− (30/96; 31%), 17p− (26/96; 27%), 11q− (21/96; 22%), 3p/3− (5/96; 5%), and trisomy 12 (4/96; 4%). Forty patients (42%) showed three or more chromosomal abnormalities. Mantle cell leukemia with complex chromosomal abnormalities correlated significantly with higher white blood cell (WBC; mean: 55 vs. 28 K/μL; $p = 0.024$) and absolute lymphocyte counts (mean: 45 vs. 19 K/μL; $p = 0.030$), and lower hemoglobin (mean: 11.3 vs. 12.4 g/dL; $p = 0.0346$), compared with mantle cell leukemia with isolated t(11;14). There were no significant differences in age, gender, and platelet count between the two groups. Mantle cell leukemia with 17p− in addition to t(11;14) also showed a trend towards lower hemoglobin (11.1 vs. 12.4 g/dL; $p = 0.1729$), although the low number of those cases with 17p− ($n = 7$) limited the statistical significance.

**Conclusions:** We identified chromosome abnormalities in addition to t(11;14) in the majority of mantle cell leukemia (65%). Deletion of 13q was most frequent, followed by 17p− and 11q−. A subset of mantle cell leukemia (42%) showed complex chromosome abnormalities. Those patients presented with higher WBC and absolute lymphocyte counts and lower hemoglobin. Our data suggest that mantle cell leukemia with complex chromosome abnormalities may behave more aggressively in contrast to the indolent variant, and the chromosomal abnormalities in addition to t(11;14) may have played a role in leukemic progression.

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**AGGRESSIVE GENE EXPRESSION SIGNATURE IN WALDENSTROM MACROGLOBULINEMIA WITH 6Q DELETION**

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**Introduction:** Waldenstrom macroglobulinemia (WM) is a rare entity of indolent B-cell lymphoma. Clinically, 6q deletion (del6q) including loss of *BLIMP-1* is reported as a one of the poor prognostic factors such as higher serum IgM level and higher risk of International Prognostic Scoring System for WM. However, it remains unclear how specific biological mechanisms contribute to the aggressiveness of WM with del6q. Thus, we conducted oligonucleotide microarray analysis to clarify the biological differences between WM with del6q and WM without del6q.

**Methods:** Archives of bone marrow samples of WM in our institute between 2010 and 2014 were utilized. To detect del6q, interphase fluorescence *in-situ* hybridization (FISH) analysis using A20/BLIMP-1/SHGC-79576 Three Color Probe (Cancer Genetics Italia®) was performed. Oligonucleotide microarray with U133 Plus 2.0 Array (Affymetrix®) were carried out. Statistical analysis and biological network analysis were performed using GeneSpring GX 13.0 software.

**Results:** A total of eight cases of WM were analyzed. Five were male, and three were female, with a median age of 71 years at diagnosis. Three of eight cases had del6q by FISH. Probe selection was performed using Welch's *t*-test ($p < 0.05$), followed by the expression level of probes of WM with del6q, which were fourfold higher than that of WM without del6q. Four hundred and twenty-eight probes were detected, and finally, gene ontology (GO) term analysis was performed ($p < 0.1$). Statistically significant GO terms including 'lymphocyte activation' and 'B-cell activation' were detected. A network analysis of GO terms regarding 'lymphocyte activation' revealed *FOXP1* was upregulated via a high expression of *BLNK* in WM with del6q. Furthermore, *IL-21R*, *CARD11*, *SYK* and *IFNγ* were also upregulated. On the other hand, the GO term 'plasma cell differentiation' was not statistically significant. Additionally, hierarchical clustering analysis failed to divide into WM with del6q and WM without del6q, which might mean that loss of *BLIMP-1* gene in WM was not due to loss of heterozygosity.

**Conclusions:** The present study revealed that *FOXP1* and *BLNK*, known as upregulated genes in the activated B-cell type of diffuse large B-cell lymphoma, had high expression levels in WM with del6q. In addition, *IL-21R* was also upregulated; IL-21R is reported to express in tumour cells in WM, and IL-21/IL-21R binding led to IgM secretion and promoted WM cell proliferation. Thus, the present result might be attributed to the aggressiveness of expression signature in WM with del6q.

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**LYMPHOMAS IN DOGS: SPONTANEOUS MODELS TO DECIPHER THE GENETICS OF LYMPHOMAGENESIS AND NEW THERAPEUTIC OPTIONS IN DOGS AND HUMANS**

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**Background and Aim:** There are over 400 genetically distinct breeds of dogs, each corresponding to a genetic isolate. The consequence of breeding practices is that most breeds naturally develop specific cancer types, reflecting the presence of predisposing alleles. This is interesting for lymphomas, as most human lymphoma subtypes are encountered in dogs, with some subtypes overrepresented in specific breeds (Pastor *et al.*, 2009; Rowell *et al.*, 2011; Marconato *et al.*, 2013). We focused on lymphoma occurring in large families of Bernese Mountain Dogs (BMDs) with the objectives to identify predisposing genetic regions and somatic alterations involved in lymphomagenesis.

**Materials and Methods:** We collected blood and tumour samples, clinical and genealogical information of affected BMDs, using the French CaniDNA biobank at CNRS, Rennes. Histopathological diagnosis was performed from formalin-fixed paraffin-embedded tumour samples by veterinary pathologists F. N. and J. A. DNA and RNA were extracted using Macherey-Nagel® kits. Dog DNAs were genotyped on the canine Illumina®, and 170 000 single-nucleotide polymorphism (SNP) arrays and RNA of matched tumour and healthy tissues were sequenced (RNAseq) using Illumina technology, by the GIGA genomic facility, University of Liege. Data were analysed by the software CRAC/CHIMCT (Philippe *et al.*, 2013) to identify translocations. The fusion point was validated on tumour DNA and cDNA, by Sanger sequencing.

**Results:** First, we demonstrated a familial segregation of lymphoma in a pedigree of BMDs. We performed a genome-wide association study using 63 lymphoma cases and 164 controls with the high-density SNP chips. Preliminary data showed a significant locus, associated with predisposition to lymphoma, on canine chromosome CFA 23. This locus does not correspond to any known locus for human lymphomas and thus represents an interesting candidate region. Second, while many chromosomal translocations are known to drive lymphomagenesis in humans, only little genetic data are available in dogs. We thus investigated somatic alterations through RNAseq on five lymphoma-affected dogs and identified a relevant translocation

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involving an immunoglobulin gene and a gene involved in cell cycle. Interestingly, this gene is known to be involved in translocations in human lymphomas, showing that similar genes are involved in both human and dog lymphomas.

**Conclusions:** Based on the collection of different types of canine lymphomas in several predisposed breeds, we first identified a potential novel locus predisposing to lymphomas. We are exploring and reducing the region of interest by using other canine breeds. Second, we discovered a novel chromosomal translocation in dogs, homologous to a translocation in the same human lymphoma type. These data clearly showed that the same key pathways are involved in lymphomas in dogs and humans, justifying the setting up of clinical trials in dogs for the benefit of both humans and dogs.

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## VDJH USAGE IN TRANSFORMED FOLLICULAR LYMPHOMA

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**Introduction:** Follicular lymphoma (FL) is the second most frequent non-Hodgkin lymphoma (NHL) in Western countries. Transformation into aggressive lymphoma continues being one of the worst events in the disease history. There is a growing interest in connecting new approaches to the biological basis of transformation and clinical application. *IGH* gene rearrangements in B lymphoproliferative disorders have identified the preferential use of certain *VDJH* gene segments, correlated in some cases with clinical prognosis. In this aspect, transformed FL (tFL) has not been yet studied.

We aimed to study the *IGHV* gene usage and clinical features in patients diagnosed with tFL and to compare with FLs that do not undergo transformation, as well as with diffuse large B-cell lymphoma (DLBCL), classified as GCB and non-GCB by immunostaining according to Hans algorithm.

**Methods:** A total of 213 NHL patients from a single centre were included in the study, distributed into three groups: (i) tFL patients ($n = 32$), most of them with paired samples at diagnosis and transformation ($n = 25$, 78%); (ii) FL with no documented transformation at a median follow-up of 66 months ($n = 74$); and (iii) DLBCL patients ($n = 107$; 44% GCB and 56% non-GCB). Clonal IGH rearrangements were amplified according to the BIOMED-2 protocol, and PCR products were sequenced. Germline *IGH* genes were identified using the IMGT/V-QUEST database. Statistical comparisons were performed using SPSS 20.0.

**Results:** Complete *VDJH* rearrangements were identified in 31 out of 32 tFL patients (97%). In addition, other cases were discarded due to the identification of a different clone, and it was recorded as a secondary NHL. A bias in the *IGHV* gene usage was observed in tFL patients, with *IGHV3-23* (20%), *IGHV3-48* (17%) and *IGHV4-34* (17%) genes accounting for 54% of the cohort. These genes are common in both FL and DLBCL. However, and despite differences being not statistically significant, a higher frequency of *IGHV3-48* was shown in tFL than in DLBCL (22% vs 6%), while *IGHV4-34* showed a higher frequency in tFL than in FL (19% vs 8%). Moreover, *IGHV4-34* was found only in the non-GCB DLBCL subgroup. Regarding the clinical outcome, the average time to transformation of tFL is 54 months (range 4–222 months). Interestingly, we found a longer time to transformation in patients with *IGHV4-34* as compared with patients using other *IGHV* (99 vs 44 months, $p = 0.023$). Finally, and interestingly, only two patients have <2% SHM, and both cases were composite of FL + DLBCL at diagnosis.

**Conclusions**: Clonality analysis is necessary to discriminate secondary NHL rather than tFL. *IGHV* in tFL gene usage is biased, similar to those of DLBCL and FL, as expected. Patients using *IGHV4-34* showed longer time to transformation than those using other *IGHV*s. This study should be considered as preliminary, requiring larger and homogeneous cohorts.

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## EXTRAFOLLICULAR PD-1 AND INTRAFOLLICULAR CD3 EXPRESSION ARE ASSOCIATED WITH SURVIVAL IN FOLLICULAR LYMPHOMA

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**Introduction:** Both microenvironment and tumour biomarkers may impact outcome in follicular lymphoma (FL). The prognostic significance of PD-1 expression in FL is debated. Nevertheless, a recent study has shown that a combination of an anti-PD-1 antibody and rituximab is effective in patients with relapsed FL. Although gene expression profiles of T cells and macrophages have been shown to predict outcome, the significance of CD3 and CD68 expression, as determined by immunohistochemistry (IHC), in FL is not clear. The importance of Ki-67 is also not well established. We aimed to study the impact of PD-1, CD68, Ki-67 and CD3 expression on outcome of patients with FL.

**Methods:** Forty-eight biopsies, performed at diagnosis, were reviewed. Available stained slides (Ki-67 and CD3) were reevaluated, and additional slides were stained for CD68 and PD-1. Expression of the different markers was correlated with clinical outcome.

**Results:** Twenty-five males and 23 females with available tissue from diagnosis were included. Twenty-seven were treated upfront with R-chemotherapy. Median age was 61, and median follow-up was 3.7 years. Thirty-three per cent had an early-stage disease, and 67% had an advanced-stage disease. FLIPI was high, intermediate and low in 21 (44%), 9 (19%) and 18 (37%) patients, respectively. Five-year overall survival (OS) and progression-free survival (PFS) of the entire cohort were 96% and 49%, respectively. Intrafollicular PD-1 expression was 0–45% (median 25%) while extrafollicular expression was 0–35% (median 10%). CD68 expression was 5–30% (median 15%) intrafollicular and 0–25% (median 10%) extrafollicular. Ki-67 expression was 0–45% (median 20%) intrafollicular and 0–20% (median 5%) extrafollicular. The intrafollicular CD3 staining level was 10–50% (median 25%), not assessed in the extrafollicular zone due to the abundance of T cells. The median values served as a cut-off point for low-expression and high-expression groups. High extrafollicular PD-1 expression predicted superior PFS compared to low expression (5-year PFS 52% vs 44%, $p = 0.04$). Five-year PFS markedly increased from 37% to 67% ($p = 0.057$) in patients with low intrafollicular CD3 expression. None of the other IHC biomarkers predicted PFS or OS. In a multivariate analysis, both intrafollicular CD3 and extrafollicular PD-1 expression significantly predicted PFS ($p = 0.03$ and $p = 0.015$, respectively).

**Conclusions:** Our data indicate that expression of extrafollicular PD-1 and intrafollicular CD3 correlates with prognosis in FL. This supports the hypothesis that survival in FL depends on immunologic crosstalk between malignant cells and the microenvironment. However, the specific types of T cells that influence the clinical behaviour of FL are still unknown. The prognostic significance of various biomarkers in FL warrants further investigation in prospective studies.

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## LEVEL OF PERIPHERAL BLOOD REGULATORY T CELLS IN NEWLY DIAGNOSED LYMPHOMA PATIENTS: IS THERE ANY CORRELATION WITH CLINICAL CHARACTERISTICS?

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**Introduction:** Lymphoma is a malignant tumour originating from lymph nodes and/or extranodal lymphoid tissue. Since lymphoma occurs in immune cells, tissues and organs, patients with immune defects tend to have increased risk for lymphoma. Lymphoma occurrence is also considered to be related with autoimmune status. In recent years, the role of regulatory T cells (Tregs) has stimulated a lot of interest as a possible cause of immunosuppression. Tregs play a role in the control of autoimmunity and transplantation rejection but may also inhibit effective anti-tumour immune responses. The relationship between Tregs and lymphoma is still controversial. In this study, our purpose was to assess the peripheral blood levels of CD4+, CD25+ and

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FOXP3+ Tregs before and after treatment in newly diagnosed patients with lymphoma and find a relationship between Treg levels and clinical characteristics.

**Methods:** The percentages of Tregs were evaluated by flow cytometry in the peripheral blood of newly diagnosed Hodgkin lymphoma (HL) patients ($n = 21$), non-HL (NHL) patients ($n = 40$) and healthy controls ($n = 30$). All NHL patients showed diffuse large B-cell lymphoma immunophenotypes.

**Results:** The median (Q1–Q3) Treg levels were 3.79% (2.31–5.29%), 4.61% (2.5–8.28%) and 3.57% (2.47–4.35%) in HL, NHL and control groups, respectively. The peripheral blood level of Tregs in HL and NHL patients was higher than that in healthy controls, but the difference was not statistically significant ($p > 0.05$). The Treg level of HL patients was positively correlated with IPS, CRP and LDH and negatively correlated with albumin and absolute lymphocyte count. Tregs were found significantly higher in male patients ($p = 0.035$) and smokers ($p = 0.044$) in the HL group. The Treg level of NHL patients was positively correlated with stage, IPS, CRP and LDH and negatively correlated with albumin, absolute lymphocyte count and survival. The difference between pre-treatment, interim and post-treatment Treg levels was not statistically significant in both HL ($p = 0.09$) and NHL ($p = 0.073$) groups.

**Conclusions:** We could not find a statistically significant difference between peripheral blood Treg levels of lymphoma patients and healthy controls. However, the relationship between Treg levels and clinical and laboratory poor prognostic parameters supported the immunosuppressive effect of Tregs in lymphoma. Further larger studies are needed to assess the exact role of Tregs in lymphoma. Concurrent intratumoural and peripheral blood evaluation and functional assessment can add new insights to current lymphoma treatment strategies.

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## CHARACTERIZATION OF THE FLOWCYTOMETRIC IMMUNOPHENOTYPE OF NATURAL KILLER LYMPHOCYTE DISORDERS

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**Introduction:** The flowcytometric (FC) phenotype of extranodal natural killer (NK) T-cell lymphoma (NKTCL) and chronic lymphoproliferative disorder of NK cells (CLPD-NK) is not well described. In a validation study of the EuroFlow eight-colour CLPD-NK panel, the NK phenotype of 10 healthy donors differed from that of three patients with clonal NK cells. The distinguishing markers were CD56, HLADR, CD57 and CD94. To our knowledge, ours is the first study to compare the FC phenotype of NKTCL, CLPD-NK and reactive NK lymphocytosis (RNKL) using eight-colour FC.

**Methods:** Patient samples analysed using the eight-colour CLPD-NK panel between 2011 and 2014 were identified from our FC database. The diagnosis, FC phenotype, EBV status and clinical data were retrieved. T cells were used as internal controls for antigen expression.

**Results:** *NK Specific Markers*: Eight NKTCL were CD56 bright, all CLPD-NK and RNKL were positive or dim. Six NKTCL were CD16 dim or negative while four CLPD-NK and six RNKL were positive.

*Markers not normally expressed on CD56+ NK cells*: Three NKTCL cases were HLADR bright or positive, only one CLPD-NK case was positive and all RNKLs were negative. CD25 was positive or dim in four NKTCL while all the CLPD-NK and RNKL cases were negative. Six NKTCL and two CLPD NK cases were CD94 positive, and only one RNKL was CD94 positive, the others being dim. CD26 was bright or positive in seven NKTCL while all the RNKL and five CLPD-NK cases were negative. Seven NKTCL, three CLPD-NK and three RNKL cases were CD2 positive. All NKTCL and RNKL cases were CD5 negative, but three CLPD-NK were positive.

*Markers of cytotoxic effector phenotype*: CD57 was negative in nine NKTCL, but positive in three CLPD-NK and 2 RNKL cases. CyPerforin was negative in eight NKTCL and positive in three CLPD-NK and four RNKL cases. Granzyme B was bright or positive in five NKTCL, three CLPD-NK and four RNKL cases.

**Conclusion:** We propose that the typical FC phenotype for NKTCL is CD56 bright, CD 16 dim with positive CD2, CD7, CD94, HLADR, CD25, CD26 and granzyme B with absent CD57.

This resembles the phenotype of the CD56 bright immunoregulatory NK cell subtype that normally comprises <1% of NK cells in healthy individuals. We hypothesize that NKTCL arises from this CD56 bright subpopulation.

**Abs 296 - Table 1.**

| | NKTCL ($n = 10$) | CLPD-NK cells ($n = 4$) | Reactive NK lymphocytosis ($n = 6$) |
|---|---|---|---|
| NK-specific markers (NK cells identified as CD45+/sm CD3−/CD19−/CD56+/dim) | | | |
| CD56 (Pe-Cy7) | 8 bright, 1 pos, 1 dim, 0 neg | 1 bright, 2 pos, 1 dim, 0 neg | 0 bright, 4 pos, 2 dim, 0 neg |
| CD16 (PB) | 0 bright, 2 pos, 3 dim, 5 neg | 1 bright, 3 pos, 0 dim, 0 neg | 0 bright, 6 pos, 0 dim, 0 neg |
| Aberrant markers | | | |
| CD2 (PB) | 1 bright, 8 pos, 0 dim, 1 neg | 0 bright, 3 pos, 1 dim, 0 neg | 0 bright, 3 pos, 2 dim, 1 neg |
| CD7 (FITC) | 1 bright, 3 pos, 2 dim, 4 neg | 0 bright, 2 pos, 0 dim, 1 neg | 0 bright, 6 pos, 0 dim, 0 neg |
| CD94 (APC) | 1 bright, 6 pos, 1 dim, 2 neg | 0 bright, 2 pos, 2 dim, 0 neg | 0 bright, 1 pos, 5 dim, 0 neg |
| HLADR (PB) | 3 bright, 2 pos, 1 dim, 4 neg | 0 bright, 1 pos, 3 dim, 0 neg | 0 bright, 0 pos, 0 dim, 6 neg |
| CD5 (APC) | 0 bright, 0 pos, 0 dim, 10 neg | 0 bright, 3 pos, 1 dim, 0 neg | 0 bright, 0 pos, 1 dim, 5 neg |
| CD25 (PE) | 0 bright, 2 pos, 2 dim, 6 neg | 0 bright, 0 pos, 0 dim, 4 neg | 0 bright, 0 pos, 0 dim, 6 neg |
| CD26 (PE) | 1 bright, 6 pos, 1 dim, 2 neg | 0 bright, 0 pos, 1 dim, 3 neg | 0 bright, 0 pos, 0 dim, 6 neg |
| Markers of cytotoxic effector phenotype | | | |
| CD57 (FITC) | 0 bright, 1 pos, 0 dim, 9 neg | 0 bright, 3 pos, 1 dim, 0 neg | 0 bright, 2 pos, 3 dim, 1 neg |
| CD11c (APC) | 1 bright, 3 pos, 2 dim, 4 neg | 0 bright, 2 pos, 1 dim, 0 neg | 0 bright, 4 pos, 1 dim, 1 neg |
| CyPerforin (FITC) | 0 bright, 2 pos, 0 dim, 8 neg | 0 bright, 3 pos, 0 dim, 1 neg | 0 bright, 4 pos, 0 dim, 2 neg |
| CyGranzyme B (PE) | 2 bright, 3 pos, 0 dim, 5 neg | 0 bright, 3 pos, 0 dim, 1 neg | 0 bright, 4 pos, 0 dim, 2 neg |
| Clinical data | | | |
| Clinical presentation | 8 fever, 1 skin lesions, 1 unknown | 1 incidental lymphocytosis, 1 lymphocytosis and thrombocytopaenia, 2 unknown | 1 PMBCL, 1 iron deficiency, 2 fever, 2 unknown |
| EBV PCR | 8 pos, 1 neg, 1 not done | 2 not done, 2 unknown | 4 not done, 2 unknown |
| Outcome | 8 dead, 2 unknown | 1 dead, 3 not known | 3 alive, 1 dead, 2 unknown |

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The distinguishing features of CLPD-NK are CD16 and CD5 positivity with weaker CD56 expression than NKTCL and absent HLADR, CD25 and CD26. RNKL is distinguished from NKTCL by the expression of CD16 and the negativity for HLADR, CD26 and CD25. RNKL can be distinguished from CLPD by the absence of CD5.

### 297
### HODGKIN LYMPHOMA PATIENTS WITH LOW NUMBER OF FOXP3 LYMPHOCYTES IN TUMOUR TISSUE—RISK PROFILE AND TREATMENT OUTCOME

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**Introduction:** The contemporary approach to treatment decisions in Hodgkin lymphoma (HL) implies that patients should be stratified into certain risk groups based on clinical stage and risk factors before selecting the appropriate therapy. Recently, a number of studies with the aim to identify reliable prognostic biomarkers for HL were performed. Several studies reported negative prognostic impact of a low number of *FOXP3* lymphocytes in tumour tissue.

**Methods:** The patients in this retrospective study were selected between the newly diagnosed ABVD-treated classical HL patients in the period 2000–2008. The ROC curve analysis determined 20 as the most appropriate cut-off value for number of *FOXP3* lymphocytes in high-power field (HPF). The analysis was performed on 42 patients with a low number (fewer than or equal to 20) of *FOXP3*/HPF. The examined parameters in survival and multivariate analysis were the presence of bulky disease, B symptoms, erythrocyte sedimentation rate $\geq 50$ mm/h, elevated lactate dehydrogenase, a high number of CD68 macrophages/HPF and a high International Prognostic Score (IPS) (3–7).

**Results:** The median follow-up was 70 months (range 2–165 months). The median age was 31 years (range 17–84). Five-year overall survival (OS) was 59.5%, and 5-year event-free survival (EFS) was 45.2%. In univariate analysis, patients with a high number of CD68 macrophages/HPF and high IPS had significantly shorter OS (5-year OS 50% vs 90%, $p = 0.0028$; 5-year OS 47.4% vs 69.6%, $p = 0.049$; respectively). None of the analysed parameters resulted in shorter EFS. Multivariate analysis identified a high number of CD68 macrophages/HPF as the independent risk factor for poor OS.

**Conclusion:** The association of a low number of FOXP3 lymphocytes and a high number of CD68 macrophages in tumour tissue resulted in poor outcome. The further stratification in high-risk groups of HL patients could be potentially useful for selecting the adequate treatment approach. Still, these have to be confirmed through randomized clinical trials.

### 298
### MAST CELLS ARE ABUNDANT IN CUTANEOUS T-CELL LYMPHOMAS AND CORRELATE WITH DISEASE STAGE

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**Introduction:** Mast cells are bone marrow-derived haematopoietic cells playing a crucial role not only in allergy and antimicrobial defence but also in tumour microenvironments with protumourigenic and antitumourigenic functions. Only little data are available on the role of mast cells in primary cutaneous T-cell lymphomas (CTCL), a heterogeneous group of non-Hodgkin lymphomas with initial presentation in the skin. The purpose of this study was to quantify the distribution of mast cells in CTCL variants and clinical stages.

**Methods:** Immunohistochemistry with a monoclonal anti-mast cell tryptase antibody was performed on formalin-fixed, paraffin-embedded biopsies of 40 patients with different CTCL variants and on control skin samples. Slides were scanned with a TissueFAXS200 Cytometer (TissueGnostics GmbH, Vienna), and dermal mast cells were quantified using HistoQuest 4.0 based on tryptase staining segmentation in selected regions of interest.

**Results:** Mast cells were detected in 37 out of 40 cases. In CTCL, mast cell density was higher in areas with tumour infiltration than in surrounding dermis. The number of mast cells was higher in CTCL (median 212, range 4–1089 mast cells/mm$^2$) compared to normal skin (median 44, range 26–65 mast cells/mm$^2$) and inflammatory skin conditions (median 111, range 89–149 mast cells/mm$^2$). In MF, advanced stages (IIB–IVA) showed higher mast cell counts (median 219, range 36–1089 mast cells/mm$^2$) than early-stage disease (IA and IB; median 149, range 4–647 mast cells/mm$^2$). With the application of image segmentation methods for mast cell quantification on whole-slide digitized sections, allowing reproducible and unbiased cell identification, our results strongly implicate a contribution of mast cells to the pathophysiology of CTCL and provide an initial basis for further research on their use as target for therapeutic intervention.

### 299
### EPSTEIN–BARR VIRUS-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA PREDICT POOR OUTCOME, REGARDLESS OF AGE

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**Introduction:** Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) is defined as patients older than 50 years alone. However, recent study showed young patients with sound immune status could also be affected.

**Methods:** In this study, we performed immunohistochemistry, *in situ* hybridization and fluorescence *in situ* hybridization in a cohort of 250 cases of DLBCL patients who were treated with R-CHOP-based therapies. We investigated the clinical features and outcome of patients with EBV-positive DLBCL in the different age groups.

**Results:** The prevalence of EBER positivity was 11.4% (19/166) and 8.3% (7/84) in the elderly and young groups, respectively. No significant difference of incidence was observed between the two groups ($p = 0.466$). The EBV-positive patients shared many unfavorable prognostic characteristics, regardless of age group. EBV-positive patients, in both the elderly and young groups, showed significantly worse overall survival (OS) (median OS, elderly group: 37.0 months vs. not reached, $p = 0.0337$; young group: 36.5 months vs. not reached, $p < 0.0001$) and progression-free survival (PFS) (median PFS: elderly group: 20.7 months vs. not reached, $p < 0.0001$; young group: 20.5 months vs. not reached, $p = 0.0010$) than negative cases.

**Conclusions:** Since EBV-positive DLBCL patients, regardless of age, shared similar poor prognostic features and showed worse outcome than negative cases, we suggest that the age criterion of EBV-positive DLBCL, and possibly the name itself, be modified in the future.

### 300
### EBV POSITIVITY HAS NEGATIVE CLINICAL IMPACT ON GERMINAL CENTER CELL ORIGIN IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** Epstein–Barr virus (EBV) positivity is an independent adverse factor for survival among elderly patients with non-Hodgkin lymphoma (NHL). EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly is defined as a histologically malignant B-cell lymphoproliferation in patients older than 50 years of age. Recently, EBV viral load in peripheral blood (PB), by real-time quantitative polymerase chain reaction (RQ-PCR), was accepted as a useful biomarker for monitoring prognosis in EBV-related NHL. In the current study, we quantified EBV viral load by RQ-PCR using whole PB samples and defined the prognostic impacts of EBV infection for R-CHOP chemotherapy in patients with DLBCL.

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**Methods:** From March 2007 to October 2013, 87 DLBCL patients who completed at least the first cycle of R-CHOP combination and underwent EBV RQ-PCR testing at diagnosis were enrolled. At diagnosis, all patients' EBV-DNA that were isolated from PB samples by manual extraction using QIAamp DNA blood Mini-Kits (Qiagen, Hilden, Germany) were examined, and RQ-PCR was performed using an ABI PRISM 7500 system. Results were expressed in copies per milliliter of total EBV DNA calculated using a standard curve.

**Results:** The EBV DNA was detected in 21 (24.1%) cases of the 87 evaluated cases. Patients who are EBV-DNA positive were more frequently male (76.2% vs. 51.5%) and trend toward having more B symptoms than those who are EBV-DNA negative. After a median follow-up duration of 32 months, estimated 3-year OS rate was significantly poorer in patients who are EBV-DNA positive compared to those who are EBV-DNA negative (93.6% ± 3.7% vs. 79.6% ± 9.2%, $p = 0.001$). EBV-DNA positivity did not markedly influence PFS or OS in patients younger than 65 years, while the estimated 3-year PFS (46.9% ± 18.7% vs. 63.5% ± 10.5%, $p = 0.025$) and OS (66.7% ± 15.7% vs. 95.2% ± 4.6%, $p < 0.001$) rates for those who are EBV-DNA positive were poorer in patients aged 65 years and older. When we analyzed survival according to histological subtype, in GCB subtype, EBV-DNA positivity had an adverse impact on the estimated 3-year PFS (31.3% ± 17.8% vs. 74.6% ± 12.8%, $p = 0.022$) and OS rates (80.0% ± 17.9% vs. 100%, $p = 0.050$), while EBV-DNA positivity did not influence PFS and OS in non-GCB DLBCL.

**Conclusions:** The present study suggests that EBV-DNA-positive DLBCL patients pursue a worse clinical course and have poorer survival when they are aged ≥65 years and, especially, in the GCB subtype.

### 301
### EXPRESSION OF THE EPSTEIN–BARR VIRUS LMP-1 PROTEIN IN GREEK PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA: FREQUENCY, CLINICAL AND LABORATORY CORRELATIONS AND PROGNOSTIC VALUE

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**Introduction:** Epstein–Barr virus (EBV) has a role in the pathogenesis in a proportion of patients with classical Hodgkin lymphoma (cHL), which is low in developed countries but considerably high in the developing ones. Different studies support an unfavourable or favourable or no prognostic significance of EBV expression in cHL. The analysis of data coming from specific geographic regions is of great interest, not only due to the differing frequency of EBV expression but also for its possible prognostic value. Additionally, no such large-scale study has yet taken place in Greece. We aimed to evaluate the frequency, clinical and laboratory correlations and prognostic significance of EBV expression in a large series of patients with cHL in Greece.

**Methods:** Two hundred seventy-five patients with cHL who received chemotherapy with ABVD or equivalent combinations with or without radiotherapy were evaluated with respect to progression-free survival (PFS) and overall survival (OS). EBV positivity was defined as immunohistochemical expression of the LMP-1 protein.

**Results:** LMP-1 was detected in 82/275 patients (29.8%). The expression was more frequent in patients ≥45 years (48% vs 25%, $p = 0.001$), in males (41% vs 19%, $p < 0.001$), and in mixed cellularity (53% vs 23% in nodular sclerosis and 22% in lymphocyte-rich cHL, $p = 0.001$), while it was less frequent in patients with leukocytosis (19% vs 40%, $p < 0.001$), possibly due to the increased frequency of leukocytosis in nodular sclerosing cHL. On the contrary, no correlation with disease stage, B symptoms or other parameters related to tumour burden was observed. Five-year PFS was 89% and 81% for LMP-1+ and LMP-1− patients, respectively, while 10-year PFS was 82% vs 80% ($p = 0.27$). The corresponding percentages of 10-year OS were 84% and 89% ($p = 0.53$). No effect of EBV expression on the outcome was observed when the analysis was restricted to different age-defined subgroups.

**Conclusions:** LMP-1 was expressed in ~30% of patients with cHL in this series of 275 Greek non-paediatric patients. The correlations with male gender, higher age and mixed cellularity subtype are in agreement with the literature. No correlation between EBV expression and outcome of patients with cHL was observed in Greece.

### 302
### DIFFERENTIAL EXPRESSED PROTEINS AT HIV DIAGNOSIS IDENTIFY PATIENTS WITH SUBSEQUENT NO, BENIGN, OR MALIGNANT LYMPHADENOPATHY

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**Introduction:** Despite highly active antiretroviral treatment (HAART), patients infected by the human immunodeficiency virus (HIV) are still at increased risk to develop malignant lymphoma. Their response to lymphoma treatment is also somewhat poorer than that of HIV-negative patients. Thus, the identification of early markers able to identify HIV-positive individuals at risk for later developing a malignant lymphoma would be a useful tool for a more risk-adapted infection management. Based on proteomic analysis, the aim of our study was to search for markers associated with later lymphoma development by comparing protein expression patterns in serum samples obtained at the time of HIV diagnosis from the following: (i) patients with no subsequent malignancy; (ii) patients with subsequent benign lymphadenopathy; and (iii) patients with subsequent malignant lymphoma.

**Methods:** Archival frozen serum samples from 21 HIV-positive individuals were analyzed. All samples were collected at HIV diagnosis. Seven of these patients did not show signs of either benign lymphadenopathy or malignant lymphoma (range: 9.9–28.3 years). Another seven patients showed benign lymphadenopathy (range: 4.9–16.2 years), and a further seven presented with malignant lymphoma (classical Hodgkin lymphoma: nodular sclerosis, $n = 2$; diffuse large B-cell lymphoma, $n = 5$; range: 0.1–27.0 years). Clinicopathological features were obtained from the Danish lymphoma registry and from patient records. Patients were matched for HIV subtype, gender, age, and treatment period (HAART vs. pre-HAART periods). Serum samples were enriched for low abundant proteins and subjected to high-resolution two-dimensional gel electrophoresis. Individual protein spots were visualized with fluorescence staining, and the expression profiles in the cohort were compared. Differentially expressed (twofold or higher, Mann–Whitney $U$-test, $p < 0.05$) proteins were identified by liquid chromatography–tandem mass spectrometry.

**Results:** The protein expression profiles of the three patient subsets showed significant and distinct differences. Fourteen differentially expressed protein spots were detected; for example, complement factor H-related protein 1 was found to be upregulated in the subcohort of HIV− patients with subsequent malignant lymphomas (fold change: 2.11; $p = 0.035$) whereas vitronectin was found to be upregulated in the HIV− patients with no subsequent malignancy (fold change: 4.17; $p = 0.013$). The identified proteins are currently under characterization and will be further investigated by immunohistochemical methods on a larger tissue microarray-based validation set and correlated to clinical parameters and outcome.

**Conclusions:** Proteomic analysis revealed differentially expressed protein markers in HIV-positive patients associated with subsequent presentation of lymphadenopathy or malignant lymphoma. Further investigation of their potential clinical usefulness as risk stratification for HIV-positive individuals with regard to subsequent presentation of malignant lymphoma is warranted.

### 303
### INITIAL C-MYC EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMAS DOES NOT AFFECT OUTCOMES AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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**Introduction:** C-myc expression in diffuse large B-cell lymphoma (DLBCL) is associated with an unfavourable prognosis with poor progression-free survival and overall survival. The degree of C-myc expression by immunohistochemistry has been associated with poorer outcomes. BCL-2 and/or BCL-6 co-expression have also demonstrated inferior prognosis.

Autologous stem cell transplantation has been known to improve the prognosis of aggressive NHL in second remission. Consolidation with stem cell transplantation after initial treatment with chemotherapy may also improve outcomes.

In patients diagnosed with DLBCL, the correlation between the degree of C-myc expression by immunohistochemistry on diagnostic biopsies, as well as BCL-2 and BCL-6 co-expression, and outcomes after autologous stem cell transplantation is undefined.

**Methods:** We conducted a retrospective analysis of all patients >18 years of age, diagnosed with C-myc-positive DLBCL by immunohistochemistry on tissue biopsies and received an autologous stem cell transplant at CR1 or CR2 from 2003 to 2014 at the Royal North Shore Hospital, Sydney, Australia. Immunohistochemistry results for C-myc (graded according to percentage, 40–80%), BCL-2 and BCL-6 on diagnostic biopsies were collected for all patients. Progression-free survival was defined as time from autologous stem cell transplant to time of relapse. Disease-free survival analysis and overall survival analysis were performed by Mantel–Cox test, and survival was estimated according to the method of Kaplan and Meier.

**Results:** A total of 40 patients were analysed. Median age was 65 years old (range 30–71 years old). Thirty-five per cent of patients were diagnosed with relapsed disease after autologous stem cell transplantation with a median time to relapse of 1.13 years. Thirty-two per cent of patients died after autologous stem cell transplantation with a median time to death of 1 year. Median follow-up from autologous transplant was 2.4 years. There was no correlation between the degree of C-myc expression and disease-free survival (C-myc 40%: $p = 0.12$, 50%: $p = 0.41$, 60%: $p = 0.67$, 70%: $p = 0.79$ or 80%: $p = 0.66$). There was no correlation between the degree of C-myc expression and overall survival (C-myc 40%: $p = 0.97$, 50%: $p = 0.75$, 60%: $p = 0.55$, 70%: $p = 0.35$ or 80%: $p = 0.76$). There was also no correlation between C-myc and BCL-2 positivity, C-myc and BCL-6 positivity or C-myc, BCL-2 and BCL-6 positivity with disease-free survival and overall survival.

**Conclusion:** The degree of C-myc expression and co-expression of BCL-2 and BCL-6 by immunohistochemistry in diagnostic biopsies does not have an effect on disease-free survival and overall survival in patients who have received autologous stem cell transplants for DLBCL in this study. Evaluating other factors may be important in determining prognosis for this subset of patients.

### 304
### CLINICOPATHOLOGICAL FEATURES AND PROGNOSIS OF AGGRESSIVE B-CELL LYMPHOMA WITH T(14;18) AND 8Q24 TRANSLOCATION

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**Introduction:** Aggressive B-cell lymphoma having both t(14;18) and 8q24, so-called double translocation lymphoma (DTL), is rare. The pathological diagnosis in most cases of DTL is B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. Patients with DTL have elevated serum LDH levels, advanced stage, bone marrow involvement, and extranodal involvement. In the present study, we evaluated the clinicopathological characteristics and prognoses of patients with aggressive B-cell lymphoma with DTL.

**Methods:** A total of 368 patients with aggressive B-cell lymphoma were treated from 2007 to 2013. Chromosomal data were available in 195 of the 368 patients. Pathologic evaluation of the materials from each patient was performed at several central review meetings by six hematopathologists in the ALTSG pathology review board. Patients were treated with cyclophosphamide, vincristine, bleomycin, etoposide, doxorubicin, and prednisolone regimen or CHOP regimen. Rituximab was administered to all patients. The median follow-up period was 44 months (range, 12–61 months).

**Results:** t(14;18) + 8q24 dual translocation was seen in 18 (9.2%) of the 195 patients with aggressive B-cell lymphoma. There were 12 males and 6 females, with a median age of 62 years. Stage III/IV was found in 56%, bone marrow infiltration was found in 39%, central nervous system infiltration was found in 17%, and high risk of International Prognostic Index (IPI) was found in 67%. Immunophenotyping analysis (CD20, CD5, CD10, BCL2, BCL6, MUM1, and Ki-67) was performed. Ki-67 staining ranged from 30% to 90%. All lymphoma cells were positive for CD20 and negative for CD5. CD10, BCL2, BCL6, and MUM1 were positive in 89%, 75%, 88%, and 19%, respectively. The 4-year overall survival (OS) rate was 72% among patients with DTL and 75% among patients in the other chromosomal abnormalities group. The 4-year progression-free survival (PFS) rate was 52% among patients with DTL and 71% among patients in the other chromosomal abnormalities group. The 4-year OS rates of the Stage I/II and Stage III/IV groups were 100% and 47%, respectively ($p = 0.016$). We next examined the survival curve of patients in whom data on serum LDH levels were available. The 4-year OS rates of the high-LDH (>2 N) and low-LDH groups (<2 N) were 33 and 100%, respectively ($p = 0.0002$). According to the IPI, the 4-year OS rates of patients with L or L-I risk and those with H-I or H risk were 100% and 50%, respectively ($p = 0.03$).

**Conclusions:** Among patients with DTL, there was one subgroup that had a good prognosis. We elucidated the clinicopathological condition of especially the subgroup of DTL with poor prognosis, and prognostic improvement of this disorder is expected in the future by considering a new treatment strategy for these subgroups.

### 305
### OVEREXPRESSION OF MMSET/NSD2, AN EPIGENETIC REGULATOR IS ASSOCIATED WITH POOR SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMA

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**Background:** The expression of MMSET (a.k.a. WHSC1 or NSD2), a SET domain-containing histone lysine methyltransferase, is deregulated in a subgroup of multiple myelomas with the t(4;14)(p16;q32) translocation. However, the expression and the significance of MMSET in diffuse large B-cell lymphoma (DLBCL) have not been fully investigated. Herein, we examined the expression of MMSET in DLBCL and studied its significance with respect to the subtypes and their prognosis.

**Design:** The expression of MMSET was evaluated by immunohistochemistry in lymphoma tissue microarrays containing 122 cases of DLBCL specimens from patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) with corresponding clinicopathological data and long-term follow-up information. The expression was recorded by a composite scoring system as negative, mild, moderate, high and very high levels of expression (0–4).

**Results:** High levels (>2) of MMSET protein expression were detected in 36% cases of germinal centre subtype DLBCL (GCB-DLBCL) and in 60% cases of non-germinal centre activated B-cell subtype DLBCL (ABC-DLBCL; $p = 0.013$). The average expression was slightly higher in the ABC-DLBCL subtype than in the GCB-DLBCL subtype, although the difference is only marginally significant ($p = 0.06$). Furthermore, the high levels of MMSET expression in ABC-DLBCL were also correlated with a poor event-free survival.

**Conclusion:** MMSET protein is highly expressed in DLBCL, and its expression correlates with poor survival in patients treated with R-CHOP, especially those with tumours of the ABC-DLBCL subtype. Further study to evaluate its prognostic and therapeutic value is warranted.

### 306
### FREQUENCY AND OUTCOME OF CD30-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMAS

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Diffuse large B-cell lymphoma (DLBCL) is the most common and one of the most heterogeneous lymphomas. Therefore, it is critical to further stratify cases of DLBCL into biologically similar and clinically meaningful subgroups, which will not only guide prognostic assessment and facilitate therapeutic decisions but also

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stimulate further research to understand the pathogenesis and develop potential novel treatments. One promising candidate is brentuximab vedotin, an antibody–drug conjugate targeting CD30-expressing cells. Previous observational studies have suggested that CD30 may be expressed in 10–20% of DLBCLs. The aim of this study was to determine the prevalence of CD30 expression in DLBCL by immunohistochemistry and to explore possible relationships with outcome. We retrospectively identified cases of DLBCL diagnosed between January 2010 and January 2013 at our institution. The following large B-cell lymphoma subtypes were excluded from this analysis: post-transplant lymphoproliferative disorders with a DLBCL morphology, primary mediastinal large-cell lymphoma and unclassifiable lymphomas with intermediate features between either DLBCL and Burkitt's lymphoma or DLBCL and Hodgkin lymphoma. Immunohistochemistry was performed as part of the routine workup (Monoclonal Mouse Anti-Human CD30, Dako), and CD30 was considered positive when ≥30% of neoplastic cells stained positive.

A total of 82 cases of *de novo* DLBCL treated with R-CHOP were included in the training set for further analysis There were 45 men and 37 women with a median age of 57 years (range, 16–84); 35 patients (43%) presented with B symptoms, and 49 (60%) had advanced Ann Arbor stages. Most of the patients had a good performance status (Eastern Cooperative Oncology Group score 0–1, 87%), elevated serum lactate dehydrogenase level (61%), and low or low-intermediate International Prognostic Index risk score (0–2, 63%). Involvement of multiple extranodal sites (≥2) was seen in 22% of cases and bulky disease in 32% of cases.

**Results:** The median follow-up time was 47 months. Among the 82 cases in the training set, CD30 was positive in 24 cases (29%). No difference in response rate was observed between CD30-positive and CD30-negative patients. Patients with CD30+ DLBCL showed a significantly superior OS and PFS compared with those with CD30−. The 3-year OS was 79% in patients with CD30+ vs 59% in CD30− ($p < 0.05$); 3-year PFS was 73% in patients with CD30+ versus 57% in CD30− ($p < 0.05$).

**Conclusions:** CD30 is expressed in approximately 29% of all DLBCL and defines a novel subgroup of DLBCLs with a more favorable prognosis. The advent of brentuximab vedotin and its well-established effectiveness in other types of relapsed lymphomas opens the possibility of its application in this subset of patients.

### 307
### OBJECTIVE QUANTIFICATION OF BCL2 PROTEIN BY IMMUNOFLUORESCENCE IN ROUTINE BIOPSY SAMPLES PREDICTS RESPONSE TO R-CHOP IN PATIENTS WITH DLBCL

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**Introduction:** Diffuse large B-cell lymphoma (DLBCL), the most common non-Hodgkin lymphoma, is pathologically and clinically heterogeneous. Therefore, biomarkers are needed to identify the substantial minority of patients whose disease is likely to progress or recur after conventional first-line therapy so that they may be offered alternative treatments. Recent studies using conventional immunohistochemistry (IHC) showed that abundant expression of BCL2 protein in lymphoma cells, especially if co-expressed with MYC protein, is associated with inferior clinical outcome. However, these studies were based on subjective, non-quantitative visual scoring of histology slides. In this study, we ascertained the abundance of BCL2 protein objectively and quantitatively using multi-channel immunofluorescence (IF) microscopy and investigated potential associations with pathological and clinical parameters, including clinical outcome.

**Methods:** Pre-treatment, formalin-fixed, paraffin-embedded biopsy samples from 66 local cases of *de novo* DLBCL treated with R-CHOP were represented on a tissue microarray (TMA). Histological sections were co-stained by IF for BCL2 and CD20, digital image files were created, and the BCL2 signal was quantified selectively in CD20-expressing cells using HALO™ software. Additional TMA sections were stained for BCL2 by conventional IHC and scored visually as 'BCL2-high' versus 'BCL2-low' using published criteria. Standard statistical methods were used to explore the relationship between BCL2 expression and pathological and clinical attributes of the cases.

**Results:** Quantification of BCL2 by IF showed acceptable run-to-run reproducibility ($r^2 = 0.72$). Cases considered BCL2-high by IHC were associated closely with more abundant BCL2 as determined by IF ($p < 0.001$). Abundant BCL2 by IF was also associated with earlier age ($p = 0.01$) and absence of extranodal disease ($p < 0.001$) at diagnosis as well as poor response to first-line R-CHOP therapy ($p = 0.031$). There were no significant associations with sex, International Prognostic Index, relapse, clinical stage, histopathological subtype or 5-year overall survival. Kaplan–Meier analysis showed a trend towards reduced disease-free survival for subjects with higher BCL2 expression, but this was not statistically significant after correction for testing of multiple hypotheses.

**Conclusions:** The abundance of BCL2 protein in lymphoma cells can be determined quantitatively, objectively, and reproducibly in routine biopsy samples using IF. Our results from this initial, exploratory study suggest that abundant BCL2 portends a poor response to R-CHOP chemotherapy in patients with *de novo* DLBCL. Additional investigations are warranted to explore potential relationships between BCL2 protein abundance and chromosomal translocations involving *BCL2* or *MYC*, MYC protein abundance, or patient survival.

### 308
### PROGNOSTIC IMPACT OF MYC/BCL-2 PROTEIN OVEREXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN THE RITUXIMAB ERA: A SINGLE-INSTITUTION EXPERIENCE

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**Introduction:** Protein overexpression and rearrangements of *myc* and *bcl-2* genes have been related with an adverse prognosis in diffuse large B-cell lymphoma (DLBCL) patients in the rituximab era. But data are not always consistent and sometimes contradictory.

**Aim:** We aimed to analyse the incidence of MYC and BCL2 protein overexpression in DLBCL as well as to evaluate the prognostic impact in terms of time to progression (TTP) and overall survival (OS).

**Methods**: We carried out a single-institution study with tissue biopsies obtained from patients diagnosed with DLBCL in the period 1994–2011. Tissue fixation and processing were performed using standard methods. Tissue microarrays that contained two representative 2-mm cores from each tumour were prepared. Immunohistochemical staining was performed using fully automated protocols. We used the following antibodies: MYC (clone Y69, Roche) and BCL2 (clone 124, DAKO). The cut-off level for BCL2 and MYC expression was 50% and 10%, respectively. MYC and BCL2 expression was evaluated by a pathologist expert as well as a haematologist. At the same time, MYC and BCL2 expression was evaluated using computerized image analysis with IMAGE-PRO PLUS 6.0. A mean number of 1000 cells were analysed per case. TTP and OS curves were built by the Kaplan–Meier method and compared by the log-rank test. Impact of TTP and OS was studied by the Cox regression test.

**Results**: One hundred and forty DLBCL patients were included, median age was 70 (23–76), male/female ratio was 73/67; 73 (52%) had Ann Arbor Stage III or IV, 72 (51%) had high LDH and 62 (44%) had high β2-microglobulin; 53 patients had low risk, 26 (19%) patients int-low risk, 31 (23%) patients int-high risk and 28 (20%) patients high risk; 59 patients (42.1%) had IPI ≥ 3. Forty-five patients were treated with CHOP-like regimens and 95 with rituximab-CHOP schedules. Median follow-up (patients alive) was 49 months (12 135). We observed MYC overexpression (≥10%) in 101 (72%), BCL2 overexpression (≥50%) in 71 (50%) and both in 56 (40%) patients. We did not observe any correlation between MYC, BCL2 and both overexpression and clinical–biological baseline characteristics. Patients with MYC and BCL2 overexpression had a lower complete response rate than the rest: 62% vs 76% ($p = 0.09$). Patients with both markers had a worse 5-year TTP, 67% vs 83% ($p = 0.09$), and also a worse 5-year OS, 60% vs 79% ($p = 0.05$). When we restricted the analysis to the rituximab-treated patients, patients with both markers had a worse 5-year TTP and OS than the rest (57% vs 73%, $p = 0.05$, and 58% vs 85%, $p = 0.002$, respectively). In the multivariate analysis including the IPI variable and *myc/bcl-2*, *myc/bcl-2* overexpression showed an independent prognostic value on OS, HR 3876 (1.58, 9.51), $p = 0.003$.

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**Conclusions:** In our experience, the protein overexpression of MYC and BCL-2 had a negative impact on the outcome, specially in the rituximab era with a lower CCR and a significantly worse TTP and OS. Further studies on these area are required.

### 309
### BCL2 STAINING IS HIGHLY PREDICTIVE FOR OUTCOME OF NEWLY DIAGNOSED CLASSICAL HODGKIN LYMPHOMA PATIENTS

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**Induction:** BCL2 as an anti-apoptotic protein may contribute to mechanisms of resistance of Hodgkin and Reed–Sternberg (HRS) cells to chemotherapy. Clinical data on the influence of BCL2 expression in HRS cells on outcome of HL patients are limited, and differential expression of BCL2 in HRS compared to surrounding lymphocytes is largely unknown. We prospectively evaluated immunohistochemical (IHC) staining for BCL2 on formalin-fixed paraffin-embedded (FFPE) tissue specimens from the initial biopsies of HL patients and compared the staining pattern with clinical outcome.

**Methods:** BCL2 protein staining was evaluated on FFPE tissue sections with monoclonal BCL2 antibody, clone 124 (Dako), using automated stainers. IHC was performed using the EnVision™ Detection Systems FLEX kit (Dako). The morphological pattern of BCL2 expression was evaluated in each case under a microscope by the pathologist blinded to patient characteristics. Patterns of BCL2 staining were scored using a 3-point scale: 0—absence or <20% of HRS cells with BCL2 staining, 1—BCL2 staining on ≥20% HRS cells comparable/lower than on surrounding lymphocytes and 2—strong BCL2 staining on HRS cells higher than on surrounding cells. Staining scores 1 and 2 were considered positive. Initial treatment was ABVD in 95% of patients, followed by radiotherapy as indicated.

**Results:** We analyzed 118 biopsy samples from patients diagnosed at our institution between 2007 and 2012. Patient characteristics were as follows: male 50%, median age (range) 34 (18–86), Ann Arbor Stage III/IV 40%, bulky disease 55%, extranodal involvement 19%, elevated LDH 41%, and low albumin 36%. BCL2 staining was scored 0 in 33%, 1 in 43%, and 2 in 24% of patients. With a median follow-up (range) of 47 (15–102) months, 3-year OS and PFS for all patients were 96% (95% CI [92%, 100%]) and 72% (95% CI [64%, 81%]), respectively. Patients with a score of 2 in BCL2 staining had significantly decreased 3-year OS and PFS of 87% (95% CI [73%, 100%], $p = 0.014$) and 28% (95% CI [11%, 45%], $p < 0.001$), respectively. In two-step Cox analysis, all clinical and laboratory variables listed above were analyzed, and only the BCL2 staining score was a significant independent prognostic factor for OS and PFS.

**Conclusions:** BCL2 was strongly expressed on HRS cells in 24% of our patient series and was significantly associated with inferior PFS and OS.

### 310
### NON-HODGKIN LYMPHOMA RACIAL DISPARITIES IN AN EQUAL-ACCESS SYSTEM IN THE USA: HIV INFECTION AND SOCIOECONOMIC DIFFERENCES AS LIKELY CAUSAL FACTORS

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**Introduction:** In the USA, African American (AA) patients with NHL fare worse than White (W) patients, although reasons for this disparity are not clear. While eliminating racial disparities in healthcare access and outcomes is the goal of healthcare reform in the USA, disparities observed in countries with equal-access healthcare systems suggest that improved health insurance access alone will not resolve racial disparities. We evaluated if racial disparities would still be present in the equal-access US Veterans Health Administration (VHA) system, where the majority of patients are of lower socioeconomic status yet have full access to state-of-the-art cancer care.

**Methods:** Patients with a new diagnosis of NHL who received care at any VHA medical center and were diagnosed between October 1998 and December 2009 were identified in the VHA central cancer registry. Information was obtained on patient age, race, disease histology, stage, comorbid conditions, and HIV status at the time of diagnosis. Survival data were obtained from VHA vital status files, linked to government pension records. Income was estimated based upon median household income within the postal code of residence at the time of diagnosis and then aggregated into quartiles. Comorbid conditions were tabulated into the Romano adaptation of the Charlson comorbidity score. Cox proportional hazards modeling was used for survival analyses, which were censored at 5 years after diagnosis.

**Results:** A cohort of 11 765 W and 1826 AA patients with NHL was identified. On univariate analyses, AA patients were significantly younger (median age 61 vs. 67 years, $p < 0.001$), more likely to be HIV+ (9% vs. 2%, $p < 0.001$), more likely to have peripheral T-cell lymphoma or cutaneous T-cell lymphoma (17% vs. 9.5%, $p < 0.001$), and more likely to have incomes in the lowest two quartiles (57.9% vs. 47.6%, $p < 0.001$), compared to W patients. The hazard ratio (HR) for mortality was significantly higher in AA patients (HR = 1.13, 95% CI 1.05–1.21), after adjusting for age alone. After additional adjustment for comorbidity score, HIV, disease histology, stage, and income, race was no longer significantly associated with mortality (HR = 1.02, 95% CI 0.95–1.1). Attributable risk was then calculated, demonstrating that 7% and 1% of deaths were attributable to HIV and 14% and 5% of deaths were attributable to income below median, among AA and W patients, respectively.

**Conclusions:** In an equal-access healthcare system in the USA, racial outcome disparities still exist among newly diagnosed NHL patients. Efforts to eliminate racial disparities among NHL patients in the VHA system should focus on broad issues related to the prevention, diagnosis, and treatment of HIV and ensuring that among Veterans of low socioeconomic status, compliance with complex NHL therapies is improved.

### 311
### DOGS AND CATS AS SPONTANEOUS ANIMAL MODELS OF HUMAN DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** The role of relevant preclinical animal models of human cancers lies in their potential to accelerate transfer of innovative therapies into human clinical practice. Non-Hodgkin lymphoma is frequent in both dogs (33/100 000) and cats (160/100 000), and diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma in dogs (59% of NHLs) and the second most common in cats (17% of NHLs) after T-cell intestinal lymphomas. In order to evaluate the interest of spontaneous canine and feline DLBCLs for preclinical studies in human, we characterized their profile using Hans' algorithm and an adapted immunohistochemical double hit score (DHS).

**Methods:** One hundred and twelve dogs and 46 cats diagnosed with DLBCL between 2005 and 2011 in the College of Veterinary Medicine of Nantes (France) were included in this retrospective study. DLBCL samples were evaluated by automated immunohistochemistry. Thresholds for positivity were 30% for CD10, BCL-6, and MUM-1; 10% for BCL-2; and either 30% (cats) or 80% (dogs) for c-MYC (clone Y69). Animals' outcomes were obtained from referring veterinarians and owners.

**Results:** The most common stage at presentation was Stage III in dogs (65%) and Stage I in cats (59%) according to the World Health Organization (WHO) staging system of canine and feline lymphomas. Histologically, the centroblastic subtype predominated in dogs (69%) whereas immunoblastic and centroblastic DLBCLs were almost equally represented in cats. Germinal center (GC) DLBCLs according to Hans' algorithm were less common than non-GC DLBCLs, in both dogs (17% vs. 83%) and cats (33% vs. 67%). Positivity for c-MYC was very common in dogs (62%) and cats (91%). A high DHS (c-MYC and BCL-2 positivity) was observed in

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27% of canine DLBCLs and 16% of feline DLBCLs. The median overall survival in cats was 51 days. The median specific survival was 64 days in dogs (47 days with palliative corticotherapy and 195 days with CHOP-like chemotherapy). By multivariate analysis in dogs, the absence of chemotherapy (HR = 4.35), a high WHO clinical stage (HR = 3.15 for Stages IV–V), a non-GC phenotype (HR = 2.00), and a high DHS (HR = 1.73 for c-MYC+ and BCL-2+ DLBCLs) were associated with shorter specific survival ($p < 0.0001$, Cox proportional hazards regression model).

**Conclusions:** The advantages of spontaneous canine and feline DLBCLs as preclinical models of human DLBCL are their high frequency, overrepresentation of aggressive subtypes (non-GC and c-MYC positive), and short natural history.

### 312
### IMMUNOPHENOTYPIC CHARACTERIZATION AND CLINICAL BEHAVIOUR IN LYMPHOPROLIFERATIVE DISORDERS MUTATED AND NON-MUTATED WITH AND WITHOUT t(11,14)

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**Introduction:** Mantle cell lymphoma (MCL) t(11;14)(q13;q32) shows a typical CD19+ CD5+ immunophenotype easily identified by flow cytometry (FC). However, typical MCL immunophenotype cases lacking t(11;14) are frequently detected in clinical routine with variable clinical presentation and different mutational status.

**Aims:** We aimed to characterize CD19+ CD5+ (non-CLL) lymphoproliferative disorders (LPD), trying to identify immunophenotypic profiles that may predict the presence of t(11;14) and find molecular differences associated to clinical behaviour.

**Methods**: We retrospectively reviewed 98 patients with CD19+ CD5+ (non-CLL) LPD detected by FC in the University Hospital of Salamanca (Spain). Immunophenotype, cytogenetics and molecular biology (MB) (bcl-1 and IGHV mutational status) data were analysed. Clonal B-cell populations were detected in peripheral blood (50), bone marrow (29) and/or lymph node (19). Erythrocyte-lysed samples were stained using panels of monoclonal antibodies (four-colour direct immunofluorescence technique), according to previously described methods, aiming to identify and characterize B neoplastic cells; complete diagnosis was performed with ancillary techniques such as MB (bcl-1 and IGHV mutational status), karyotype and fluorescent *in situ* hybridization (FISH) for t(11;14).

**Results:** Out of 98 samples CD19+ CD5+ by immunophenotype, 52% of them were t(11/14) positive (+), with FISH being the most useful technique. Of the t(11;14)+ cases, 84% showed typical CD22+/CD23− immunophenotype and bright expression of CD20+ and FCM7+; CD38 was negative in 53% of the cases. Negative (−) cases for t(11;14) showed CD22+/CD23− in a slightly lower frequency (62%), being more heterogeneous, but with similar expression of CD20; CD38 was positive in 33% of these cases.

Mutated cases were higher among leukaemic cases (88%) than among nodal cases (35%), both with and without t(11;14). Despite no differences in OS, unmutated cases could be associated with more aggressive behaviour due to the need for earlier treatment (TTT median 15 days vs 29 months; $p < 0.001$) and a higher progression risk (SLP median 52 months vs NR; $p < 0.001$). Among the 98 CD19+ CD5+ cases (non-CLL), 8 were non-classifiable chronic-LPD, 4 were classified as SMZL, 4 as SLL, 2 as DLBCL, 1 as FL and 79 as MCL.

Overall, 75% of cases received therapy (of which 73% had nodal behaviour and 27% leukaemic behaviour). Time to treatment was longer in the leukaemic cases than in the nodal cases (median 82 months vs 15 days; $p < 0.001$).

**Conclusions:** Although typical MCL immunophenotype cases are frequently detected, t(11;14) is present only in half of them without clear differences in immunophenotype between cases t(11;14)+ and t(11;14)−. Despite no differences in OS (patients were not treated uniformly), unmutated cases could be linked to more aggressive behaviour due to the need for earlier treatment and to an increased risk of progression.

### 313
### CD5-POSITIVE CHRONIC B-CELL LYMPHOPROLIFERATIVE DISORDERS: DIAGNOSTIC CRITERIA AND PROGNOSIS

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**Introduction:** Differential diagnosis of chronic B-cell lymphoproliferative diseases (B-CLPD) presenting with peripheral clonal lymphocytosis considers a biologically heterogeneous group including chronic lymphocytic leukemia (CLL) and the leukemic phase of non-Hodgkin lymphomas. Patients without expression of CD5 will usually have nodal or splenic marginal zone lymphoma (MZL), lymphoplasmacytic lymphoma (LPL), follicular lymphoma (FL), or hairy cell leukemia, while establishing diagnosis of CD5-positive patients who do not have characteristic features of CLL or mantle cell lymphoma (MCL) can be more difficult. Patients with CD5+ B-cell clones that do not have the characteristic features of CLL or MCL have frequently been considered to have 'atypical' CLL. Therefore, our aim was to determine if there is a difference in the clinical courses of patients with CD5+ unclassified B-CLPD and patients with CLL.

**Methods:** Patients were included in the analysis if their blood contained a monoclonal CD5-positive B-cell population identified by flow cytometry but did not have the characteristic immunophenotype of CLL or MCL using immunophenotypic criteria. All patients had bone marrow biopsy, while patients with enlarged lymph nodes or spleen underwent lymph node biopsy or splenectomy. If findings did not satisfy criteria for CLL/SLL, MCL, MZL, LPL, or FL, patients were considered to be unclassified CD5+ B-CLPD.

**Results:** We analyzed 38 patients (median age 69, M : F ratio 2:1) with CD5-positive lymphocytosis but without immunophenotypic criteria for CLL or MCL and compared them with an age-matched and sex-matched control group of 38 CLL patients. In the group of CD5+ B-CLPD, 12 patients underwent splenectomy, while 7 patients had lymph node biopsy. In all splenectomized patients, diagnosis corresponded to splenic MZL while all patients with lymph node biopsy had nodal MZL. The remaining 19 patients had insufficient tissue for diagnostic evaluation, and their bone marrow analysis was nonanalytical. We considered them as unclassified CD5+ B-CLPD. There is no significant difference in physical examination and laboratory data between two groups on presentation. During follow-up, 13/38 (34%) CLL patients received chemotherapy (fludarabine-based regimen), and 16/19 (84%) patients with CD5+ B-CLPD also received chemotherapy (eight patients with CHOP, four patients with chlorambucil, four patients with COP, and one patient with a fludarabine-based regimen). During follow-up, five CLL patients and eight with CD5+ B-CLPD died. In CD5+ B-CLPD patients, median overall survival was 43 months while median is not reached in the CLL group ($p = 0.004$).

**Conclusion:** Our analysis showed that patients with CD5+ B-CLPD had worse clinical course and significantly shorter survival than patients with CLL. Moreover, an accurate diagnosis of the underlying lymphoid malignancy in patients with CD5+ B-CLPD requires surgical biopsy of a lymph node or other involved non-BM tissues.

### 314
### SIGNIFICANCE OF SMUDGE CELL ON BLOOD SMEAR IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: A PROSPECTIVE INSTITUTIONAL STUDY FROM INDIA

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**Introduction:** Smudge cells are ruptured lymphocytes seen on routine blood smears of chronic lymphocytic leukemia (CLL) patients. We evaluated significance of smudge cells percentage on a blood smear in CLL patients.

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**Methods:** We calculated smudge cell percentages (ratio of smudged to intact cells plus smudged lymphocyte) on blood smears of 165 consecutive untreated CLL patients registered at IRCH, AIIMS, New Delhi, over a period of 5 years (2006–2010).

**Results:** There were 125 males and 40 females. The median age was 59 years (30–88). Median absolute lymphocyte count was $40 \times 10^9$/L. Clinical Rai stage distribution was as follows: Stage 0, 5%; Stage I, 25%; Stage II, 40%; Stage III, 10%; and Stage IV, 20%. The median smudge cell percentage was 28% (4–76%). There was no correlation of proportion of smudge cells with age, sex, lymphocyte count, lymphocyte doubling time, beta 2 microglobulin, organomegaly, ZAP70+ or CD38 + CLL patients, but there was a significant correlation with stage of disease. Median smudge cell percentages were as follows: Stages 0 and I, 36% (12–76); Stage II, 30% (12–61); and Stages III and IV, 20% (4–51) [$p < 0.001$]. Eighty-five patients of early-stage (0, I, and II) disease required treatment during follow-up (65% required treatment with smudge cell <30%, against 35% patients requiring treatment with smudge cells >30%, $p = 0.01$). The percentage of smudge cells as a continuous variable correlated with OS (HR 0.96, $p < 0.001$). The 5-year survival rate was 51% for patients with 30% or less smudge cells compared with 76% for patients with more than 30% smudge cells. Median OS was 4.8 years with a median follow-up period of 3.6 years. Smudge cell percentage (<30% vs. >30%) had significant association with OS (HR 0.97, 95% CI (0.62–1.21), $p = 0.001$).

**Conclusions:** Simple and inexpensive detection of smudge cells on blood smears on routine diagnostic test is useful in predicting progression-free survival and OS in CLL patients and may be beneficial in countries with limited recourses.

### 315
### DIAGNOSTIC CYTOLOGICAL FEATURES OF BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE-CELL LYMPHOMA COMPARED TO BENIGN LATE-ONSET EFFUSIONS

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**Background:** Breast implant-associated anaplastic large-cell lymphoma (BIA-ALCL) is an uncommon entity that could manifest as a solid mass infiltrating the peri-prosthesis fibrotic capsule, or as small clusters of tumour cells within the peri-prosthesis effusion and lining the surface of the capsule. The most common presenting complaint is a seroma occurring more than 1 year after implantation. Therefore, needle aspiration and cytological examination of all late seroma are crucial for a prompt diagnosis. However, its diagnosis might be challenging for pathologists with no experience in hematopathology. Since the cytological features of BI-ALCL compared to the non-neoplastic late peri-prosthesis effusions have been reported in only few cases, an accurate description is still warranted.

**Methods:** All late-onset peri-implant effusions were collected at our institution from 2013 to 2015. Fine-needle aspiration cytospins of 22 peri-implant effusions from 16 patients were screened with Papanicolaou stain and immunocytochemistry for CD30 (clone Ber-H2, Dako). Fourteen out of 16 patients had a history of breast carcinoma, two had acute breast inflammation signs and two had ultrasound evidence of implant rupture.

**Results:** In 4 out of 16 (25%) patients, a BIA-ALCL diagnosis was made. One patient had contralateral axillary lymph node infiltration. Neoplastic smears showed the classical 'hallmark' tumour cells with large irregularly shaped nuclei, prominent nucleoli and abundant clear cytoplasm. Secondary common features were serous fibrinous background, absence of neutrophils and presence of apoptotic cells. The cellularity varied from cases with few large neoplastic cells to cases with a mixture of large-sized to medium-sized atypical elements with dark and irregularly shaped nuclei. Mitoses were evident in all but one case. Neoplastic cells were CD30 positive in all the samples. Only one sample showed a larger number of small mature lymphocytes and eosinophils admixed with tumour cells. On the other hand, the cytological appearance of the reactive effusions was mutable and in the same patient changed over time. Based on the cellular composition, benign effusions could be grouped into three main patterns: (i) neutrophil rich (three samples, of which two had fever and signs of acute breast inflammation); (ii) small mature lymphocyte rich (four samples); (iii) mixed chronic infiltrate with lymphocytes, macrophages and occasional eosinophils (11 samples, among which 2 had implant rupture). CD30+ small reactive blasts were very rare or absent in all the benign seroma.

**Conclusion:** In our experience, BI-ALCL cases accounted for 25% of unselected peri-implant effusions. The screening of all late seromas and the knowledge of the appropriate cytological features together with CD30 immunostaining could allow the early recognition of the disease. Moreover, this might help to establish the real incidence of BI-ALCL and to avoid an eventual systemic spread of the disease.

### 316
### PRESYMPTOMATIC IDENTIFICATION OF CANCER/LYMPHOMA DURING NON-INVASIVE PRENATAL DIAGNOSIS

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**Introduction:** Over the past years, non-invasive prenatal testing (NIPT) for fetal aneuploidy detection has become a clinical reality. NIPT is based on the analysis of circulating cell-free DNA (ccfDNA) in maternal plasma by either whole-genome or targeted sequencing methods. Most NIPT providers focus on the detection of only the most common aneuploidies, trisomies 13, 18, and 21, respectively.

**Methods:** We recently optimized a massive parallel sequencing-based NIPT analysis pipeline, which not only interrogates the common trisomies but also allows for genome-wide discrimination of fetal and maternal segmental aneuploidies. Thus far, this analysis pipeline was applied on ccfDNA of 4000 pregnant women. Genome representation (GR) profiles of the reported cases were validated on biopsy specimens using FISH and array CGH.

**Results:** During routine NIPT for fetal aneuploidies, three samples showed aberrant (GR) profiles that could not be attributed either to the maternal genomic constitution or to the fetal genomic constitution. Whole-body diffusion-weighted magnetic resonance imaging and subsequent pathologic and genetic investigations uncovered the presence of asymptomatic maternal malignancies diagnosed as ovarian carcinoma (OC), t(14;18)-positive follicular lymphoma (FL), and early-stage nodular sclerosis classical Hodgkin lymphoma, respectively. Further FISH and array CGH studies of biopsy specimens showed matched patterns compared to the genomic imbalances detected in ccfDNA. Whereas the patient with an indolent FL was not treated during pregnancy, the remaining two patients were successfully treated with a standard therapy for advanced-stage OC and an ABVD-based regimen. It is worth stressing that in the follow-up blood samples collected after the first cycle of chemotherapy, the aberrant GR profile 'normalized' in both cases and the profile remained within normal range in all successive samples.

**Conclusions:** Non-invasive massive parallel sequencing of ccfDNA allows for presymptomatic identification of cancers during NIPT. It opens the perspective towards applications outside pregnancy, particularly in diagnostics, monitoring of therapeutic response, and physiopathological understanding of cancer genetics. In addition, this novel discovery will facilitate the development of biomarkers and may facilitate targeted and precision anticancer therapy.

### 317
### PLASMA OMEGA-3 FATTY ACID LEVELS IN PATIENTS WITH UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** The identification of modifiable dietary factors that can reduce cancer risk or affect prognosis is a priority for patients as well as clinicians. Our center has shown that diets high in omega-3 fatty acids (n-3 FA) are associated with reduced lymphoma risk (*J Nutr.* 2013 May; 143(5): 672–681). However, there have been no studies of blood n-3 FA levels in patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL) and their relationship to lymphoma prognosis.

**Methods:** Patients with DLBCL were prospectively enrolled in the University of Iowa/Mayo Clinic SPORE Molecular Epidemiology Resource. For this pilot study, patients treated with R-CHOP or similar rituximab-containing immunochemotherapy with a pre-treatment plasma sample were included. n-3 FA levels, specifically the marine n-3 FAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and the vegetable n-3 FA alpha linoleic acid (ALA), were assessed on stored research plasma samples using a gas chromatography–mass spectrometry stable isotope dilution method in the Mayo Clinic clinical reference laboratory. Event-free survival (EFS) was defined as the time from diagnosis to relapse, re-treatment, or death due to any cause; EFS24 was defined using EFS status at 24 months from diagnosis. Comparisons of n-3 FA levels between outcome groups were assessed using Wilcoxon rank-sum tests.

**Results:** Plasma samples from 25 patients were evaluated. The median age was 57 years (range 41–84), and 44% were male. IPI was 0–1 in 8 patients, 2 in 11 patients, and 3 in 6 patients. Thirteen patients achieved EFS24, and 12 patients failed to achieve EFS24. Median n-3 levels were DHA = 128 μmol/L (reference range: 30–250), EPA = 55 μmol/L (14–100), and ALA = 57 μmol/L (50–130). In comparison to the clinical reference range, all patients had normal DHA, five patients (20%) had elevated EPA, and six patients (24%) had low ALA. There were no significant associations between EPA, DHA, or ALA and the clinical characteristics age, sex, and IPI (all $p > 0.10$). ALA was lower in patients failing to achieve EFS24 (median ALA = 45) compared to patients who achieved EFS24 (median ALA = 70), $p = 0.053$. EPA (median = 109 vs. 148) and DHA (median = 38 vs. 70) were also lower in patients failing to achieve EFS24, but these were not statistically significant ($p = 0.33$ and $p = 0.17$, respectively).

**Conclusions:** This pilot study is the first to demonstrate that plasma n-3 FA levels are low in a subset of patients with DLBCL. Lower n-3 FA levels, specifically ALA, in DLBCL patients is associated with poor outcome as assessed by EFS24. This provides the rationale to test n-3 FA levels in a larger group of DLBCL patients to confirm these findings.

### 318
### ALPHA-1 GLOBULIN AND M PROTEIN ARE NEW PROGNOSTIC FACTORS IN PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** Several studies concerning primary extranodal diffuse large B-cell lymphoma (DLBCL) have been reported sporadically. However, no new, valuable prognostic factors have been reported since several risk factors, such as high international prognostic index score, elevated lactate dehydrogenase (LDH) level, poor Eastern Cooperative Oncology Group performance status (PS), and advanced stage, were identified.

**Methods:** To identify new and valuable prognostic factors, we reviewed the medical records of patients with primary extranodal DLBCL who were newly diagnosed at the Cancer Institute Hospital of the Japanese Foundation for Cancer Research from February 2005 to September 2014 and retrospectively analyzed the data of a total of 593 consecutive DLBCL patients, including 291 primary extranodal DLBCL patients. We investigated the relationship between the overall survival (OS) rate and the albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin percentages and M protein of the serum protein fraction electrophoresis at diagnosis. Univariate and multivariate analyses of estimated risk factors for OS in primary extranodal DLBCL patients were performed using the log-rank test and Cox proportional hazard regression analysis.

**Results:** Sites of the disease were lymph nodal (302 cases, 50.9%) and extranodal sites (291 cases, 49.1%). Primary extranodal origin cases were the stomach, 87 cases; bone, 29 cases; paranasal sinus, 25 cases; small intestine, 19 cases; breast, 18 cases; oral cavity, 17 cases; orbital cavity, 14 cases; colon, 12 cases; thyroid, 12 cases; skin, 10 cases; nasal cavity, 8 cases; testis, 6 cases; muscle, 6 cases; and others, 27 cases. The median age was 67 years (range, 20–89 years). The median follow-up was 41 months (range, 1–115 months). To adjust the impact of the albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin percentages and the presence of M protein of the serum protein fraction electrophoresis at diagnosis for other significant factors for OS, we identified the following risk factors by univariate analysis: clinical Stages III and IV, PS ≥ 2, elevated soluble IL-2 receptor level, elevated LDH level, low albumin percentage, and high α1-globulin and α2-globulin percentage along with the presence of M protein. Then we performed multivariate analyses by using all these factors in the Cox proportional hazard model. High α1-globulin percentage (HR 3.58, 95% CI 1.97–6.51, $p < 0.001$), M protein (HR 6.01, 95% CI 2.10–17.21, $p < 0.001$), and Stages III and IV (HR 2.30, 95% CI 1.27–4.16, $p = 0.0062$) were identified as independent significant prognostic factors for OS.

**Conclusions:** These results suggest that the higher percentage of α1-globulin and the presence of M protein in the serum protein fraction electrophoresis were significantly associated with poor OS in patients with primary extranodal DLBCL. α-1-Globulin and M protein appear to be new, robust prognostic factors in patients with primary extranodal DLBCL.

### 319
### HIGH APELIN LEVELS COULD BE USED AS A DIAGNOSTIC MARKER IN MULTIPLE MYELOMA

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**Introduction:** Apelin is an endogenous peptide. The apelin/APJ system regulates angiogenesis and is overexpressed in some malignancies. Recent studies suggested that apelin also induced lymphangiogenesis and lymph node metastasis. No study has evaluated apelin level in multiple myeloma (MM) and non-Hodgkin lymphoma (NHL). In this study, we evaluated apelin level in MM and NHL; and we analyzed the association between apelin levels and clinical findings.

**Methods:** We included consecutive 29 MM, 31 NHL, and 19 healthy controls. Patients' demographic and clinical features, treatment modalities, and responses were recorded from hospital records. Serum apelin was determined by enzyme-linked immunosorbent assay (ELISA) method.

**Results:** Apelin levels in MM, NHL, and healthy control groups are seen in Table 1. The groups were similar in age and sex ($p > 0.05$). MM patients had significantly higher serum apelin level than NHL and healthy control groups ($p < 0.001$). Apelin level in NHL group was significantly higher than that in controls ($p < 0.001$). In the MM group, apelin level correlated negatively with serum monoclonal protein ($r = -0.45$, $p = 0.016$) and LDH ($r = -0.39$, $p = 0.038$). ROC curve analysis showed that the area under the curve value for apelin level in MM was 0.842 ng/ml (95% CI 0.739–0.945, $p < 0.001$). Serum apelin level ≥0.827 ng/ml had a 76% sensitivity and a 86% specificity for the diagnosis of MM. In the NHL group, apelin level correlated with hemoglobin level ($r = 0.47$, $p = 0.009$). Ann Arbor Stage IV NHL patients had significantly lower apelin level than patients in other stages (0.39 ± 0.23 vs. 0.74 ± 0.73 ng/ml, $p = 0.029$).

**Conclusions:** Serum apelin level was found to be significantly increased in MM and NHL, similar to other malignancies. Significantly high apelin level in MM could be

**Abs 319 - Table 1.** The comparison of demographic and biochemical features of MM, NHL, and healthy control groups

| | MM | NHL | Controls |
|---|---|---|---|
| *n* (M/F) | 29 (18/11) | 31 (17/14) | 19 (13/6) |
| Age (mean ± SD) | 64.3 ± 9.9 | 60.5 ± 11.4 | 59.1 ± 9.4 |
| Apelin (ng/ml) | 1.99 ± 1.1 | 0.56 ± 0.56 | 0.42 ± 0.16 |

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used as a diagnostic biomarker and could be playing a role in pathogenesis. Significantly decreased apelin level in NHL in advanced stages was a noteworthy finding.

## HODGKIN LYMPHOMA

### 320
### CLINICAL CHARACTERISTICS OF HODGKIN LYMPHOMA PATIENTS—A PRELIMINARY REPORT FROM THE BRAZILIAN HODGKIN LYMPHOMA REGISTRY

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**Introduction:** The outcome of Hodgkin lymphoma (HL) has markedly improved over the past few decades. However, data about HL in developing countries are scarce and come mostly from retrospective series. In 2009, a prospective registry of HL was implemented to gather data on demographic features, clinical presentation, treatment modalities and outcomes in Brazil.

**Methods:** Clinical, demographic and socio-economic status (SES) information were collected prospectively in a Web-based registry. SES stratification was done using an index widely used in publicity and political polls in Brazil.

**Results**: A total of 577 patients are currently registered. Twenty institutions take part in the registry. The median age was 30 years old (3–80), and 53% were male. The median time from the onset of symptoms until diagnosis was 5.5 months (0–60). According to GHSG classification, 49 patients (9%) were classified as limited stage, 160 (26%) as intermediate stage and 364 (63%) as advanced disease. Missing data precluded subclassification of localized disease in 14 patients (2%). A high IPS was found in 227 patients (40%). B symptoms were present in 379 patients (66%), and 79 (14%) presented a poor performance status. No differences in clinical presentation were found between lower-SES and higher-SES patients regarding stage, IPS, presence of B symptoms or bulky mediastinal mass, except for poor performance status, which was more prevalent in patients with lower SES (21% × 10%, $p = 0.002$). Regarding histopathological diagnosis, 76% were classified as nodular sclerosis and 12% as mixed cellularity. Bone marrow biopsy was performed in 93% of patients and was positive in 11%. Reasons for not performing a biopsy were physician's choice in 27 patients (5%), difficulty in obtaining an adequate sample in 8 patients and lack of needle to perform it in 4 patients. PET was used for staging in 8% and for evaluation of response in 35% of patients. First-line treatment was ABVD in 90% of patients.

**Conclusions:** The organization of a national registry of HL provides a reliable portrait of the disease in Brazil. This preliminary analysis confirms the diagnosis of HL at more advanced stages in Brazil, and more frequently associated with poor prognostic factors. Data on treatment outcomes are currently being collected.

### 321
### STUDY OF PROTEIN Z AND PLASMINOGEN ACTIVATOR INHIBITOR-1 IN PATIENTS WITH HODGKIN LYMPHOMA

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**Introduction:** Hemostasis is a homeostatic mechanism interacting with other defence mechanisms, such as inflammatory response, but it is also associated with pathological conditions, such as malignancy. Inflammation and cancer are nowadays considered as prothrombotic states. The aim of this work was the study of protein Z (PZ) and plasminogen activator inhibitor 1 (PAI-1) in patients with Hodgkin lymphoma (HL), a hematological malignancy characterized by an intense inflammatory background.

**Methods:** Sixteen patients newly diagnosed with HL and treated with ABVD combination and 16 controls were studied prospectively. All the participants had provided informed consent. Blood samples were collected using a free-flowing technique, including four samples from each patient before $I_A$, $I_B$, $II_A$ and $II_B$ ABVD and one sample from each control. PZ and PAI-1 plasma levels were measured using the ELISA technique. Their levels were compared between patients and controls, while correlations with patients' clinical and laboratory characteristics, prognosis and outcome were also assessed.

**Results:** The median age of the patients was 38.5 years and 44% were males, while the median age of controls was 39 years and 44% of them were males. No patient or control was receiving anticoagulants or hormone therapy. Patients' PZ plasma levels before $I_A$ ABVD were not significantly higher than those of controls (mean value = 1869.9 vs 1721.1 ng/ml, $p = 0.546$) and did not correlate with most of patients' characteristics (age, gender, histological subtype, stage, IPS, PCT, CRP, ESR, interim PET, Hb, WBC, lymphocyte count, PLT count, albumin, g-globulins, $\alpha_2$-globulins, haptoglobins and immunoglobulins). However, plasma PZ levels marginally correlated with $\beta_2$-microglobulin levels (cc = −0.478, $p = 0.061$). Additionally, baseline PZ levels were significantly lower in patients with B symptoms ($p = 0.029$) and marginally lower in patients with positive final PET ($p = 0.062$), who finally relapsed ($p = 0.057$). PZ did not change significantly during the first two cycles with ABVD, although it presented a declining trend. Patients' plasma PAI-1 levels before $I_A$ ABVD were significantly higher compared to those of controls (mean value = 39 vs 15.2 ng/ml, $p = 0.035$), correlating with platelet counts (cc = 0.522, $p = 0.038$). Moreover, baseline PAI-1 levels were marginally higher in patients with positive final PET ($p = 0.062$), who finally relapsed ($p = 0.057$). Furthermore, PAI-1 levels, after an increasing trend, declined significantly after $I_B$ ABVD ($p = 0.020$).

**Conclusions:** In the present study, PAI-1 reflected better the prothrombotic state that was expected to accompany HL than did PZ. Both markers did not correlate with most of patients' characteristics. However, a possible correlation between baseline PZ or PAI-1 levels and a positive final PET and relapse did emerge and needs further evaluation.

### 322
### CIRCULATING ARGINASE-1 IN SERUM IS A NOVEL BIOMARKER IN HODGKIN LYMPHOMA

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**Background:** Arginase-1 (Arg-1) is a key enzyme contained in neutrophils' granules to mediate immunosuppression in Hodgkin lymphoma (HL). Our previous work showed that Arg-1 is a potential biomarker in HL. To define its value as a marker to monitor treatment response, we correlated serial Arg levels with clinical response in newly diagnosed and relapsed classical HL patients.

**Material and Methods:** Serum was collected from 61 (39 early stage and 21 advanced stage) newly diagnosed HL patients before, during and after treatment and from 10 refractory/relapsed patients before and after treatment with brentuximab. s-Arg-1 was determined by enzyme-linked immunosorbent assay and was related to pre-treatment SUVmax and SULpeak, as measured by quantification of 2-[$^{18}$F] fluoro-2-deoxyglucose positron emission tomography (PET) images, and to treatment response.

**Results:** Baseline s-Arg-1 correlated with stage of disease and bulky disease and weakly with SUVmax and SULpeak. s-Arg-1 was positively correlated to the absolute count of neutrophils (ANC) and Arg-1 detected in neutrophils by RT-PCR.

Since neutrophilia could affect the amount of s-Arg-1, we considered the normalized value (norm-s-Arg-1) defined as s-Arg-1/ANC to explore any correlation with semiquantitative parameters of PET at diagnosis. Medians of SUVmax and SULpeak were respectively 12.1 (range 2.7–23.2) and 7.8 (range 1.6–15.1).

SUVmax and SULpeak were weakly positively correlated to norm-s-Arg-1 (respectively, $r = 0.32$, $p = 0.03$, and $r = 0.31$, $p = 0.03$).

Response to treatment was observed in 17 of 21 early-stage and 25 of 39 advanced-stage patients. A level of 200 ng/mL s-Arg-1 resulted in 68% (95% CI 58–87) sensitivity and 61% (95% CI 42–86) specificity in predicting response status at 30 months (area under curve, 0.69, $p = 0.01$).

Reduction in s-Arg-1 could be observed as early as after two cycles of chemotherapy in all responsive patients, while s-Arg-1 remained elevated during and after treatment in non-responsive patients. s-Arg-1 was elevated in all relapsed patients at time of relapse and remained elevated after salvage treatment in the four non-responsive patients, while it was suppressed in brentuximab responders.

**Conclusion:** Baseline s-Arg-1 correlates with classical HL tumour burden, and serial levels correlate with response to treatment.

### 323
### VALIDATION OF CLINICAL PROGNOSTIC SYSTEMS FOR BONE MARROW INVOLVEMENT IN HODGKIN LYMPHOMA

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**Introduction:** Bone marrow involvement (BMI) is observed in 5–6% of patients with Hodgkin lymphoma (HL). In the era of positron emission tomography, PET/CT bone marrow biopsy (BMB) can be omitted. However, in case a baseline PET evaluation is not possible for various reasons, the prediction of BMI based on clinical characteristics is still relevant in order to avoid BMB in patients at low risk for BMI. The aim of this study was to evaluate and validate two clinical prognostic systems for BMI in patients with HL.

**Methods:** Five hundred and thirty-nine patients with HL were studied retrospectively. Patients were classified as being at low risk, intermediate risk and high risk for BMI based on a published prognostic system (Vassilakopoulos *et al*., *Blood* 2005), which takes into account age (<35 vs. ≥35 years), B symptoms, clinical Stage III/IV before BMB, iliac/inguinal involvement, anemia, and white blood cell (WBC) count ($<6 \times 10^9$/L). In addition, patients were scored as 0, 1, 2, and 3–5 based on another prognostic score (Levis *et al*., *Clin Lymphoma Myeloma* 2004), which takes into account B symptoms, liver involvement, involvement of four or more nodal regions, MC/LD histology, and presence of infradiaphragmatic disease. The accuracy of the two prognostic systems was evaluated based on BMB findings.

**Results:** Of 539 patients, 508 (94%) had a negative BMB and 31 (6%) had a positive one. When patients with a positive BMB were evaluated based on the first of these systems, it was found that age ≥35 years, presence of B symptoms, clinical Stage III/IV before BMB, iliac/inguinal involvement, anemia, and low WBC were significantly correlated with higher percentages of BMI. Among evaluable patients, 48%, 32%, and 20% were classified as low, intermediate and high risk, respectively. The corresponding risk of BMI was 0/251 (0%), 7/172 (4.1%), and 24/110 (21.8%), which were similar to those of the original publication (0.3%, 4.2%, and 25.5% in low-risk, intermediate-risk, and high-risk patients, respectively). The prognostic system of Levis *et al*. was also effective in predicting the risk of BMI, as B symptoms, liver involvement, involvement of four or more nodal regions, and infradiaphragmatic disease, but not MC/LD histology, significantly correlated with increased risk of BMI. Among evaluable patients, 42%, 26%, 15%, and 16% were scored as 0, 1, 2, and 3–5, respectively. BMI was observed in 0/214 (0%), 5/134 (3.7%), 7/78 (9%), and 13/81 (16%) patients, respectively. These figures are in agreement with those of the original publication (0.3%, 2.5%, 7.6%, and 27% in patients with scores 0, 1, 2, and 3–5, respectively).

**Conclusions:** The performance of the tested clinical prognostic systems for BMI in HL was validated in this study. The first prognostic system appeared to be superior, because it defined a larger subgroup of patients with minimal risk for BMI (48% vs. 42%). BMB could be safely omitted in these low (minimal)-risk patients. This observation can be particularly important whenever baseline PET/CT is not feasible, due to financial, geographic, or social reasons.

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### NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA: EARLY OUTCOMES

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**Introduction:** To evaluate treatment response, patterns of failure and prognostic factors for patients with nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) treated at the Tata Memorial Hospital (TMH) were analyzed.

**Materials and Methods:** Between January 2007 and July 2013, 61 patients with histologically proven NLPHL in the age group of 6–70 years (median 30.5 years) were treated at TMH. Forty-four (72%) were males. Majority had Stage I [29 patients (47%)] and Stage II [15 patients (25%)] disease. Fifteen (25%) had bulky disease at presentation. Sixteen (26%) were treated with involved field radiation therapy (IFRT) alone, 18 (30%) received chemotherapy (CTh) alone, while 23 (38%) received a combination of CTh followed by IFRT. Four patients underwent surgery as the local treatment. The IFRT doses were in the range of 20–36 Gy. Thirty-five (80%) patients received ABVD CTh. Five (8%) patients received rituximab. Primary MINE CTh was used for four (6%) patients.

**Results:** After a mean and median follow-up of 30 and 26 months, respectively, the 3-year disease-free survival (DFS) and overall survival were 83% and 98%, respectively. Complete response at completion of primary treatment was 92%. At the last follow-up, 54 (89%) were alive without disease. Two (3%) patients each had residual disease and in-field and out-of-field relapse. Four (6%) had disseminated relapse, and one (2%) had transformation to DLBCL. Seven (63%) out of 11 patients with disease relapse received salvage treatment (three IFRT, three CTh, and one both), of which four were disease free at last FU. Two patients have been planned for autologous stem cell transplantation. On univariate analysis, early stage (86% vs. 76%), absence of B symptoms (85% vs. 71%), and use of IFRT (82% vs. 64%) resulted in superior DFS. For patients with early-stage disease (Stages I and II), there was no difference in DFS (94%) between patients receiving IFRT alone and CTh + IFRT. The use of IFRT was associated with improved 3-year DFS (82% vs. 64%, $p = 0.57$). All patients tolerated treatment well without any Grade III or IV toxicities.

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**Conclusion:** NLPHL is associated with excellent overall survival. For patients with early-stage disease, IFRT alone results in similar outcomes compared to CTh + IFRT. Early stage at presentation, absence of B symptoms, and the use of IFRT confer superior outcome.

### 325
### TREATMENT OUTCOMES FOR PATIENTS WITH HODGKIN LYMPHOMA AND HIV INFECTION

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**Introduction:** Although the incidence and prevalence of AIDS-defining malignancies have decreased in the era of highly active antiretroviral therapy (HAART), the incidence and prevalence of Hodgkin lymphoma (HL) in the HIV-infected population continue to rise. Compared with the general population, HIV-infected patients exhibit up to a 15-fold increased risk for developing HL.

**Methods:** A retrospective analysis was performed to evaluate the outcome in the management of patients with HL and HIV/AIDS infection, diagnosed and treated at the National Institute of Oncology and Radiobiology and in the Institute for Tropical Disease 'Pedro Kouri'.

**Results:** Between 2000 and 2012, 33 patients were diagnosed with HL. Majority of patients were male (90%), with a median age of 39 years. Of these patients, 54.5% were diagnosed with advanced stage (Stages III and IV) and 42.6% had extranodal involvement. Mixed cellularity was the most frequent histological subtype (72.8%). ABVD regimen concomitant with HAART was used in the treatment of 96.7% of patients, with a median of six cycles. Complete response was achieved in 92.8% of the patients. The 5- and 10-year overall survival rates were 75.5% and 67.8%, respectively. The 5-year HL-specific survival was 83.5%. No significant impact in overall survival was found for age $\geq$ 40 years, CD4 cell count $<$ 200 cells/mm, hemoglobin $<$ 10.5 g/dl, and IPS.

**Conclusions:** In our series, the use of ABVD regimen concomitant with HAART has shown excellent results similar to those achieved in immunocompetent patients.

### 326
### EXCELLENT PROGNOSIS IN STAGE III AND IV HODGKIN LYMPHOMA TREATED WITH ABVD WITH COMPLETE RESPONSE ON PET

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**Introduction:** Combination chemotherapy such as doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) is the mainstay of treatment in advanced Hodgkin lymphoma (HL). The indications for consolidative radiotherapy (RT), however, are less well defined. Positron emission tomography (PET), which is now considered standard for staging and response assessment, was not routinely used in published studies examining the role of RT. Therefore, we conducted a study to evaluate survival outcomes and to identify prognostic factors in patients with advanced HL treated with ABVD in the PET era to guide patient selection for RT.

**Methods:** Patients were included in the study if they were over 18 years old with newly diagnosed Stage III or IV HL treated with ABVD at our institution from January 2005 to December 2012. All patients were staged with PET prior to chemotherapy and had a post-chemotherapy PET to assess response. Patients were given consolidative RT at the discretion of treating clinicians. Kaplan–Meier survival estimates were used to evaluate disease-free survival (DFS) [measured from PET complete response (CR) to disease recurrence or death], progression-free survival (PFS; measured from first day of chemotherapy to lymphoma progression or death), and overall survival (OS; measured from first day of chemotherapy to death of any cause). Cox regression models were used to assess association of potential prognostic factors (age, bulky disease defined as a single nodal mass of 10 cm or greater, stage, and presence of B symptoms) with survival outcomes.

**Results:** Forty-three patients were included in the study. The median age was 42 years (range 18–74 years) with males comprising 58.1%. The predominant histological subtype was nodular sclerosing (44.2%) followed by mixed cellularity (30.2%). The majority of patients had Stage III disease (62.8%) and B symptoms (65.1%). Five patients (11.6%) had bulky disease at presentation. Six patients (14.0%) completed consolidative RT.

The median follow-up was 45.7 months (range 6.6–102.1 months). PET CR after ABVD was seen in 35 patients (81.4%), partial response in three patients (7.0%), and progression in five patients (11.6%). Five-year DFS, PFS, and OS were 88.0%, 73.8%, and 86.2%, respectively. Five-year OS for patients with PET CR was 94.1%. Three of these patients relapsed, all in initial sites of disease, with one patient also relapsing at new sites. Age, bulky disease, stage, and the presence of B symptoms were not significant prognostic factors for DFS, PFS, or OS.

**Conclusions:** Patients with advanced-stage HL who achieve PET CR following ABVD chemotherapy have an excellent prognosis with a 5-year OS of 94.1%. We were unable to identify prognostic factors for relapse or survival due to the small number of patients and events. Large prospective studies are required for further clarification of risk factors to help select appropriate patients for consolidative RT.

### 327
### PROTON THERAPY FOR MEDIASTINAL LYMPHOMAS: AN 8-YEAR, SINGLE-INSTITUTION REPORT

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**Purpose/Objective(s):** Long-term cardiopulmonary toxicity and second malignancies are well-recognized sequelae of mediastinal radiation therapy (RT) for patients with lymphoma. Proton RT (PRT) has been used for years in other tumour subtypes to reduce dose to normal tissues. For patients with mediastinal lymphomas, efforts to reduce the lung volume, cardiac and spinal cord dose are needed to improve the therapeutic ratio. Herein, we report our single-institution experience using PRT for treatment of mediastinal tumours in patients with Hodgkin lymphoma (HL) or non-HL (NHL).

**Materials/Methods:** Adult patients (age $\geq$ 18) who received PRT for mediastinal lymphoma between 2006 and 2014 were included in this analysis. Patients completing definitive or salvage radiation were evaluated. For a subset of patients ($n$ = 20), 3D conformal photon plans were generated and compared to protons for target coverage, organs at risk and integral dose. Cox univariate analyses were performed for overall survival (OS), progression-free survival (PFS) and toxicity-free survival (TFS). Recorded toxicities included side effects related to both systemic and local therapies.

**Results:** Forty-six patients with HL ($n$ = 34) and NHL ($n$ = 12) were eligible for inclusion; the median follow-up for this study is 50.5 months. The cohort is comprised of 24 males and 22 females; median age at the time of treatment was 30.5 years (18–65 years). Most patients ($n$ = 33) had PRT as part of an initial course of therapy. However, 13 (28%) received PRT as part of salvage therapy, 4 having received prior mediastinal RT with photons. A median dose of 36 GyE (25.5–39.6 GyE) was delivered at 1.5–2.0 Gy per fraction. PRT reduced mean lung dose by 52% and V20 by 49%. Right coronary artery dose did not change significantly with PRT due to proximity of the target volumes; however, left coronary artery dose decreased by 30–90% (mean dose 12 GyE), and left ventricle dose was reduced by 72%. Mean dose to spinal cord with PRT was 8 Gy (5–30 Gy). At 5 years, OS was 98% (CI 0.85–0.99), PFS 80% (CI 0.63–0.90) and TFS 78% (CI 0.58–0.89). All relapsed patients went on to receive salvage therapy. Nine patients developed late toxicities related to treatment; four patients developed hypothyroidism following PRT, four patients reported neuropathy due to chemotherapy and two patients had significant cardiac and pulmonary toxicity related to prior photon RT before starting PRT.

**Conclusions:** This is the largest single-institution experience using PRT for patients with HL or NHL involving the mediastinum. PRT significantly reduces cardiac, lung, spinal cord and integral dose. It is well tolerated and has excellent local control. Additional follow-up is needed to assess long-term control and late toxicity.

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**HODGKIN LYMPHOMA: 5-YEAR OUTCOME AFTER ABVD IN ROUTINE CLINICAL PRACTICE FROM A SINGLE INSTITUTION**

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**Introduction:** Hodgkin lymphoma (HL) is a highly curable disease of young patients. ABVD is an effective regimen with low toxicity, allowing patients to maintain a good quality of life during treatment administered on an outpatient basis. Therefore, from the mid-1970s, ABVD is the preferred first-line treatment chemotherapy for patients with all disease stages. As we use ABVD at our institution uniformly, we retrospectively evaluated outcome of HL patients diagnosed between January 2007 and December 2011.

**Methods:** Two hundred and twenty-eight patients with histopathologic diagnosis of classical variant of HL were identified. The median age (range) at diagnosis was 33 (17–77), 112 (49%) patients were male, 143/225 (63%) had Stage I/II disease and bulky disease was present in 121/224 (54%) patients. Extranodal sites were involved in 42/226 (18%) cases including bone marrow in 5/223 (2%) and spleen in 26/226 (11%). B symptoms were present in 121/224 (54%) of patients, and there were low hemoglobin and albumin levels in 96/222 (43%) and 55/213 (26%) of patients, respectively. Elevated LDH was present in 72/218 (33%) cases.

Two hundred and nineteen (96%) patients had induction treatment ABVD followed by radiotherapy if indicated. Other regimens were EVA, CHOP, COPP and COPP/ABV administered due to medical condition accessed by physician. Patient data were collected from paper and electronic charts. Diagnoses were verified by a local pathologist (GR). OS and PFS were calculated using the Kaplan–Meier method. Multivariate Cox's analysis at the 5% level of significance was used to identify significant predictors for OS and PFS.

**Results:** Overall response according to Cheson (1999) was 92% (209/228), including complete response in 73% (166/228) of patients. Of 227 patients, 37 (15%) had dose reductions due to adverse events on treatment. Doses of doxorubicin and vinblastine were most often reduced. Among 62 events of progression (PD), primary refractory disease (progression up to 3 months after completion of induction treatment) was present in 15 (6%) cases. Thirty-five patients underwent high-dose therapy followed by autotransplantation. In 13 cases, PD occurred after transplantation. Twenty patients died in the follow-up period, mostly due to HL. One fatal cardiovascular event occurred during ABVD.

For the whole group, median follow-up was 57 months (range: 2.3–114), 5-year OS was 90% (95% CI [86%, 94%]) and 5-year PFS was 72% (95% CI [66%, 78%]). Age below 33 years ($p = 0.012$), clinical Stage I/II ($p = 0.001$) and normal albumin level ($p = 0.005$) were predictive for longer OS in multivariate analysis. Age above 33 ($p = 0.43$), clinical Stage III/IV ($p < 0.001$), spleen involvement ($p < 0.001$) and B symptoms ($p = 0.033$) were negative prognostic factors for PFS.

**Conclusions:** Our 5-year PFS and OS data on ABVD are consistent with the results reported by others.

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**UPDATED REVIEW OF NAMED PATIENT PROGRAM OUTCOMES WITH BRENTUXIMAB VEDOTIN FOR RELAPSED/REFRACTORY HODGKIN LYMPHOMA AND SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA**

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**Abs 329 - Table 1.**

| NPP cohort, *N* (NPP) | R/R disease: HL (*n*) | R/R disease: ALCL (*n*) | R/R disease: Other (*n*) | Median age, years (range) | Median prior therapies (range) | Primary refractory/no response to last prior therapy (%) | Prior autologous/prior allogeneic stem cell transplant (%) |
|---|---|---|---|---|---|---|---|
| Italy | | | | | | | |
| 65 (65) | 65 | — | — | 27.5 (12–66) | 4 (2–13) | 69/80 | 88/5 |
| 2 (2) | — | 2 | — | 65/73 | 3.5 (3–4) | NR | NR |
| 1 (1) | 1 | — | — | 21 | 2 | NR | 100/— |
| Germany | | | | | | | |
| 45 (34) | 34 | — | — | 35 | 4 (2–12) | 62/64 | 87 |
| 16 (16) | 14 | 2 | — | 48.5 (HL: 45 [24–74]) | 3 (HL: 3 [2–6]) | 69/— (HL: 64/—) | 0/— |
| 4 (4) | 4 | — | — | 19.5 (18–39) | 6 (4–8) | NR | 25/100 |
| 1 (1) | — | 1 | — | 29 | 4 | —/100 | 0/— |
| France | | | | | | | |
| 45 (35) | 32 | 13 | — | 38 (20–71) | 4 (2–8) | 87 | 88/5 |
| 24 (24) | 24 | — | — | 35 (20–60) | 2 (2–4) | 42 | 42/17 |
| 9 (9) | 6 | 3 | — | 36 (21–59) | 3 (2–5) | NR | 78/— |
| UK | | | | | | | |
| 24 (24) | 18 | 5 | 1 | 41.5 (21–78) | 3 (2–8) | —/71 | 33/— |
| Turkey | | | | | | | |
| 10 (NR) | 10 | — | — | 26 (22–30) | 4 (3–5) | 50/80 | 100/— |
| 58 (58) | 58 | — | — | 26 (13–62) | 4 (2–7) | 49/72 | 80/2 |
| Switzerland | | | | | | | |
| 3 (NR) | 3 | — | — | 26 (25–32) | 3 (3–7) | NR | 67/— |
| China | | | | | | | |
| 1 (1) | 1 | — | — | 17 | 4 | —/100 | 100/— |
| Asia | | | | | | | |
| 22 (22) | 22 | — | — | 30 (16–57) | NR | 55/NR | 77 |

NR, not reported.

[a]Combined percentage for any patient receiving hematopoietic stem cell transplant.

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**Introduction:** Brentuximab vedotin (ADCETRIS®), a novel anti-CD30 antibody–drug conjugate, received accelerated approval in the USA in 2011 for the treatment of relapsed Hodgkin lymphoma (HL) and systemic anaplastic large-cell lymphoma (sALCL). In ~60 countries outside of the USA/Canada, patients who meet the US label criteria are able to receive brentuximab vedotin via the named patient program (NPP). We re-evaluated published reports of NPP outcomes with brentuximab vedotin, including the latest reports of experience in Turkey and Asia, to assess brentuximab vedotin's efficacy and safety in routine clinical practice in the context of the pivotal Phase 2 trials in relapsed/refractory (R/R) HL and sALCL on which US approval was based.

**Methods:** A systematic literature review of PubMed articles and pre-specified congress abstract books (data cutoff: 26 Sep 2014) identified NPP publications. Patient demographics, treatment histories, response, survival, and safety data were reviewed. For all NPP cohorts reporting data, overall response rates (ORR) and complete remission (CR) rates were pooled and summed by indication. Adverse event, dose reduction, and discontinuation rates were calculated as a proportion of the total number of patients within the NPP cohorts reporting the specific event.

**Results:** Twenty-three NPP publications were identified describing 330 patients in 16 unique cohorts treated in 8 countries; 309 received brentuximab vedotin in the NPP. Of the 319 patients with a specified diagnosis, 292 had R/R HL, 26 had R/R sALCL, and 1 had CD30+ T-cell lymphoma (not specified). Key characteristics of patients treated under the NPP, by country, are shown in Table 1. The ORR and CR rate were 64% and 25%, respectively, for R/R HL and 76% each for R/R ALCL. Results in patients from Turkey (ORR, 64%; CR 27%) and Asia (ORR, 73%; CR 18%) were comparable to those observed in Western patients. Overall, incidences of Grade 3/4 neurologic and hematologic toxicities were 5% and 12%, respectively.

**Conclusions:** Review of updated NPP data suggests that the efficacy and tolerability of brentuximab vedotin in R/R HL and sALCL in 'real-world' practice are similar to those reported in the two pivotal Phase 2 trials. Results in Eastern patients, who only accounted for a small proportion of the pivotal Phase 2 trial populations, appear similar to those of Western patients in routine clinical practice.

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### MEDIAN OVERALL SURVIVAL META-ANALYSIS COMPARING BRENTUXIMAB VEDOTIN WITH OTHER THERAPIES IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA POST-AUTOLOGOUS STEM CELL TRANSPLANT

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**Introduction:** Brentuximab vedotin received conditional approval in Europe and accelerated approval in the USA for the treatment of CD30+ relapsed/refractory (R/R) Hodgkin lymphoma (HL) and relapsed HL, respectively. Currently, no head-to-head trial data are available to compare brentuximab vedotin with other therapies. This meta-analysis compared the median overall survival (mOS) of brentuximab vedotin reported in long-term follow-up data from a pivotal Phase 2 trial (NCT00848926) with published results of other therapies for the treatment of R/R HL post-autologous stem cell transplant (ASCT).

**Methods:** A systematic literature review identified studies (Feb 1993–Jan 2014) that reported survival outcomes following conventional and experimental therapies in R/R HL. Studies were included where ≥50% of patients had failed ≥1 ASCT. Kaplan–Meier curves were used to reconstruct individual patient-level survival data. Patients were grouped by type of post-ASCT treatment received; reconstructed datasets were used to estimate mOS. A censored median regression model was used to compare mOS in these groups with the mOS observed in the pivotal brentuximab vedotin trial. A sensitivity analysis was conducted among studies reporting a 100% prior-ASCT patient population.

**Results:** Forty studies ($N = 2518$) reporting treatments other than brentuximab vedotin were identified; 1 Phase I/II, 10 Phase II, 8 prospective cohort, and 21 retrospective. Reported study treatments were chemotherapy ($n = 8$), allogeneic stem cell transplantation (allo-SCT, $n = 21$), and other therapies ($n = 11$). The median age of all patients ranged from 25 to 51 years, the median number of prior regimens ranged from ≤2 to 5, and the number of patients with prior-ASCT ranged from 52% to 100%. In the pivotal brentuximab vedotin study, the median age was 31 (range: 15–77), the median number of prior chemotherapy regimens was 3.5 (range: 1–13), and all patients had prior ASCT. The mOS, estimated from the start of treatment under evaluation, was 26.4 months (95% CI [23.5, 28.5]) across all 40 studies compared with 40.5 months (95% CI [30.8, NA]) reported for brentuximab vedotin ($p < 0.0001$). The difference in mOS between brentuximab vedotin and chemotherapy, allo-SCT, and other therapies was 17.7 (95% CI [10.6, 24.7]; $p < 0.0001$), 12.5 (95% CI [8.2, 16.9]; $p < 0.0001$), and 15.2 months (95% CI [4.9, 25.5]; $p = 0.0037$), respectively. For the 11 studies reporting a 100% prior-ASCT rate ($n = 662$), the mOS was 28.1 months (95% CI [23.9, 34.5]), and the difference in mOS between brentuximab vedotin and chemotherapy, allo-SCT, and other therapies was 19.0 (95% CI [12.9, 25.1]; $p < 0.0001$), 9.4 ($p > 0.05$), and 6.8 months (95% CI [1.2, 12.5]; $p = 0.0018$), respectively.

**Conclusions:** In the absence of randomized controlled trial data, these results suggest brentuximab vedotin is associated with longer mOS compared with other therapies and improves long-term survival in R/R HL post-ASCT.

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### FOUR DRUGS AND 4 DAYS IN AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA PATIENTS

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**Introduction:** Due to the lack of evidence regarding the best conditioning regimen for the realization of HSCT in patients with lymphoma, new schemes have been proposed in an attempt to reduce toxicities and hospitalization times and costs, without compromising survival. We evaluated a conditioning protocol with lomustine, etoposide, cytarabine and melphalan (LEAM) for a short period of time for autologous stem cell transplantation in patients with lymphoma.

**Method:** In the first step, the maximum tolerated dose of lomustine was set at 300 mg/m$^2$, by means of a 3:3 scale. The second step consisted of evaluating the toxicity of the protocol with the use of lomustine (300 mg/m$^2$ D-4), followed by etoposide (1 g/m$^2$ D-3), cytarabine (4 g/m$^2$ D-2) and melphalan (140 mg/m$^2$ D-1), in 30 patients. The results were compared with the historical series of 63 patients submitted to conditioning with carmustine, cyclophosphamide and etoposide (CBV). The onset and duration of neutropenia, toxicities according to the WHO criteria, mortality related to treatment (MRT), progression-free survival (PFS), overall survival (OS) and response duration (RD) were evaluated.

**Results:** Of the 93 patients evaluated, 60.2% were patients with Hodgkin lymphoma (HL) with a homogeneous distribution between the two protocols (CBV: 61.9%; LEAM: 51.7%; $p = 0.6$), as well as for age ($p = 0.79$), presence of refractory disease at the time of auto-HSCT ($p = 0.6$) and the number of prior chemotherapy regimens ($p = 0.39$). The patients in the historical control had an earlier onset of neutropenia ($p = 0.02$), which lasted longer than in those who received LEAM ($p = 0.008$), with

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a trend towards lower average hospitalization time ($p = 0.08$). Although there were no statistically significant differences between the main toxicities studied, there was a trend to lower MRT in the LEAM group (6.6% vs 19%), which resulted in 84% of OS after 30 months for the LEAM group and 56% for the CBV group ($p = 0.02$). When evaluating the RD, there was no difference between the two treatment groups for patients who had not died in the first 100 days ($p = 0.72$), indicating no difference in PFS ($p = 0.22$).

**Conclusion:** LEAM proved to be a secure protocol, of short duration, with no lower survival rates than the protocol used previously in our environment. It did, however, delay the time of neutropenia onset and reduced its duration. OS at 30 months was higher in the LEAM group, probably as a result of the lower MRT with this protocol, which demonstrated a good response quality as evidenced by a similar PFS to the CBV conditioning.

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**TREATMENT OUTCOMES OF RELAPSED/REFRACTORY HODGKIN AND AGGRESSIVE NON-HODGKIN LYMPHOMA PATIENTS**

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**Introduction:** Refractory or relapsed (RR) disease after frontline therapy dramatically increases morbidity and mortality in patients diagnosed with Hodgkin lymphoma (HL) or aggressive non-HL (NHL). Different salvage therapeutic approaches are defined with a highly variable therapeutic outcome. We aimed to analyze therapeutic outcomes of our patients who had RR disease after frontline therapy.

**Methods:** We have retrospectively analyzed our lymphoma database and selected HL or aggressive NHL patients who had RR disease to be included in the analysis. Patients' demographics, clinical features, and therapeutic outcomes were recorded.

**Results:** Fifty-six patients with a median age of 45.5 (17–72) were included in the study. Of these patients, 44.6% were diagnosed with HL, and 33.9%, 14.3%, and 7.1% were diagnosed with diffuse large B-cell NHL, peripheral T-cell lymphoma, and mantle cell lymphoma, respectively. Of the patients diagnosed with HL, 3.6% had Stage 1, 12.5% had Stage 2, 25% had Stage 3, and 58.9% had Stage 4 disease. Of these patients, 9.3% were able to achieve complete remission (CR) after frontline chemoradiotherapy, 19.6% could achieve partial remission (PR), and 21.4% had a stable disease (SD); 19.6% had a progressive disease after frontline treatment. Of the patients, 55.4% had a progressive disease less than 6 months, and 12.5% of the patients had a progressive disease after more than 24 months. ESHAP regimen was received by 85.7% of patients as a second-line therapy. Other second-line therapeutic combinations were ICE regimen (7.1%), bendamustine (3.6%), and DHAP regimen (1.8%). All second-line regimens were applied with the addition of rituximab when needed. The treatment of 42.9% of patients was high-dose chemotherapy with autologous stem cell transplantation. Overall response rate of second-line therapy was 41.1% (16.1% CR and 25% PR). Twenty-five per cent of patients had SD, and 33.9% had progressive disease after second-line therapy. A third-line treatment was received by 32.2% of patients. Gemcitabine-based and vinorelbine-based regimens were the most common third-line treatment options. Fifty per cent of patients received gemcitabine and vinorelbine as a third-line therapeutic option, and 27.8% received IGEV or IGEV–bortezomib combination regimens. Remaining patients received bendamustine (11.1%), hyper-CVAD (5.6%), or ESHAP (5.6) regimens. None of the patients were able to achieve a CR after third-line therapy, 27.7% of patients could achieve PR and 38.9% had a stable disease after third-line therapy, and 33.3% of patients had a progressive disease even after third-line therapy. Overall, 22 patients (39.3) had died during the follow-up.

**Conclusions:** Our results indicate that the responses achieved after first-line therapies are still limited considering the patients diagnosed with HL or aggressive NHL who had a refractory or relapsed disease. There is an ongoing need for more targeted therapies considering the relatively low response rates despite growing numbers of different therapeutic approaches and high-dose chemotherapies applied with autologous stem cell transplantation.

**333**
**CLINICAL OUTCOME OF PATIENTS WITH HODGKIN LYMPHOMA REFRACTORY OR IN RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION**

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Although Hodgkin lymphoma (HL) is largely curable with first-line therapy, approximately one-third of patients will not have a complete response to frontline treatment or will subsequently relapse. Only in 50% of these patients will effective salvage be achieved with conventional therapies. The prognosis is particularly poor for those patients who are refractory or relapse following an autologous stem cell transplant (ASCT). Reduced-intensity conditioning (RIC) is increasingly used as a potentially curative option. We performed a retrospective analysis of 25 adult patients (15 women/10 men) with HL, who relapsed within 12 months or had progressive disease after ASCT, in order to analyze clinical outcome. Disease status at ASCT was complete remission in 7 patients (28%), sensitive disease in 14 (56%), and refractory disease in 4 (16%). Median time from ASCT to relapse was 7 months (range 0.5–11); 12 patients (48%) had Stages 3 and 4 at relapse/progression. B symptoms were present in 40% of the patients, bulky disease in 20%, and extranodal involvement in 56%. Treatments following relapse/progression were the standard BEACOPP regimen in 10 patients (40%), second ASCT in 5 (20%), brentuximab vedotin without further therapy in 2 (8%), and RIC in 8 (32%); the therapeutic regimens used as a 'bridge' to RIC were brentuximab vedotin in 6 (24%) and bendamustine in 2 (8%).

**Results:** Overall response rate was 64%, including 36% complete remission and 28% partial response. Progression occurred in 24%, and 12% had stable disease. No difference in response rate was found among the treatment regimens. RIC was administered in eight patients (four in complete and four in partial remission). Estimated overall survival at 40 months was 72% for the whole population, 88% for patients who underwent RIC, and 58% for those who did not. Estimated progression-free survival at 40 months was 50%, 62% for the RIC group, and 42% for the non RIC group. After a median follow-up of 36 months (range 3–84), 18 of the 25 patients are alive (72%) and 11 (44%) still in complete remission (45% of them underwent RIC).

**Conclusions:** Patients with HL who fail ASCT have a very poor prognosis, and the goal of treatment should be to ultimately refer them to RIC. Even if several questions are still open, this approach should be proposed for these poor-prognosis patients. Brentuximab vedotin is useful in order to reduce the disease burden before RIC.

**334**
**OUTCOMES IN RELAPSED HODGKIN LYMPHOMA: RESULTS FROM THE WEST OF SCOTLAND HAEMATO-ONCOLOGY NETWORK**

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**Introduction:** There is consensus across international guidelines that standard treatment of relapsed Hodgkin lymphoma (HL) should include salvage chemotherapy and autologous transplantation. Increasingly, a risk-adapted strategy is employed with consideration of more aggressive transplant approaches and/or additional radiotherapy in higher-risk cohorts. Early relapse and advanced stage at relapse define the worst prognostic group. Our network employs telelinked multidisciplinary team discussion to agree to risk-adapted management plans for our patients. We report here the outcomes for relapsed HL patients from our network.

**Methods**: The West of Scotland Haemato-oncology Network covers a population of 2.6 million. We collected data on patients with relapsed HL between 2007 and 2010, excluding those with primary refractory disease or relapse within 90 days of completion. Patients had to be eligible for further treatment. Patient demographics, initial stage of disease and time to relapse were recorded alongside the modality of second-line treatment. We measured outcomes including ongoing remission, time to second relapse and time to death.

**Results**: Between 2007 and 2010, 267 patients were diagnosed with HL. Thirteen patients relapsed and were eligible for further treatment: seven women and six men, aged 18–72 years (median 36). Time to relapse ranged from 15 to 143 weeks (median 58). Second-line treatment included involved field radiotherapy (IFRT)

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alone (three patients), salvage chemotherapy and autologous BMT (eight patients) and salvage chemotherapy and allogeneic BMT (two patients). With IFRT alone, two remain in remission at 5 years. Neither received salvage chemotherapy, and both had Stage 1A disease at relapse. One patient relapsed with cutaneous disease at 58 weeks after radiation but had not been fit for BMT at relapse and survived a further 78 weeks from his second relapse.

In those treated with autologous BMT, six remain in remission (median follow-up 183 weeks, range 146–248). One relapsed at 30 weeks, was poorly compliant with further treatment and died at 110 weeks post-BMT. One patient relapsed at 6 weeks post-BMT and died at 40 weeks. Of the two patients who received an allogeneic BMT, one developed respiratory complications and died at 8 weeks. The other relapsed at 30 weeks, received a donor lymphocyte infusion and remains under active surveillance. Overall progression-free survival (PFS) at 4 years in this relapse cohort was 69% with one treatment-related death. Overall survival (OS) was 77%.

**Conclusion**: PFS and OS for patients with relapsed HL in our network compares favourably with internationally published data. Our treatments ranged from radiotherapy alone to allogeneic transplant in the poor-risk candidates. Of note, IFRT alone proved effective in selected good-risk patients.

## INDOLENT LYMPHOMA AND CLL

### 335
### SURVIVAL ANALYSIS OF FOLLICULAR LYMPHOMA IN A NATIONAL REGISTRY FROM THE SPANISH ONCOLOGY LYMPHOMA GROUP

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**Background:** In Spain, between 3000 and 5000 new cases of follicular lymphoma are diagnosed each year. It is the second most common tumour of lymphoid lineage. The development of national registers has helped us to understand the clinical and pathological characteristics of the patients in our area. Treatment for follicular lymphoma is not well established, and we need to have better knowledge of the survival patterns of these patients.

**Material and Methods:** One thousand and seventy-six patients diagnosed with follicular lymphoma, between 1986 and 2012, and treated at the oncology department of 17 Spanish hospitals were reviewed. A survival analysis was made by using SPSS v19.

**Results:** Fifty-three per cent were women. The mean age at diagnosis was 58 years, with Stages III–IV in 72%, B symptoms in 21%, ECOG > 1 in 10%, bulky disease in 24%, bone marrow involvement in 39%, and extranodal involvement in 15%. In first-line treatment, 69% of the patients received chemotherapy associated with rituximab (59% with anthracycline-based chemotherapy and 10% without anthracyclines), 24% of the patients received chemotherapy without rituximab (15% based on anthracycline and 9% non-anthracycline) and 3.4% of patients received rituximab monotherapy. In only 2.2% of the patients was observation at diagnosis the first approach. Intermediate or high FLIPI, ECOG > 1, B symptoms, and treatment with rituximab (monotherapy?; any of the patients in this registry received maintenance?) were worse factors in multivariate analysis. The median overall survival was 234 months (95% CI 212–255 months).

**Conclusion:** The median overall survival of patients diagnosed with follicular lymphoma is over 20 years in our series. FLIPI and factors-linked clinical general status are adverse factors. First-line rituximab increases survival.

### 336
### ROLE OF HLA SPECIFICITIES IN FOLLICULAR LYMPHOMA

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**Introduction:** Follicular lymphoma (FL) is the most common indolent lymphoma, accounting for ~20–30% of all non-Hodgkin lymphoma (NHL). It is an incurable disease with frequent relapses and shorter response to further treatments, developing drug resistance. Recently, using GWAS, polymorphisms in the 6p21.3 region were related to susceptibility to developing NHL, including FL. The role of the HLA system in antigen presentation could be related to susceptibility and disease control. Previous studies show an association between HLA alleles and/or haplotypes and NHL. However, information regarding FL is little. We aimed to analyse the role of HLA specificities in the development and prognosis of FL.

**Patients and Methods:** A total of 149 consecutive patients from a single centre who were diagnosed with FL between 2000 and 2010 were included in the study. Grade 3b FL or those cases with DLBCL areas were not considered. Healthy donors ($n = 1940$) from the CyL Bone Marrow Donors Registry were used as the control group. HLA typing of Classes I (A, B and C) and II (DRB1 and DQB1) at a low resolution level was performed according to the EFI methodology. Allelic frequencies were estimated by direct counting. Phenotypic frequencies between groups were compared with the Fisher test, considering $p < 0.05$ as statistically significant. $p$-Values were corrected ($pc$) according to the number of valid comparisons (Bonferroni correction). Survival analyses were carried out using Kaplan–Meier. Differences between curves were estimated using the log-rank test (SPSS 20.0).

**Results:** There were no statistical differences in specificity frequencies of HLA Class I. However, a higher incidence of HLA-DRB1*01 was observed in patients than in donors (46% vs 19.5%, $p < 0.0001$, $pc < 0.0013$). A total of 82% of the patients received treatment for FL, 68% of them with rituximab. Those patients receiving a CHOP-like plus rituximab regimen and carrying HLA-DRB1*13 had worse 10-year OFS (54% vs 85%, $p = 0.05$) compared with patients without this specificity.

**Conclusions:** The present study suggests an association between HLA-DRB1*01 and FL development, in line with previous studies. This study should be considered as preliminary, requiring a higher sample size.

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**These authors had equal contribution in this work.*

### 337
### SERUM ALBUMIN RETAINS INDEPENDENT PROGNOSTIC SIGNIFICANCE IN ADVANCED-STAGE MARGINAL ZONE LYMPHOMA PATIENTS TREATED WITH R-CVP

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**Introduction:** Marginal zone lymphoma (MZL) is a distinct subgroup of non-Hodgkin lymphoma that typically follows an indolent clinical course and long survival. Although successful results were reported with local treatment or several antibiotics for localized MZL, it presents as a disseminated disease in one-third of the cases at diagnosis. Rituximab plus CVP (R-CVP) regimen is one of the effective treatments for patients with advanced-stage MZL. In this study, we conducted retrospective analyses of treatment outcomes of advanced-stage MZL patients who are treated with R-CVP as a first-line treatment.

**Patients and Methods:** Between August 2006 and June 2013, patients with histologic diagnosis of advanced-stage MZL from 15 different institutions in Korea were included for analysis in this study. R-CVP combination was administrated for patients with previously untreated Ann Arbor Stage III and IV MZL. Treatment consisted of rituximab 375 mg/m$^2$, cyclophosphamide 750 mg/m$^2$ and vincristine 1.4 mg/m$^2$ (maximum 2.0 mg), given intravenously on Day 1, and oral prednisone 100 mg on Days 1–5. The following clinical data were collected from the record: patient demographics, complete blood count, lactic dehydrogenase (LDH) level, Ann Arbor stage, IPI, bone marrow findings, the presence of B symptoms, performance status, date of diagnosis, type of treatment, treatment response, date of relapse, date of last follow-up and cause of death.

**Results:** The study population consisted of 80 advanced-stage MZL patients. They comprised 44 males and 36 females, with a median age of 55 years (range, 16–81 years). The stages of patients were III and IV in 23.8% and 76.2%, respectively. The IPIs $< 3$ and IPIs $\geq 3$ were 72.5% and 27.5%, respectively. BM involvement was observed in 30%. Twelve patients (15%) were nodal MZL. Multiple MALT sites involving patients were 41.3%. Overall response rate was 91.3% including 73.8% of CR. Three-year progression-free survival rate was 70%. Using multivariate analysis, we found that serum albumin $< 3.9$ g/dL had an independent influence on poor PFS.

**Conclusion:** Our study shows that serum albumin $< 3.9$ g/dL is an independent prognostic marker in advanced-stage MZL patients treated with R-CVP.

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### MAST CELL AND FOLLICULAR LYMPHOMA: ASSOCIATION OR RELATIONSHIP?

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**Background:** Follicular lymphoma (FL), a well-defined entity, delineated in current classification schemes, is said to be very difficult to cure. After several years, it may recur, often in the form of a higher-grade lymphoma. In the early stage, its diagnosis may be challenging, as reactive follicular hyperplasia shares many of its morphological patterns. This well-differentiated lymphoma tends to keep markers of centrifollicular normal cells, that is, CD10 and BCl6. A better understanding of its pathophysiology and evolution might come from the knowledge of its microenvironment, such as that reported in Hodgkin lymphoma and large B-cell lymphomas. In this study, we describe the amount and localization of mast cells in a prospective monocentric series of FL, stained with CD117 and tryptase antibodies.

**Material and Methods:** Starting from October 2010, two antibodies were added to the panel of immunohistochemical markers in suspected lymph node, bone marrow and mediastinal lymphomas, namely CD117 (rabbit polyclonal, 1/50, Dako, Trappes, France) and tryptase (clone AA1, 1/100, Serotec, France) in order to describe the amount and distribution of mast cells in formalin-fixed paraffin-embedded material.

Four-micrometre sections were mounted on glass slides, dried and processed in Benchmark Ultra (Ventana-Roche Diagnostics, France). Immunostaining was performed in 45 FL patients; 38 were low grade, and 7 high grade.

**Results:** In follicular cases (36 cases), few mast cells (less than three per nodule) were found inside the neoplastic follicles. In diffuse (three cases) or nodular and diffuse (six cases) cases, very few mast cells (less than 0.1% of cells) were observed. Conversely, numerous mast cells were observed around the neoplastic follicles, even at low-power examination, sometimes in a continuous ribbon pattern. In partially diffuse cases, numerous mast cells were present along the margin of tumour. In three cases, mast cells were associated with numerous thin-walled venules, suggestive of angiogenesis. Correlations with clinical outcome are to be presented in the poster.

**Comments**: This perinodular or peridiffuse distribution of mast cells in FL has not yet been reported, to our knowledge. It was alluded to by Duse *et al*., in a paper on angiogenesis and mast cell in FL. Taskinen *et al*. counted mast cells in two or three HPF, in a series of TMA and whole sections of FL, with discordant results and prognostic correlation. We believe whole sections are preferable in this setting.

**Conclusion:** This unreported distribution of mast cells in FL may assist in diagnosis of FL and opens avenues for understanding the relationship between FL and its environment.

### 339
### THE IMPACT OF A WATCHFUL WAIT STRATEGY IN PATIENTS WITH NEWLY DIAGNOSED FOLLICULAR LYMPHOMA

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**Background:** Recent clinical studies indicate that rituximab (R) and/or chemotherapy improve overall survival of CD20-positive B-cell malignant lymphoma including follicular lymphoma (FL). In contrast, previous clinical studies reveal that a watchful wait (WW) strategy is one of the initial therapies for patients with low-grade lymphomas, especially with FL. Although tumour burden is an important factor in selecting the WW strategy, it is still controversial, so that we retrospectively analysed 126 patients with newly diagnosed FL including 22 patients in the WW group.

**Methods:** Data of 126 patients between 2002 and 2014 were gathered at our institution, and OS, PFS and prognosis factors were analysed. Survival analysis and comparisons between groups were performed by the Kaplan–Meier procedure and logistic analysis using JMP10 (JMP Company, Tokyo, Japan).

**Results:** The median age of the 126 patients was 58.7 years (range, 27–82 years). The sex ratio was 60:67 (male : female). Fourteen patients were in Stage 1, 18 in Stage 2, 37 in Stage 3 and 57 in Stage 4. Pathological diagnosis was Grade 1 for 42 patients, Grade 2 for 38 patients, Grade 3a for 16 patients and Grade 3b for 28 patients. Median follow-up duration of all patients was 5.8 years (range, 0.3–20.2 years). The OS rates at 5 and 10 years were 81.7% and 70%, respectively, and the PFS rates at 5 and 10 years were 61% and 43%, respectively. Although there was no significant difference in OS between the stages or between four pathological subtypes (Grades 1, 2, 3a and 3b), elevated soluble interleukin 2 (sIL-2) levels and ages were found to be prognostic factors. In terms of the initial therapy, 104 patients were treated with at least one treatment (Tx), and 22 patients were followed without any medications as WW. The OS of WW was better than that of Tx (10-year OS: 86% and 72%, respectively), and the same PFS was observed among the two groups (10 years: 43%). Two patients were dead in WW because of disease activity and acute myocardial infarction. Focusing on the patients' background, the median age and the population of the pathological subtypes were almost similar between the two groups; meanwhile, the ratio of Stages 3 and 4 in Tx was higher than that in WW (81% and 43%, respectively). The serum LDH and the serum sIL-2 in Tx were significantly higher than those in WW. The median total number of therapies during the clinical course was 1.78 in Tx (range: 1–5) and 0.54 in WW (range: 0–3).

**Conclusions:** In this study, the initial treatment was selected based on the physician's judgment, and the patients with lower tumour burden were collected in WW. Our data revealed that only one patient in WW was dead due to disease activity, resulting in better OS of WW. However, the stages and serum LDH were not detected as prognostic factors, so that tumour burden might not be important in

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considering the initial therapy. To investigate the superiority of the WW strategy, a prospective study will be required with the consideration of several factors including serum sIL-2.

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### A PHASE II TRIAL OF SHORT-COURSE FLUDARABINE, MITOXANTRONE AND RITUXIMAB FOLLOWED BY $^{90}$Y-IBRITUMOMAB TIUXETAN IN UNTREATED FOLLICULAR LYMPHOMAS: 7-YEAR FOLLOW-UP

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**Introduction:** As follicular lymphoma is characterized by an indolent clinical course, affected patients generally experience frequent disease relapse with shorter duration of remission at every disease recurrence. The addition of consolidative radioimmunotherapy to standard chemoimmunotherapy approaches has led to enhanced results in terms of response and progression-free survival (PFS) rates. The application of short-course inductions has helped in reducing chemo-related toxic effects. Benefits deriving from combined-strategy regimens require however a confirmation in the long term.

**Methods:** From December 2006 to November 2008, 55 untreated patients with intermediate/high-risk follicular non-Hodgkin lymphoma were enrolled in a non-randomized multicentre Phase II trial of four induction cycles of fludarabine, mitoxantrone and rituximab (FMR) and a subsequent consolidating single administration of $^{90}$Y-ibritumomab tiuxetan, given 8–14 weeks later (Zinzani *et al*., *Annals of Oncology*, 2011). Patients' median age was 56 (range, 26–84) years; 19 of 55 (34.5%) patients had a high risk, FLIPI score (≥3). All patients obtaining at least a partial response have been followed through a whole-body computed tomography scan every 6 months for the first 2 years and every year thereafter.

**Results:** The complete response (CR) rate after the entire treatment regimen was 89.0% (49/55 patients). With a median follow-up of 7 years, 30/49 (61.2%) patients are still in continuous CR, whereas 21/55 (38.1%) experienced disease progression (PD). The disease-free survival (DFS) at 6.4 years was 68%, with a PFS at 6.7 years of 50.1%. The overall survival at 7.8 years was 72.7%. Death occurred in eight (14.5%) patients; among them, four died of a secondary acute myeloid leukaemia, developed after 3 (in three cases) and 4 years since enrolment.

**Conclusions:** Short-course FMR with $^{90}$Y-ibritumomab tiuxetan consolidation confirms its efficacy in patients with intermediate/high-risk follicular lymphoma also in the long term, as demonstrated by favourable 7-year DFS and PFS rates. These results are comparable with those obtained with six FMR cycles in larger patient series.

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### FINAL RESULTS OF A SHORT COURSE OF BENDAMUSTINE + RITUXIMAB FOLLOWED BY $^{90}$Y-IBRITUMOMAB TIUXETAN FOR CHEMOTHERAPY-NAÏVE FOLLICULAR LYMPHOMA

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**Background:** $^{90}$Y ibritumomab tiuxetan (Zevalin®, $^{90}$YIT) is approved for use for follicular lymphoma (FL) patients who have achieved a complete or partial response to frontline chemotherapy; however, its use after bendamustine (Treanda®) and rituximab (BR) has never been studied. In this prospective, single-arm, multicenter Phase II trial, we assessed the response rate and safety of a short induction course of BR followed by consolidation with $^{90}$YIT for chemotherapy-naïve patients with FL.

**Methods:** Patients older than 18 years with Stage II–IV FL requiring treatment were eligible for this study. Treatment consisted of an initial dose of rituximab 375 mg/m$^2$. BR (90 mg/m$^2$ Days 1–2, 375 mg/m$^2$ Day 1) was given for four cycles every 28 days. Patients received $^{90}$YIT if they obtained at least a partial response (PR) after induction, had adequate hematologic function, and had bone marrow infiltration of less than 25%. The primary endpoint of this study was the determination of the complete and unconfirmed complete response (CR/CRu) rate after sequential therapy. Secondary endpoints were overall response rate (ORR) after four cycles of BR, conversion rate from PR to CR/CRu after $^{90}$YIT, progression-free survival, and safety.

**Results:** Thirty-nine patients initiated study treatment. Median age was 57 years (31–75). The study enrolled patients with low (18%), intermediate (44%), and high (38%) FLIPI; Grade 3a (15%); Stage III/IV (90%); and bone marrow involvement (44%).

Thirty-nine patients completed four cycles of BR. Twenty-two of 39 evaluable patients achieved a CR/CRu (56%), and 16 patients had a PR (41%) for an ORR of 97%. Thirty-five of 38 BR responders received $^{90}$YIT; two patients in CR did not due to low platelets, and one in CR declined. After $^{90}$YIT, 30 of 35 evaluable patients were in CR/CRu (86%), 4 patients remained in PR (11%), and 1 patient progressed during $^{90}$YIT. Of the 16 patients who had a PR after BR, 8 (50%) converted to a CR/CRu immediately after $^{90}$YIT, with five additional conversions to CR/CRu in follow-up (range 6–23 months). With a median follow-up of 21 months, the estimated 2-year PFS is 85%.

The incidence of Grade 3–4 hematologic toxicities during BR included lymphopenia (33%), neutropenia (8%), and thrombocytopenia (3%). Non-hematologic toxicities included Grade 2 phlebitis (18%); Grade 3 hyperglycemia (8%); and infusion-related reaction, headache, and bleeding (3%).

Grade 3–4 hematologic toxicities after $^{90}$YIT included neutropenia (74%), leukopenia (36%), thrombocytopenia (65%), lymphopenia (35%), and anemia (9%). Median neutrophil and platelet recovery was 8 weeks after $^{90}$YIT.

There were no incidences of neutropenic fever. One patient developed progressive multifocal leukoencephalopathy 13 months after completing treatment. One patient developed chronic myelogenous leukemia 11 months after treatment. There have been no cases of myelodysplasia or acute myelogenous leukemia to date.

**Conclusions:** In this final analysis for response, the CR/CRu and ORR were 82% and 95%, respectively. The conversion rate from PR to CR/CRu was 50% after $^{90}$YIT. Sequential treatment with four cycles of BR followed by $^{90}$YIT is highly effective and safe and should be considered as a frontline treatment.

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### LENALIDOMIDE AND OBINUTUZUMAB IN INDOLENT NHL, RESULTS OF A PHASE I STUDY

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**Background:** Patients with advanced indolent non-Hodgkin lymphoma (iNHL) can develop chemoresistance, and most relapse following standard therapy. Lenalidomide activates NK cells ± T cells and leads to *in vivo* expansion of immune effector cells in NHL models. In preclinical studies, we have shown the synergistic anti-tumour effect of combining lenalidomide with α-CD20 mAbs (Wang 2007). The combination of rituximab and lenalidomide (R2) in relapsed and untreated iNHL is highly active (Fowler 2014). We hypothesize that these responses are related to augmentation of immune response and ADCC through alteration of immune cell subsets in tumour and peripheral blood. Obinutuzumab is a defucosylated Type II α-CD20 mAb with higher affinity for the FcγRIIIa receptors, leading to enhanced ADCC. The primary objective of this study was to determine the safety and maximum tolerated dose of lenalidomide and obinutuzumab.

**Methods**: Patients with relapsed SLL, marginal zone and follicular lymphoma (Grades 1–3a) were eligible. Patients were enrolled in three predefined dose cohorts of oral lenalidomide (10, 15 and 20 mg) given on Days 2–22 of a 28-day cycle. Obinutuzumab was given at a fixed-dose (1000 mg) IV on Days 1, 8, 15 and 22

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of Cycle 1 and Day 1 of subsequent cycles. All patients received prophylactic steroids prior to obinutuzumab. In the absence of toxicity or progression, the combination was continued for up to 12 cycles. Traditional 3 × 3 dose escalation was used with dose-limiting toxicities (DLT) assessed during Cycle 1. Patients attaining partial response or more continued obinutuzumab every 2 months for up to 24 months. Prophylactic growth factors were not used. Adverse events were graded using CTCAE version 4.03.

**Results:** Nine patients were enrolled in the study and received treatment; all were evaluable for safety and five (with first restaging scans done) for efficacy. The median age was 60 (36–72) years, and six (67%) were male. All patients had follicular lymphoma with low, intermediate and high FLIPI 1 (11%), 3 (33%) and 5 (55%), respectively, at study entry. No DLTs were observed. In nine patients, the most common Grade 1–2 non-hematologic toxicities were fatigue in seven (78%), diarrhoea in four (44%) and dyspnoea in five (55%). The only Grade 3+ events were one instance of Grade 3 thrombocytopenia during Cycle 1 and one instance of Grade 4 neutropenia during Cycle 2. Two infusion-related reactions occurred, both during the first dose of obinutuzumab. The overall response rate was five of five (100%) with two of five (40%) complete responses. All patients remained on therapy without progression after a median follow-up of 6 (2.1–8.4) months.

**Conclusion:** The combination of 20 mg lenalidomide and 1000 mg obinutuzumab is safe and effective in patients with relapsed iNHL. Correlative efforts are ongoing to study the immunomodulatory potential of the combination and potentially identify biomarkers of response. The Phase II portion of this study is currently enrolling patients with dose expansions in relapsed iNHL.

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### THE LONG-TERM RESULTS AND EFFECTIVENESS OF ANTIVIRAL THERAPY IN PATIENTS WITH HEPATITIS C-ASSOCIATED INDOLENT LYMPHOMA (IL+C)

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The incidence of HCV infection in patients with B-cell indolent non-Hodgkin lymphomas is approximately 15%.

**Materials:** Our study included 93 patients with indolent lymphoma and hepatitis C markers (IL+C) and a control group of 146 patients with indolent lymphomas without markers of hepatitis C (IL−C). Subtypes of lymphoma were 71% follicular lymphoma, 22% marginal zone lymphoma and 7% chronic lymphocytic leukaemia.

Age of patients with IL+C ranged from 28 to 82 years (median 50). The age of patients with IL−C was 28–79 years (median 61; $p = 0.0002$).

Male/female ratio was 1:1.2 in the IL+C group and 1:2 in the IL−C group ($p = 0.0002$).

Stages of disease were as follows: I–II 3%, III 20% and IV 77% in group IL+C and I + II 26%, III 16% and IV 58% in group IL−C ($p = 0.00002$).

Extranodal lesions were 23% in the IL+C group and 10% in the IL− C group ($p = 0.00001$). Involvement was spleen 53% in the IL+ C group and 19% in the control group ($p = 0.00001$), liver 17% and 8% in comparable groups ($p = 0.02$) and bone marrow 56% in the IL+C group and 34% in the control group ($p = 0.001$).

**Method:** In 93 patients with IL+C, 43 patients received antiviral therapy as the first treatment. Fifty patients with IL+C received polychemotherapy as the first line of therapy.

**Results**: Patients with IL+C who underwent antiviral therapy (AVT) had complete remission (CR, 77%), partial remission (PR, 11%), stabilization (4%) and progression (8%). In all patients in whom antiviral therapy was not effective (stabilization + progression), virus proteins of hepatitis C on tumour cells were not detected.

On chemotherapy in patients with IL+C, CR was achieved in 64%, PR in 23%, stabilization in 9% and progression in 4%. On chemotherapy in patients in the control group with IL−C, CR was achieved in 53%, PR in 31%, stabilization in 5% and progression in 11%.

The median relapse-free survival (RFS) in patients with IL+C on AVT was 36 months. The median RFS in patients with IL+C on chemotherapy was 19 months. The median RFS in patients with IL-C on chemotherapy was 33 months.

Thirty-seven patients with relapse IL+C after chemotherapy received AVT. CR was achieved in 81% of patients, PR in 11% and stabilization/progression in +8% of patients. The median RFS in patients with IL+C recurrence after chemotherapy for AVT was 31 months.

**Conclusion:** IL + C with markers of viral hepatitis C is a separate group of lymphomas with characteristic clinical, morphological features. This allows us to separate a hepatitis C-associated IL+C. The effectiveness of AVT was significantly higher than that of chemotherapy in patients with IL+ . AVT should be the first-line therapy in patients with IL+C.

### 344
### LONG-TERM ACTIVITY AND SAFETY OF SINGLE-AGENT IBRUTINIB IN PATIENTS WITH RELAPSED/REFRACTORY WALDENSTRÖM'S MACROGLOBULINEMIA

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**Introduction**: Bruton's tyrosine kinase (BTK) is a crucial signaling molecule in the B-cell receptor (BCR) pathway and is essential for BCR-mediated proliferation, adhesion, and survival of B cells. Ibrutinib, a first-in-class, once-daily, oral inhibitor of BTK, has emerged as an attractive treatment option for Waldenström's macroglobulinemia (WM). Recently, ibrutinib was approved by the FDA for the treatment of WM, representing the first FDA-approved agent in WM. The first-in-human trial of ibrutinib was an open-label, Phase 1 study in patients with relapsed/refractory (R/R) B-cell malignancies including WM (Advani, *J Clin Oncol.* 2013). Herein, we report long-term activity and safety outcomes with ibrutinib in four patients with R/R WM enrolled in this study.

**Methods:** Four patients with R/R, histologically confirmed WM, IgM levels ≥1000 mg/dL (with bone marrow infiltration), and adequate hematologic, renal, and hepatic functions received oral ibrutinib between 560 and 12.5 mg/kg/day until progressive disease or unacceptable toxicity. Patients in the parent study (PCYC-04753) with objective response or stable disease after 6 months of therapy could continue into the extension study (PCYC-1103-CA) at a fixed dose of ibrutinib of 560 mg daily. Response was assessed per the Third International Workshop of WM (IWWM). Adverse events (AEs) were assessed by NCI CTCAE v3.0. In the extension study, only AEs Grade ≥3, serious AEs (SAEs), and AEs leading to dose modification or discontinuation were captured.

**Results:** Durable partial responses, which are ongoing after 4 years of therapy, were seen in three of the four WM patients. Clinical improvements included early and rapid reductions from baseline in IgM levels (~80% to 90%), reaching a plateau after 1 year of therapy; reduction in lymphadenopathy (present in three of four patients at baseline); sustained increases or stabilization in hemoglobin levels (without the use of erythropoietic growth factors or transfusion); and improvement in hematocrit over time. Grade 3/4 AEs included neutropenia (one patient) and thrombocytopenia (one patient), and SAEs included febrile neutropenia (two patients), pneumonia and pneumonitis (one patient), and atrial fibrillation (one patient). In addition, the same patient experienced a second episode of Grade 2 atrial fibrillation (not an SAE), leading to dose modification to ibrutinib of 420 mg daily. All Grade 3/4 AEs and SAEs resolved without sequelae and were assessed by the investigator as unrelated to ibrutinib.

**Conclusions:** Single-agent ibrutinib was well tolerated and produced profound and durable responses in patients with R/R WM—consistent with findings of a Phase 2 trial (Treon, *IWWM*, 2014). This case series is the first to demonstrate extended activity and tolerability of single-agent ibrutinib in WM and supports its long-term use in this difficult-to-treat patient population.

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### FIRST-LINE TREATMENT AND SURVIVAL OF TRANSFORMED FOLLICULAR LYMPHOMA IN THE NETHERLANDS IN THE RITUXIMAB ERA; A POPULATION-BASED ANALYSIS

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**Introduction:** Treatment of transformed follicular lymphoma (TFL) is diverse as there are no randomized studies to guide therapy. Patients are treated with rituximab (R)-chemotherapy only or with R-chemo and upfront autologous stem cell transplantation (ASCT) at their physicians' discretion. We investigated different treatment modalities and outcome of patients registered in the Population-based Haematological Registry for Observational Studies (PHAROS), covering 40% of the Dutch population.

**Methods:** From the PHAROS registry, we extracted all patients with a diagnosis of both follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) between January 2004 and July 2013 and checked their histology. Only patients with biopsy-proven DLBCL and underlying FL (previously or simultaneously diagnosed) were included. A separate analysis was performed on patients below 68 years, since upfront ASCT was given up to 67 years.

**Results**: One hundred and sixty-one patients were included, median age at transformation was 63 years (34–91), median time to TFL was 14 months (0–101) and median follow-up after transformation was 17 months (1–111).

*Former treatment for FL*: Sixty-one per cent had been treated with chemotherapy for FL (66% of them with R), 2% had received radiotherapy only, 24% were under a watch-and-wait strategy and, in 13%, FL and DLBCL were diagnosed simultaneously.

*Treatment for TFL*: Ten patients were unable to receive any treatment. All 151 treated patients received R-chemo as induction therapy: 62 patients (33, <68 years) received R-chemo only, and 32 (all <68 years) were in remission after induction R-chemo and received upfront ASCT [23 with R and 9 preceded by $^{90}$Y ibritumomab tiuxetan (Z)]. One patient received upfront consolidation with allogeneic SCT. Fifty-six patients (40, <68 years) were primary refractory to induction treatment, of whom 15 (all <68 years) could be rescued by ASCT.

When patients had been treated for FL before, significantly more often upfront ASCT was given as treatment of TFL: 33% of untreated patients received upfront ASCT versus 63% of pretreated patients ($p < 0.01$). In the R-chemo-only group, 39% had been pretreated with chemo and 54% with R, versus 69% and 77% in the upfront ASCT group ($p < 0.01$).

Median OS of all TFL patients was 52 months, with a 2-year OS of 55%.

To compare survival between treatment groups, we analysed 109 patients <68 years. After successful R-chemo, 2-year OS was 90%, rising to 96% after upfront ASCT (with or without Z). Two-year OS was 40% after salvage Z-ASCT and 0% when patient was R-chemo refractory and unable to reach salvage ASCT.

**Conclusion:** Although TFL is considered to have a poor prognosis, our data show that when induction R-chemotherapy for TFL is successful, OS is very high. Refractoriness to induction is the main cause of mortality. Physicians offer upfront ASCT more often to patients pretreated with R-chemo for FL. Upfront Z-ASCT seems to result in the highest OS rates, despite more pretreated patients in this group. Studies are needed to identify the patients benefitting most.

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**LOW INCIDENCE OF TRANSFORMATION IN FOLLICULAR LYMPHOMA IN THE RITUXIMAB ERA. A RETROSPECTIVE ANALYSIS FROM THE CZECH LYMPHOMA STUDY GROUP DATABASE**

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**Introduction:** Follicular lymphoma (FL) can transform into more aggressive disease, which is usually associated with rapid progression and poor outcome. Because an improvement in overall survival of FL patients was reported during the last years, our analysis sought to describe the incidence, risk factors and treatment outcome of transformation in the era of wide use of rituximab.

**Patients and Methods:** Between 2002 and 2012, through the prospectively maintained multicentric Czech Lymphoma Study Group database, patients with newly diagnosed indolent FL were included in the analysis. Patients with Grade 3B and primarily transformed FLs were excluded. Cumulative incidence of transformation was determined by using death as a competing risk. Time to transformation was defined as the time from the date of diagnosis (diagnostic biopsy) to the date of transformation (biopsy with histological confirmation).

**Results:** There were 1131 patients with newly diagnosed FL Grades 1–3A, who had a median age of 58 years (range, 26–87 years) with preponderant proportion of females (60.6%) and a median follow-up of 4.52 years (range, 0.4–12.7 years). Majority (84%) of patients received rituximab in first or subsequent lines of therapy, and anthracycline-based regimens (CHOP-like) were administered in 750/1131 (66%) cases. The histological confirmation rate at relapses (1–4) ranged from 56% to 66%. One hundred and fifty-nine patients had died without transformation, whereas 29 patients developed histologically proven transformation. Median time to transformation was 3.26 years (range, 0.33–10.5 years). Transformation occurred in first and subsequent relapses in 69%, 17%, 14% and 0% of FLs. The overall transformation rate (TR) at 5 years was 2.53%; when only relapsed cases ($n = 536$) and histologically confirmed cases ($n = 332$) are counted, TR was 5.4% and 8.7%, respectively. The bulky tumour (≥10 cm), increased lactate dehydrogenase, performance status (≥1) and FLIPI (low vs intermediate or high risk) were associated with transformation ($p < 0.05$). The male gender tended to be also associated with higher risk of transformation ($p = 0.09$). Whereas the global survival was similar between the group of transforming and the group of relapsing but never transforming patients (median 4.31, range 1.55–9.2 years vs 3.77, range 0.19–11.5 years), 16/29 (55%) patients died after transformation with median time of 1.2 years (range 0–5.3 years).

**Conclusion:** Transformation rate in FL seems to be much lower than in previous series, which can be influenced by the fact that only histologically confirmed cases were included. The very low transformation incidence can also be explained by the high use of rituximab prior to transformation and also due to a high proportion of females (who are more sensitive to rituximab treatment) as compared to published data. The prognosis and treatment outcome of transformed patients remained poor with generally short survival.

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**MAINTENANCE RITUXIMAB IN THE MANAGEMENT OF COMPOSITE AND DISCORDANT B-CELL LYMPHOMAS FOLLOWING R-CHOP INDUCTION**

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**Introduction:** Patients with follicular lymphoma (FL) who achieve a complete (CR) or partial (PR) response to rituximab-containing chemotherapy benefit from 2 years of maintenance rituximab (MR). In contrast, MR is not beneficial for chemosensitive patients with diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP. The impact of MR on patients presenting with indolent and aggressive histology lymphoma simultaneously in the same biopsy [composite (COM)] or two separate sites [discordant (DIS)] is unknown.

**Methods:** In British Columbia, patients with COM/DIS lymphomas who achieve a CR/PR following R-CHOP induction are eligible for MR. The BCCA Lymphoid Cancer Database was screened to identify all patients with newly diagnosed COM/DIS lymphoma composed of an indolent B-cell non-Hodgkin lymphoma

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(excluding CLL/SLL, mantle cell lymphoma, and Grade 3B FL) and DLBCL who were in a CR/PR following R-CHOP and received MR (rituximab 375 mg/m$^2$ IV every 3 months × 2 years). Patients with CNS involvement at diagnosis were excluded. Progression-free survival (PFS) and overall survival (OS) were calculated from the time of diagnosis.

**Results:** A total of 55 patients were identified with a median age of 64 years (range 22–86), 51% male, 25% elevated LDH, 20% performance status ≥ 2, 85% Stage III/IV, and 29% two or more extranodal sites. Forty (73%) had COM and 15 (27%) had DIS involvement. Indolent histologies included 43 (78%) FL, 10 (18%) low-grade lymphoma not otherwise specified, 1 (2%) lymphoplasmacytic lymphoma, and 1 (2%) marginal zone lymphoma.

Following R-CHOP, 41 (75%) patients achieved a CR and 14 (25%) a PR. Thirty-nine (71%) completed 2 years of MR; for the remainder, MR was interrupted due to progressive disease ($n = 8$), toxicity ($n = 6$), loss to follow-up ($n = 1$), and secondary malignancy ($n = 1$).

With a median follow-up of 6 years for living patients (range 1–10), the 6-year PFS was 63% (SE 8%) and 6-year OS was 73% (SE 7%). A total of 15 (27.3%) patients experienced relapse: six indolent, five DLBCL, and four undetermined. Of these, eight (53%) died from lymphoma. There were five additional deaths: three due to secondary malignancies, one due to infection, and one due to accident. In univariate analysis, elevated LDH was associated with a worse OS. All other factors, including the IPI, were not associated with PFS or OS.

**Conclusion:** Patients with COM or DIS B-cell lymphomas treated with R-CHOP followed by MR have excellent outcomes, with a 6-year PFS of 63% and OS of 73%. Comparing these estimates to those treated with R-CHOP alone may clarify whether MR beyond R-CHOP provides a significant benefit.

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**PHARMACOKINETIC DATA, CLINICAL CHARACTERISTICS AND OUTCOME IN FOLLICULAR LYMPHOMA PATIENTS IN MAINTENANCE WITH RITUXIMAB: AN ANALYSIS OF THE FONDAZIONE ITALIANA LINFOMI**

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**Introduction:** Rituximab (R)-chemotherapy induction followed by R maintenance is the current standard of care for patients with follicular lymphoma (FL). In recent years, several studies have investigated the optimal dose and schedule of R based on pharmacokinetic (PK) data. These studies have proposed a presumptive 'active' level of 25 μg/ml. However, scanty data are available with regard to the PK of R during maintenance and its possible relationship with the patients' characteristics and outcome.

**Methods:** Patients with Grade 1, 2, or 3a FL in maintenance therapy with iv R (375 mg/m$^2$) administered every 2 (patients treated frontline) or 3 months (after salvage immunochemotherapy) were investigated. R plasma trough concentrations ($C_{tr}$) and the area under the curve (AUC) were determined using a sensitive validated ELISA assay. PK data were then correlated with the patients' clinical characteristics and outcome.

**Results:** From March 2013 to March 2014, 101 patients have been recruited by 12 FIL centers. The median age was 60 years (IQR 30–84), and 50 (48.5%) were males. According to body mass index (BMI), 44 (43%) patients were overweight and 10.8% were obese. At study entry, 92 patients were in first complete response (CR), 19/92 in second CR, and 9 in partial response (PR), of whom 2 achieved PR after salvage therapy. In 85/101 (Group 1) and in 16/101 (Group 2) patients, R was administered every 2 or 3 months, respectively. After a median follow-up of 23 months from the start of R maintenance, 91 patients were in CR, 3 were in PR, and 7 had relapsed. The median $C_{tr}$ level was 32 μg/ml (0.0–200 μg/ml) in all patients, 33 μg/ml (0.0–188 μg/ml) in patients treated with R every 2 months, and 22.8 μg/ml (0.0–200 μg/ml) in patients treated every 3 months ($p = 0.7$). A higher median R $C_{tr}$ level (36 vs. 22 μg/ml, $p = 0.04$) and a higher AUC (61.23 vs. 41.9, $p = 0.011$) were found in females. No correlation between $C_{tr}$, AUC, and BMI were documented, but a trend towards higher levels were found in patients with a BMI <30 ($C_{tr} = 40.38$ vs. 31.96 μg/ml, $p = 0.23$; AUC = 54.19 vs. 42.55, $p = 0.13$). This preliminary analysis did not show any relationship between the quality of response (CR vs. PR), the outcome (response vs. relapse), and the $C_{tr}$ levels and AUC; however, in a subanalysis of Group 1, more patients with lower $C_{tr}$ relapsed during R maintenance (38.8 vs. 33.9 μg/ml, $p = 0.01$), and notably, four of six who relapsed had $C_{tr}$ levels under 25 μg/ml.

**Conclusions:** Our study confirms the favorable R PK profile in female patients and in the 2-month administration scheme. At present, probably because of the relatively short follow-up and small number of events during maintenance, no relationship between PK data and outcome has so far emerged, and a longer observation time is required.

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**ALLOGENEIC TRANSPLANTATION: A SUITABLE OPTION FOR PATIENTS WITH RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA? A CLINICAL EXPERIENCE FROM A SINGLE INSTITUTION ON 37 PATIENTS**

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**Introduction:** Major issues in the treatment of follicular lymphoma (FL) consist in choice of the most appropriate therapy at time of relapse. Treatment options are still controversial, especially allogeneic stem cell transplantation (Allo-SCT).

**Methods:** Thirty-seven consecutive patients who underwent Allo-SCT between April 1999 and September 2013 were analyzed. Indications for Allo-SCT were relapse after autologous stem cell transplantation (Auto-SCT) and primary chemotherapy refractory patients (PRP). Strategy consisted of high-dose chemotherapy supported by Auto-SCT followed by Allo-SCT for patients relapsing after a previous salvage therapy (18 patients). Allo-SCT without previous Auto-SCT was performed in 19 patients: 11 patients relapsing after a previous Auto-SCT, 3 PRP, and 5 patients with stem cell harvest failure.

**Results:** Median ages at diagnosis and at transplantation were 50 (range 36–62) and 55 years (range 38–66), respectively. All patients were previously heavily pretreated, with 59% receiving three or more previous lines of treatment. All patients received a rituximab-containing regimen. Median time from diagnosis to transplantation was 50 months (range 14–211). Median time from last relapse to transplantation was 8 months (range 2–147). At transplant, 41% of patients were in complete remission (CR), 48% in partial response (PR), and 11% had less than PR. All patients received a reduced-intensity conditioning regimen (RIC), including fludarabine, busulfan, and anti-thymoglobulin for most of them. Donor was alternative in 24% of patients. With a median follow-up of 51 months, 26 patients are alive in CR. Six patients have relapsed or progressed after transplant, and three of them died. Non-relapse mortality (NRM) was 21%. Sixty-four patients presented an acute graft-versus-host disease (GVHD; 16% of Grade 3/4). Incidence of severe chronic GVHD was present in 24%. Incidence of extensive chronic GVHD was 35%. The 5-year overall survival (OS) and progression-free survival (PFS) were at 71% and 61%, respectively. There was no significant difference in OS and PFS in patients treated with tandem Auto-SCT–Allo-SCT versus patients receiving Allo-SCT alone ($p = 0.9$). Disease status at transplant influenced outcome with a significant improved survival for patients in CR/PR compared to PRP ($p = 0.024$).

**Conclusions:** RIC–Allo-SCT may represent a suitable option for patients with relapsed or refractory FL. Allo-SCT is a potential curative strategy due to the graft-versus-lymphoma effect. The benefit of Auto-SCT preceding Allo-SCT remains

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questionable in previously heavily pre-treated patients. Larger series are needed to confirm these results. Other strategies are also requested to reduce NRM and GVHD rate.

## MCL

**350**
**MANAGEMENT TRENDS AND OUTCOMES FOR STAGE I AND II MANTLE CELL LYMPHOMA USING THE NATIONAL CANCER DATA BASE: ASCERTAINING THE IDEAL TREATMENT PARADIGM**

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**Introduction:** Early-stage mantle cell lymphoma is a rare albeit aggressive subset of non-Hodgkin lymphoma, resulting in varied treatment approaches. Given a paucity of data defining the optimal approach, NCCN guidelines remain ambiguous, recommending treatment options of chemotherapy (CT) with or without radiotherapy (RT) or RT alone. We conducted an exploratory analysis using the National Cancer Data Base (NCDB) to identify management patterns in the USA and subsequent outcomes among these patients.

**Methods:** The NCDB was queried for patients with Stage I–II mantle cell lymphoma diagnosed from 1998 to 2012 and receiving CT and/or RT. Factors associated with treatment selection were assessed through binomial regression. Propensity scores using inverse probability weights were constructed using multivariable logistic regression with forward conditional selection; scores were validated by assessing standardized differences in propensity score between treatment arms. Log rank testing and Cox proportional hazards modeling with propensity score adjustment were conducted for survival analyses.

**Results:** In total, 2539 patients were identified with a median age of 68 years. Key characteristics are as follows: 69.1% male, 71.0% age ≥60, 51.4% Stage I disease, and 72.2% without extranodal involvement. A majority of patients received CT alone (69.8%), RT alone (11.5%), or combined modality therapy (CT + RT, 18.7%). Utilization of CT + RT decreased over time from 23.1% to 14.1% in 1998–2002 and 2010–2012, respectively ($p < 0.001$). CT + RT utilization was lower among patients ≥60 years old, of female gender, with Stage II disease, with B symptoms, or diagnosed in later years. With a median follow-up of 42.8 months (interquartile range 20.4–75.6), the unadjusted median survival for CT, RT, and CT + RT was 87.0 (95% CI 82.5–91.5), 88.5 (95% CI 79.1–97.9), and 103.2 (95% CI 96.0–110.4) months, respectively ($p < 0.001$). Following propensity score adjustment and correction of other covariates, combined modality therapy resulted in a significant reduction in the risk of mortality compared to monotherapy (HR 0.38, 95% CI 0.19–0.76, $p = 0.007$).

**Conclusions:** As seen with other subtypes of lymphoma, integration of radiotherapy with chemotherapy continues to decline in the USA. For patients with Stage I–II mantle cell lymphoma, combined modality therapy results in a reduction in mortality, suggesting the need for careful consideration before selecting single-modality approaches.

**351**
**FIRST-LINE TREATMENT WITH R-CHOP-BASED PRIMARY THERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION AND RITUXIMAB MAINTENANCE IN PATIENTS WITH MANTLE CELL LYMPHOMA**

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**Introduction:** Mantle cell lymphoma (MCL) is an aggressive lymphoma and remains largely incurable with standard therapy. High-dose chemotherapy (HDT) followed by ASCT as consolidation after primary chemotherapy has become the standard of care in eligible patients. Relapse remains the main cause of treatment failure, and strategies such as intensifying induction therapy with high-dose cytarabine or adding rituximab maintenance (RM) may reduce the relapse rate (RR) post-ASCT.

**Methods:** We conducted a retrospective analysis of sequential MCL patients who underwent ASCT after first-line chemotherapy at the Princess Margaret Cancer Centre between 2000 and 2013. Patients received induction with CHOP, RCHOP, or RCHOP alternating with RDHAP (RCHOP/RDHAP), followed by HDT with or without total-body irradiation (TBI). All patients had a documented response to

**Abs 351 - Table 1.** Patient characteristics

| | ***n* = 97 (%)** |
|---|---|
| Age at diagnosis | |
| Median (min, max) | 56 (36, 66) |
| Gender | |
| Female | 28 (29) |
| Male | 69 (71) |
| Subtype | |
| Other subtypes | 82 (85) |
| Blastoid variant | 13 (13) |
| Pleomorphic | 2 (2) |
| Stage at diagnosis | |
| 2 | 5 (5) |
| 3 | 4 (4) |
| 4 | 88 (91) |
| Bone marrow involvement | |
| No | 12 (13) |
| Yes | 81 (87) |
| Not available | 4 |
| LDH at diagnosis | |
| Normal | 38 (39) |
| Elevated | 39 (40) |
| Not available | 20 (21) |
| MIPI | |
| Low risk | 40 (52) |
| Intermediate risk | 19 (25) |
| High risk | 18 (23) |
| Not available | 20 |
| Time from diagnosis to transplant (months) | |
| Median (min, max) | 7.5 (2.5, 33.4) |
| Not available | 1 |
| Primary chemotherapy | |
| CHOP | 14 (14) |
| RCHOP | 57 (59) |
| RCHOP/RDHAP | 26 (27) |
| TBI (10–12 Gy in 6 fractions) | |
| No | 21 (22) |
| Yes | 76 (78) |
| Conditioning regimen | |
| Melphalan + VP16 | 45 (57) |
| Cytarabine + melphalan | 29 (37) |
| Other | 5 (6) |
| Not available | 18 |
| Maintenance rituximab | |
| No | 16 (19) |
| Yes | 70 (81) |
| Not available | 11 |
| Maintenance rituximab schedule | |
| No maintenance | 16 (16) |
| Every 3 months for 2 years | 12 (12) |
| 4× weekly at 3 and 9 months | 32 (33) |
| Other | 9 (9) |
| Given, schedule not available | 17 (18) |

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induction. After ASCT, patients received RM with single-agent rituximab 375 mg/m$^2$ or were simply observed.

**Results:** Ninety-seven MCL patients were treated (Table 1). Induction therapy was as follows: CHOP 14%, RCHOP 59%, and RCHOP/RDHAP 27%. Response evaluation was per Cheson (*JCO* 1999; CT scan and bone marrow morphologic assessment). After induction, CR/CRu was obtained in 44% and PR in 56% patients. CR rates were similar for the three induction strategies: CHOP 50%, RCHOP 43%, and RCHOP/RDHAP 42% ($p$ = ns). HDT was melphalan + etoposide for 57% patients and cytarabine + melphalan for 37% patients; 78% also received TBI. Post-ASCT responses were as follows: CR 94%, PR 4%, and 2% PD (one unavailable). Maintenance data were available for 86/97 patients. RM was given to 81% patients. Median follow-up for the entire cohort was 2.9 years (range 0.1–14.1). Median PFS was 4.7 years (95% CI: 4.2–NR) with 2- and 5-year PFS of 85.1% (75.7–91.1) and 49.1% (33.7–62.8), respectively. Twenty-seven patients relapsed after ASCT (29%). Median time to relapse was 9 years (95% CI: 4.6–12.1). Two- and 5-year RR were 13.8% and 42.9%, respectively. Relapse occurred in 12% patients after RCHOP/RDHAP, 26% after RCHOP, and 71% after CHOP. Univariate (UVA) and multivariate (MVA) analyses remained significant for PFS stratified by MCL subtype and RM. Median OS was 9.16 years (95% CI: 7.31–NR), 2-year OS was 88.3% (79.3–93.5), and 5-year was OS 75.3% (61.6–84.7). For patients on observation, median PFS was 2.73 years (1.16–4.64) and median OS 5.99 years (1.61–NR). For those receiving RM, PFS and OS were 9.07 (4.65–NR) and 9.16 (7.72–NR) years, respectively. There was no difference in OS according to induction therapy received, while RM was statistically significant in UVA ($p$ = 0.019), but not in MVA.

**Conclusions:** The outcomes of responding patients following ASCT appear superior to those of previous strategies. Within the limits of a retrospective study, the response rate and EFS were similar among three different induction regimens, while OS may be improved by adding RM.

### 352
### OXALIPLATIN IS A KEY DRUG IN THE ACTIVITY OF GEMOX-R IN MANTLE CELL LYMPHOMA

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**Introduction:** Mantle cell lymphoma (MCL) is mostly incurable. The current standard therapy achieves a high rate of complete remission (CR), but the pattern of continuous relapses still marks this disease as a challenge. We previously reported the efficacy of GemOx-R, a combination regimen of gemcitabine, oxaliplatin and rituximab, in patients with refractory and relapsing MCL. Our aim is to confirm our previous results in a larger retrospective series and evaluate the efficacy of each component of GemOx-R in a panel of MCL cell lines and in patient-derived primary cells.

**Methods:** Between 2003 and 2014, 28 patients with MCL were included in a retrospective study of treatment with GemOx-R from the University Hospital Son Espases. The translational study was performed in established cell lines as well as primary MCL lines from patients by cell viability, cell cycle, apoptosis and western blot analysis. Drug synergy was determined by the isobologram and combination-index methods.

**Results:** Overall response rate was 86% with a CR of 71% and 62% for patients in the frontline and salvage cohorts, respectively. Median progression-free survival was 28 months in the entire series: 30 and 22 months, respectively, for the two cohorts. Median overall survival was 34 months in the entire series: not reached and 20 months, respectively, for the two cohorts. Cell viability and apoptosis analysis showed that oxaliplatin is the most effective drug in this regimen in contrast to the poor responses induced by gemcitabine and rituximab. Oxaliplatin had a profound effect on cellular viability, consistent with the induction of caspase activity and the downregulation of pro-survival proteins. We further present synergistic efficacy of oxaliplatin combined with cytarabine in MCL cells. Interestingly, this synergistic effect was not seen when cisplatin and cytarabine were combined, indicating that among the platinum-derived agents, oxaliplatin may be the preferred approach.

**Conclusions:** (1) Oxaliplatin is the most effective drug in GemOx-R; (2) oxaliplatin has a robust *in vitro* activity comparable to that of cytarabine, and the combination of both oxaliplatin and cytarabine shows a significant synergism; (3) taken together, our findings suggest that oxaliplatin alone or combined with cytarabine could constitute a new or alternative backbone for promising new regimens in MCL.

### 353
### MANTLE CELL LYMPHOMA MANAGEMENT AND OUTCOME IN THE UK'S POPULATION-BASED HAEMATOLOGICAL MALIGNANCY RESEARCH NETWORK

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**Introduction:** Mantle cell lymphoma (MCL) is generally associated with an aggressive clinical course and poor prognosis, but few studies have evaluated its management and outcome in an unselected, non-trial general population. We investigated this using data from a specialist UK population-based registry (www.hmrn.org), examining outcome in relation to first-line therapy, as well as second-line therapy given for refractory/relapsed disease.

**Methods:** All 244 patients newly diagnosed in 2004–2012 with MCL were followed up until April 2014; and demographic, prognostic, treatment and outcome data were analysed.

**Results**: With a median diagnostic age of 73.7 years (range 39–96), 65% patients were male, 39% had B symptoms and 47% had a high MIPI score. One hundred and eighty-seven (73%) received first-line chemotherapy (median age 73.2). In addition, 11 (5%) went on to have autologous transplantation (ASCT). These patients were significantly younger (median age 57.3; Table 1).

Five-year overall survival (OS) was 25% (median OS 2.2 years), and 5-year relative survival was 31%. The MIPI score was predictive with OS for low, intermediate and high scores being 4.6, 2.9 and 1.2 years, respectively. Patients who had an ASCT had the best survival, followed by those remaining on a watch-and-wait strategy (Table 1). Of the 43 patients initially on the watch-and-wait strategy, 25 subsequently received chemotherapy, on average 442 days after diagnosis; their median OS was 3.0 years (95% CI 1.7–3.9). For patients whose regimen included rituximab, OS was significantly increased (hazard ratio = 0.60, 95% confidence intervals = 0.41–0.86). For the 118/198 patients who achieved a response to first-line treatment, median progression-free survival was 3.8 years (95% CI 3.3–4.6).

**Abs 353 - Table 1.** First-line treatment, age and overall survival

| | Total | Median age (years) | Median survival (95% confidence intervals) |
|---|---|---|---|
| Total | 244 | 73.7 | 2.2 (1.7–2.8) |
| Chemotherapy without autologous stem cell transplant (ASCT)[a] | 187 | 73.2 | 2.5 (1.9–3.1) |
| With rituximab | 78 | 71.5 | 3.1 (2.4–3.8) |
| Without rituximab | 109 | 74.1 | 1.7 (1.4–2.2) |
| Chemotherapy with autologous stem cell transplant (ASCT) | 11 | 57.3 | 5.6 (4.8–) |
| Radiotherapy only | 4 | 74.6 | 2.2 (0.1–) |
| Palliative approach | 23 | 82.2 | — |
| Watch-and-wait approach only | 18 | 78.2 | 4.5 (2.1–) |

[a]Forty-three patients were initially on a watch-and-wait approach.

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Ninety-one patients had second-line therapy; 34 had refractory disease, and 57 had relapsed; and the median survival from the start of second-line therapy was 0.3 (95% CI 0.2–0.6) and 0.7 (95% CI 0.4–0.8) years, respectively. No variations with regimen were detected.

**Conclusions:** Outside trials in the unselected patient population, survival from MCL is very poor, averaging just over 2 years. Only the small proportion of younger patients receiving ASCT (5%) and those whose disease did not progress to the stage where treatment was required (7%) had a median survival in excess of 4 years. Our data also confirm the benefit of rituximab in the general patient population and demonstrate the fact that patients treated with second-line therapy have very poor outcomes, highlighting the need for new therapeutic options.

**354**
**INCREASE OF NK-CELL SUBSETS IS A RESPONSE INDICATOR AND PHARMACODYNAMIC MARKER OF LONGER PROGRESSION-FREE SURVIVAL IN MANTLE CELL LYMPHOMA PATIENTS TREATED WITH LENALIDOMIDE**

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**Introduction:** Lenalidomide, an immunomodulatory drug, has shown activity in single-arm Phase II studies of patients with relapsed/refractory (R/R) mantle cell lymphoma (MCL) and is indicated for the treatment of R/R MCL in the USA and Switzerland. Lenalidomide binding to the $CRL4^{CRBN}$ E3 ligase complex results in ubiquitination and subsequent proteasomal degradation of Aiolos and Ikaros, leading to stimulation of immune cells, such as T cells. We investigated the effects of lenalidomide treatment on immune-cell subsets in MCL patients enrolled in a Phase II clinical trial.

**Methods:** Study MCL-002 (NCT00875667), a randomized open-label Phase II study enrolled R/R MCL patients with up to three or more relapses, who were ineligible for stem cell transplantation. Lenalidomide was given orally at 25 mg/day on Days 1–21 of each 28-day cycle until progression ($N = 170$). The control arm consisted of investigator choice of single-agent rituximab, gemcitabine, fludarabine, chlorambucil, or cytarabine ($N = 84$). Peripheral blood samples for exploratory analysis were collected at Cycle 1 Day 1 (C1D1, pre-treatment), at Cycle 1 Day 4 (C1D4), at Cycle 2 Day 15 (C2D15), and at treatment discontinuation. Flow cytometry profiling of T-cell, B-cell, and NK-cell subsets was performed. In this study, we examined the effects of treatment on immune subsets such as T cells (CD3/CD4/CD8/CD45RA/CD25/CD27), B cells (CD19/CD20/CD5/CD23/CD40/CD22/kappa/lambda), and NK cells (CD56/CD16/CD3/CD69), which were analyzed for correlation with clinical outcome [response to lenalidomide and progression-free survival (PFS)].

**Results:** At baseline, no significant differences were observed in the levels of immune subsets when comparing non-responders (NR) and responders (R) in either the lenalidomide ($N = 50$) or control ($N = 18$) arm. In the lenalidomide arm, significantly elevated (adj. $p < 0.05$) proportions of NK-cell subsets CD56bright+ CD16+ (difference of means = 6.08; 95% CI [3.12, 9.04]), CD56+ CD16+ (difference of means = 8.73; 95% CI [4.48, 12.98]) were observed after 4 days of treatment (C1D4) in the R ($N = 19$) outcome sub-group compared to NR ($N = 11$). A similar trend in levels of NK subsets was observed at C2D15; however, the difference between R ($N = 19$) and NR ($N = 6$) was not significant. In addition, elevated proportions of CD3− CD56dim+ CD16+ CD69− ($p \leq 0.006$), CD56dim+ CD16+ ($p \leq 0.006$), and CD56+ CD16+ ($p \leq 0.016$) NK cells at C1D4 relative to total lymphocytes correlate significantly to longer PFS in the lenalidomide arm. In contrast, subset analysis in the control arm did not show any correlation to response or PFS at any time point.

**Conclusions**: A significant increase in proportions of NK-cell subsets (vs. total lymphocytes) at C1D4 is a potential response indicator of favorable clinical outcome in R/R MCL patients treated with lenalidomide.

**355**
**COMPLETE METABOLIC RESPONSE AFTER THERAPY INDEPENDENTLY PREDICTS OUTCOME IN MANTLE CELL LYMPHOMA PATIENTS**

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**Introduction:** Mantle cell lymphoma (MCL) is a rare subtype of non-Hodgkin lymphoma with heterogeneous clinical, pathological and genetic features. Data analysing prognostic value of the metabolic response after therapy using positron emission tomography (fPET) are controversial. A role of total metabolic tumour volume measured at time of diagnosis (TMTV0) has not been evaluated yet.

**Methods:** We analysed 46 consecutive MCL patients treated in a single centre from 4/2006 to 4/2014. The median age at diagnosis was 65.4 (40.9–81.7) years; male-to-female ratio was 2.1:1. Ann Arbor Stages I through IV were observed in 2, 4, 3 and 37 patients, respectively. MIPI scores were low in 2 (4%), intermediate in 18 (39%) and high in 26 (57%) patients, respectively. Most of the patients were treated with R-CHOP-21 regimen ($n = 27$, 59%), and 18 patients (39%) received an intensive sequential protocol (three cycles of etoposide–anthracycline regimen, one cycle of methotrexate and one cycle of a high-dose AraC-containing regimen). Five patients (11%) were treated with R-AraC/R-CHOP-21 regimen, and two (4%) with FCR therapy. Sixteen (35%) patients were consolidated with high-dose therapy and ASCT. PET-CT fusion scans were done before chemotherapy and after treatment completion (fPET). In one patient, fPET was not assessable (death during induction). The PET results were expressed as positive/negative using a 5-point Deauville (D1–5) scale.

**Results:** Treatment responses were as follows: complete (CR), partial (PR) and stable disease in 26 (57%), 14 (30%) and 2 (4%) patients, respectively. Four (9%) patients progressed on therapy. After a median follow-up of 33.5 months, 22 (48%) patients relapsed or progressed and 16 (35%) of them died. Five-year overall survival (5-year OS) reached 68.0% (95% CI 0.53–0.83), and 5-year progression survival (5-year PFS) was 38.9% (95% CI 0.22–0.56). Using visual criteria, fPET was negative in 27/45 (60%) cases. With Deauville criteria, fPET scores were as follows: D1–2 in 23 (50%), D3 in 8 (17%) and D4–5 in 14 (30%). A low Deauville score (D1–2) was associated with superior 5-year PFS (65.0%) compared to D3–4 (22.2%, $p = 0.007$). Visually negative fPET led to superior 5-year PFS (18.3% vs 64.3%, $p = 0.005$). There was a trend to superior OS in D1–2 and visually negative groups ($p = 0.10$ and $p = 0.086$, respectively). Negative (NPV) and positive predictive values (PPV) of fPET for PFS were 74.1% (vis) and 79.3% (D) and 77.8% (vis) and 68.2% (D), respectively. Cox regression analysis identified fPET as predictor for lymphoma relapse/progression independent of the MIPI score (HR 0.49, $p = 0.018$). Mean TMTV0 was $810.8 \pm 871.7\ cm^3$; we found a difference in PFS in patients with low versus high tumour burden ($p = 0.50$).

**Conclusions:** Complete metabolic response after therapy is associated with superior PFS in MCL patients. TMTV0 seems not to be important for a prognostic assessment.

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## AGGRESSIVE LYMPHOMA

### 356
### IMPACT OF BODY MASS INDEX ON PROGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH IMMUNOCHEMOTHERAPY

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**Introduction:** While several studies have suggested that increased BMI (defined as weight in kilograms divided by height in square meters) is associated with better overall survival in DLBCL, the association with lymphoma-specific endpoints (continuous event-free survival, event-free survival at 24 months, and lymphoma-specific mortality) and stratified by gender has not been comprehensively evaluated in the immunochemotherapy era. We therefore evaluated the association of BMI ($kg/m^2$), defined as normal weight (18.5–24.9), overweight (25.0–29.9), and obese (≥30.0), with these endpoints as well as overall survival (OS) in a cohort of DLBCL patients treated with immunochemotherapy.

**Methods:** Newly diagnosed patients with DLBCL were prospectively enrolled from 2002 to 2012 into the Molecular Epidemiology Resource at the Mayo Clinic and University of Iowa. Baseline clinical, pathology (including cell of origin by Hans algorithm), and treatment data were abstracted, and all patients were actively followed for disease progression, retreatment, and death. Deaths were reviewed and coded as whether related to lymphoma/treatment, which was used to define lymphoma-specific survival (LSS). Cox proportional hazards regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association of BMI with EFS, LSS, and OS, stratified on sex and adjusted for age, age-adjusted IPI, and study center; odds ratios (ORs) were used to estimate the association of BMI with EFS24, adjusted for the same factors.

**Results:** This analysis was restricted to DLBCL patients treated with immunochemotherapy ($N = 844$). The mean age at diagnosis was 60 years (range 18–92); 54% were male; 25% reported B symptoms; 18% had a performance status >2; and the IPI distribution was 33% with 0–1, 29% with 2, 25% with 3, and 14% with 4–5. At diagnosis, 25% of DLBCL patients had normal BMI, 39% were overweight, and 35% were obese; <1% ($N = 9$) were underweight, and they were excluded from further analysis. During a median follow-up of 4 years, there were 338 events (247 failed EFS24) and 257 deaths (164 due to lymphoma). Compared to normal-BMI cases, obese men (HR = 0.88, 95% CI 0.58–1.34) or women (HR = 1.18, 95% CI 0.80–1.76) had no significant associations with EFS. Results were similar for EFS24 for obese men (OR = 0.71, 95% CI 0.38–1.30) and women (OR = 1.31, 95% CI 0.71–2.41). There were also no significant associations with OS for obese men (HR = 0.80, 95% CI 0.50–1.28) or women (HR = 1.16, 95% CI 0.74–1.78); results were similar for LSS. Using smoothing splines, there was no evidence for any nonlinear associations. After excluding patients with >10% weight loss in 6 months preceding lymphoma diagnosis ($N = 115$), all results were largely unchanged. There were no differences in analyses stratified on cell of origin.

**Conclusion:** In a large and well-characterized cohort of DLBCL patients treated with immunochemotherapy, we found no evidence of an effect of BMI on disease-related outcomes or overall survival in either men or women.

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### COMPARISON OF IPI AND NCCN-IPI IN 324 DE NOVO DLBCL PATIENTS: MULTICENTER RETROSPECTIVE ANALYSIS

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**Introduction:** International Prognostic Index (IPI) was developed in the pre-rituximab era to predict the prognosis of aggressive lymphomas. Due to limitations of IPI, we need a better prognosis-indicating system to anticipate the outcome of patients receiving CHOP + R. The National Comprehensive Cancer Network (NCCN)-IPI was recently claimed to be a better predictor of prognosis of diffuse large B-cell lymphoma (DLBCL). In this retrospective multicenter analysis, we aimed to compare the prognostic significance of IPI and NCCN-IPI in DLBCL patients treated with anthracycline-based chemotherapy in the rituximab era.

**Methods:** Three hundred twenty-four *de novo* DLBCL patients who have no known malignancy, treated with R-CHOP-like chemoimmunotherapy between 2002 and 2013, were included in the study. Initial age, LDH level, ECOG performance status, Ann Arbor stage, and extranodal involvement status were analyzed to determine IPI and NCCN-IPI. We classified the patients into four risk groups for both IPI and NCCN-IPI, and we compared progression-free survival (PFS) and overall survival (OS) rates between these scoring systems.

**Results:** The mean age was 53 years old (range: 17–90). Fifty-two per cent of the cohort had Stage III or IV disease, and 50% of patients had high LDH levels. Extranodal involvement of major organs and systems (i.e., bone marrow, CNS, liver, GI tract, and lung) was observed in 35.5% of patients. The cohort was categorized as low-risk, low intermediate-risk, high intermediate-risk, and high-risk groups for both IPI ($n$ for each categories was 140, 76, 66 and 42, respectively) and NCCN-IPI ($n$ for each categories was 79, 131, 92 and 22, respectively). Median follow-up was 44 months. PFS and OS were compared between risk categories. NCCN-IPI was able to discriminate more accurately high-risk category OS compared to IPI; 5-year OS was 29% for NCCN-IPI and 44% for IPI (Table 1). No difference was observed between OS rates of low-risk groups (both 91% at 5 years).

**Conclusion:** Although these scoring systems are essential predictors of prognosis in DLBCL patients, neither IPI nor NCCN-IPI covers molecular subgroups like double-hit and double-expressor lymphomas. The molecular parameters are still not part of scoring systems due to standardization and accessibility issues. We had many scoring systems to predict the prognosis of aggressive lymphomas, and we need to develop better ones. Nonetheless, our 'real-life' cohort showed that, although we can discriminate more accurately the high-risk patients with NCCN-IPI, it is not superior to IPI in low-risk patients in the rituximab era.

### 358
### LACK OF PROGNOSTIC VALUE FOR PERIPHERAL ABSOLUTE MONOCYTE COUNT IN A POPULATION OF DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA

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**Abs 357 - Table 1.** Kaplan–Meier survival estimates with hazard ratios (HR) for PFS and OS in 5 years

| | IPI | | | | NCCN-IPI | | | |
|---|---|---|---|---|---|---|---|---|
| | PFS (%) | HR (95% CI) | OS (%) | HR (95% CI) | PFS (%) | HR (95% CI) | OS (%) | HR (95% CI) |
| Low risk | 85 | 1.0 | 91 | 1.0 | 90 | 1.0 | 91 | 1.0 |
| Low-int | 72 | 2.2 (1.17–4.12) | 82 | 2.1 (0.93–4.79) | 74 | 2.1 (0.98–4.47) | 86 | 1.6 (0.62–4.13) |
| High-int | 52 | 3.6 (2.00–6.72) | 67 | 4.3 (2.04–9.18) | 54 | 5.1 (2.49–10.77) | 64 | 3.2 (2.34–13.57) |
| High risk | 46 | 6.6 (3.55–12.25) | 44 | 10.3 (5.00–21.45) | 38 | 10.7 (4.51–25.84) | 29 | 13.2 (4.94–35.53) |

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**Abs 358 - Table 1.**

| | | Univariate analysis | | |
|---|---|---|---|---|
| | | OS | CSS | PFS |
| **Feature at diagnosis** | **No. of patients (%)** | ***p*-value** | ***p*-value** | ***p*-value** |
| Median age years (range) | 67 (22–89) | | | |
| Age > 60 years | 218/326 (67) | <0.0001 | 0.004 | <0.0001 |
| Sex male | 168/326 (52) | n.s. | 0.030 | n.s. |
| B symptoms present | 88/324 (27) | 0.003 | 0.0001 | 0.0001 |
| ECOG PS 2–4 | 54/322 (17) | <0.0001 | <0.0001 | <0.0001 |
| Ann Arbor Stages III and IV | 193/326 (59) | 0.010 | 0.0003 | <0.0001 |
| LDH serum level > UNL | 167/315 (53) | 0.0002 | 0.0001 | <0.0001 |
| Bone marrow involved | 45/326 (14) | n.s. | 0.027 | 0.020 |
| Extranodal sites > 1 | 138/326 (42) | n.s. | 0.012 | 0.003 |
| IPI risk int-high/high | 153/322 (48) | <0.0001 | <0.0001 | <0.0001 |
| RIPI risk 0 vs 1–2 vs >2 RF | 29/137/148 | <0.0001 | <0.0001 | <0.0001 |
| B2M serum level > UNL | 190/309 (61) | <0.0001 | 0.0004 | <0.0001 |
| AMC median (IQR range) | 561/μl (400–710) | n.s. | n.s. | n.s. |
| AMC cont. variable | — | n.s. | n.s. | n.s. |
| ALC median (IQR range) | 1400/μl (900–1800) | 0.007 | n.s. | 0.007 |
| ALC cont. variable | — | n.s. | n.s. | 0.054 |
| ALC/AMC cont. variable | — | n.s. | n.s. | n.s. |

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**Introduction:** An increased absolute monocyte count (AMC) at diagnosis has been associated with inferior outcome in different lymphomas, including DLBCL. This study aimed to assess the prognostic impact of different clinical variables at diagnosis, including AMC in this setting.

**Methods:** This is a retrospective analysis of a population of 326 DLBCL patients diagnosed from 2001 to 2011 and treated with immunochemotherapy including RCHOP/CHOP-like regimens whose clinical information were included in the joint database of the Hematology Division of the Amedeo Avogadro University of Eastern Piedmont and the Oncology Institute of Southern Switzerland.

**Results:** Median follow-up for the whole population was 6 years (years), median overall survival (OS) was 10 years (IQR: 3.3–nr), and median cause-specific survival (CSS) was not reached yet; median progression-free survival (PFS) was 7.7 years (IQR: 1.6–nr).

Table 1 shows the main clinical features at diagnosis and their prognostic impact on OS, CSS, and PFS at univariate analysis. At multivariate analysis, only risk defined according to the Revised International Prognostic Index (R-IPI) criteria and β2-microglobulin (B2M) serum level retained prognostic impact on OS, whereas only R-IPI independently predicted CSS and PFS. No role for AMC and ALC/AMC ratio was demonstrated in the definition of prognosis of DLBCL.

**Conclusions:** In our retrospective study on DLBCL treated with R-based regimens, R-IPI represented the best predictor of outcome. Also, B2M showed a strong prognostic impact on survival. On the other hand, AMC was not confirmed as a useful prognostic tool.

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**SUBJECTIVE GLOBAL ASSESSMENT OF MALNUTRITION IS A SIMPLE AND INDEPENDENT PREDICTIVE AND PROGNOSTIC BIOMARKER FOR DIFFUSE LARGE B-CELL LYMPHOMA**

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**Introduction**: Malnutrition is common in patients with lymphomas. International Prognostic Index (IPI), used to prognosticate patients with diffuse large B-cell lymphoma (DLBCL) does not include malnutrition. We hypothesized that malnutrition is an independent poor prognostic factor in patients with DLBCL. Our aims were to determine the complete response rates and overall survival of patients with DLBCL by malnutrition scores.

**Methods**: This was a single-centre, IRB-approved prospective observational study. We included 297 patients with DLBCL among the 496 consecutive new patients enrolled in the Lymphoma Clinic from January to December 2010. Nutritional statuses of all patients were assessed before starting treatment using subjective global assessment (SGA). Patients were followed up until June 2014. Each patient's EMR was reviewed for adverse events, response to treatment, relapse and death. Tumour response was transformed as a bivariate variable (complete response, yes or no). Multivariate analysis was used to adjust for confounding. Actuarial survival was estimated using the Kaplan–Meier method.

**Results:** There were 198 men and 99 women with a mean age of 49.8 years. IPI scores of low, low intermediate, intermediate high and high risk were found in 70, 103, 70 and 54 patients, respectively. SGA scores A, B and C were found in 145, 98 and 54 patients, respectively. There was a statistically significant association between IPI groups and SGA groups and absolute lymphocyte counts and SGA scores. The complete response rates were 84.8%, 51.0% and 22.2% in SGA scores A, B and C groups, respectively ($p < 0.0001$). The independent risk factors for complete response included IPI score, SGA score, use of rituximab, absolute lymphocyte count and private medical care. The overall actuarial survival (OAS) at 4 years was 63% for all patients. The OAS at 4 years was 75.7%, 82.5%, 48.6% and 27.8% in IPI groups low, low intermediate, intermediate high and high in risk, respectively. The OAS at 4 years was 81.4%, 55.1% and 29.7% for SGA groups A, B and C, respectively ($p < 0.001$).

**Conclusions:** Malnutrition as determined by SGA is simple and independent predictive biomarker for complete response as well as a prognostic biomarker for overall survival in patients with DLBCL. The role of adjunct nutrition therapy with chemotherapy for improving outcomes of patients with DLBCL needs to be evaluated in patients with moderate and severe malnutrition.

**360**
**EVALUATION OF NCCN-IPI COMPARED TO IPI IN 312 CONSECUTIVE DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED AT A SINGLE INSTITUTION AND FOLLOWED FOR MORE THAN 3 YEARS**

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**Introduction:** The International Prognostic Index (IPI) was designed for patients with aggressive non-Hodgkin lymphoma before the rituximab era. The new enhanced National Comprehensive Cancer Network (NCCN)-IPI was developed with the intent to better risk-stratify patients uniformly treated with immunochemotherapy. We retrospectively evaluated the outcome of diffuse large B-cell lymphoma (DLBCL) patients treated at our institution between 2007 and 2011. We adopted both IPI and NCCN-IPI risk categories to stratify patients and compare the outcome.

**Methods:** We indentified 312 patients with *de novo* diagnosed DLBCL. Patient characteristics were as follows: male, 157; female, 155; median age (range), 67 (17–90); Stage III/IV, 147 (47%); ECOG performance status more than 1, 107 (34%); critical extranodal sites involved (liver/gastrointestinal tract, lung, CNS, and bone marrow), 97 patients (31%); and elevated LDH, 165 (52%) patients. Two hundred eighty-eight patients received R-CHOP, and 22 patients were treated with R-CHOP-like therapy. In 92 patients (29%), chemotherapy dose reduction was applied. We evaluated patient outcome stratified in four IPI or NCCN-IPI risk categories: low, low-intermediate, high-intermediate, and high.

**Results:** In 294/312 patients evaluable for response, the overall response rate was 92%, and complete response rate was 78%. Six patients died early. After a median (range) follow-up of 42 (1–116) months, median progression-free survival (PFS) and overall survival (OS) were not reached. Five-year PFS and OS rates were 62% (95% CI [58%, 67%]) and 65% (95% CI [60%, 70%]). In patients with low, low-intermediate, high-intermediate, and high NCCN-IPI risk, 5-year OS was 92%, 78%, 53%, and 29%, respectively ($p < 0.0001$). For IPI risk groups with low, low-intermediate, high-intermediate, and high risk, 5-year OS was 81%, 67%, 40%, and 40%, respectively ($p < 0.001$). Dose reduction was a negative prognostic factor for OS: 5-year OS was 57% vs. 67% for patients with reduced-dose and full-dose therapy, respectively.

**Conclusion:** Our data confirm utility of the NCCN-IPI for identifying DLBCL patients with a very poor outcome of less than 30% 5-year survival after R-CHOP therapy. We also confirm an adverse effect of chemotherapy dose reduction on the overall survival.

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### QUALITY OF LIFE PREDICTS SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** Outcomes for patients with diffuse large B-cell lymphoma (DLBCL) are heterogeneous. The rate of cure has improved with chemoimmunotherapy; however, for those with refractory DLBCL or those who cannot receive standard treatment, outcomes are very poor. The current prognostic model, the International Prognostic Index, incorporates patient characteristics including performance status (PS) and additional measures of disease burden. PS is assessed by the healthcare provider and can be subjective. Quality-of-life (QOL) scores can provide an objective measure of the general health status of individuals. We conducted this study to evaluate the association between QOL score and overall survival (OS) in patients with DLBCL, hypothesizing that high QOL scores may predict improved survival.

**Methods:** We identified 1408 patients with DLBCL who completed baseline QOL questionnaires at the MD Anderson Cancer Center from 1999 to 2012; 982 of these had not received prior treatment. We measured QOL by two dependent variables: short-form 12 (SF-12) physical component summary (PCS) and mental component summary (MCS) ratings. Higher scores on the SF-12 PCS and MCS indicate better health status. The QOL scores were described as median values and were analyzed as continuous and categorical variables. Survival was estimated by the Kaplan–Meier method and compared by the log-rank test. The Cox proportional hazards regression model was used to explore the association between QOL and OS.

**Results:** Of the 982 patients with untreated DLBCL included in these analyses, with mean age of 56 years, 53% were male, and 77% were Caucasian. Both PCS [higher than median score, hazard ratio (HR) 0.37; 95% confidence interval (CI), 0.28–0.50] and MCS (higher than median score, HR 0.61; 95% CI, 0.46–0.81) QOL scores were significantly associated with improved OS. There is a significant trend for longer OS with increasing score. Racial disparities were identified, with Caucasian patient QOL scores being associated with survival (PCS HR 0.38; 95% CI, 0.30–0.49; MCS HR 0.65; 95% CI, 0.52–0.82). For Hispanic patients, high PCS (HR 0.44; 95% CI, 0.23–0.82) QOL scores were associated with improved survival; however, MCS (HR 0.67; 95% CI, 0.35–1.27) scores did not have a significant association with survival. For Black patients, PCS and MCS scores were not associated with survival (PCS HR 0.92; 95% CI, 0.31–2.72; MCS HR 1.24; 95% CI, 0.47–3.26).

**Conclusions:** In this large sample of DLBCL patients, QOL at diagnosis is predictive of OS. PCS ratings appear to have a stronger association with OS than MCS. The implications of racial disparities warrant further exploration.

### 362
### PREDICTIVE AND PROGNOSTIC SIGNIFICANCE OF SELECTED FACTORS IN DLBCL IN THE RITUXIMAB ERA: A POLISH LYMPHOMA RESEARCH GROUP MULTICENTER RETROSPECTIVE ANALYSIS

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**Introduction:** Diffuse large B-cell lymphoma (DLBCL) is a heterogenous entity with patients exhibiting a wide range of outcomes. The addition of rituximab to CHOP chemotherapy markedly improved the results of treatment. The roles of previously recognized prognostic factors remain unclear in the rituximab era. Literature data are divergent. The aim of our study was to determine the impact of IPI, peripheral blood absolute lymphocyte count (ALC), Ki-67 proliferation index, and immunohistochemical expression of BCL2 and BCL6 at diagnosis on complete response (CR) rate and survival in patients with newly diagnosed DLBCL who were treated with six cycles of R-CHOP-21.

**Methods:** This is a retrospective analysis of medical records of patients diagnosed with DLBCL who were treated between September 2003 and December 2014 in six hematology and oncology centers of the Polish Lymphoma Research Group.

**Results:** The study group consisted of 398 patients (F/M 201/197) with Stages I–IV who were treated with six cycles of R-CHOP-21 between September 2003 and December 2014. The median age was 57 (range 29–86) years. The CR rate was 66%. In multivariate logistic regression, two variables were associated with failure to achieve CR, ALC < 1.0 G/L (HR = 1.9, 95% CI 1.1–3.1, $p = 0.013$), and IPI (HR = 2.3, 95% CI 1.4–3.7, $p = 0.001$). After the median follow-up time of 32 months (0–116), the 5-year progression-free survival (PFS) and overall survival (OS) estimated with the Kaplan–Meier method were 56% (95% CI 46–60) and 64% (95% CI 56–69), respectively. In univariate analysis, high IPI (log-rank test, $p < 0.001$), ALC < 1.3 G/L (log-rank test, $p = 0.005$), Ki-67 index > 80% (log-rank test, $p < 0.001$), and BCL2(+)/BCL6(−) expression (log-rank test, $p = 0.019$) were significant for OS. It is noteworthy that there were no significant differences in OS in either patients with BCL2(+) versus BCL2(−) or patients with BCL6(+) versus BCL6(−). Similarly, the subgroups defined according to the Hans algorithm as GCB and non-GCB did not show any differences in OS. In the multivariate analysis with Cox model, high IPI (HR 2.9, 95% CI 1.1–7.3, $p = 0.024$) and Ki-67 > 80% (HR 1.7, $p = 0.022$) remained significant for OS. Similarly, the independent negative prognostic factors for PFS were high IPI (HR = 5.00, $p < 0.001$) and Ki-67 > 80% (HR = 1.55, $p = 0.037$).

**Conclusions:** Our results indicate that low ALC and high IPI at diagnosis are independent predictors of poor response to R-CHOP-21 for patients with DLBCL. Moreover, in the rituximab era, both high IPI and high Ki-67 proliferation index remain independent adverse prognostic factors for PFS and OS. There is no difference in

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OS in the groups of patients belonging to GCB versus those belonging to non-GCB defined according to the Hans algorithm. Additionally, neither the expression of BCL2 nor BCL6 has any impact on OS when considered as single markers. Patients who have expression of BCL2 and lack Bcl-6 expression on the lymphoma cells have markedly decreased survival when compared with the other groups.

### 363
### EVALUATION OF PROGNOSTIC SCORES IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB PLUS CHEMOTHERAPY

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**Introduction:** The aim of this study was to evaluate the utility of the current prognostic scores including the revised IPI (R-IPI), National Comprehensive Cancer Network (NCCN)-IPI and European modified scoring m(NCCN)-IPI (for elderly patients) to predict the outcome of DLBCL patients treated with chemotherapy and rituximab from a single institution.

**Methods:** Eighty-nine DLBCL patients, 49 males and 40 females with a median age of 68 years (range 23–92), treated with R-chemotherapy between November 1999 and June 2014 and followed up in a single institution, were retrospectively analyzed. Prognostic scores of R-IPI and NCCN-IPI were calculated in all patients as in the original references. Alternative NCCN-IPI and m(NCCN)-IPI scores were calculated according to a report by Melchart *et al*. (*British Journal of Haematology*, 2015, 168, 239–245) in patients older than 60 years. This m-IPI prognostic index assigned 2 additional points for lower levels of albumin <3.5 mg/dl in the group of patients >60 years. Group risks were defined according to the score (in points) obtained in each prognostic index. Categorical data were compared using Fisher's exact test and two-sided *p*-value. The actuarial survival analysis was carried out according to the method by Kaplan and Meier.

**Results:** Median follow-up duration was 34 months (1–160 months). At DLBCL diagnosis, 12% had more than one extranodal localization (32% had bone marrow disease, CNS, liver/GI tract, or lung), and 50% had an ECOG performance status of 2 or greater. Sixty-four per cent presented Stage III or IV, and 32.6% had elevated LDH (1–3: 25% and >3 times: 8%). Most patients (94%) received R-CHOP as a first-line therapy. Overall response rate was 79% (40% complete remission). The distribution according to the risk group after applying all the prognostic scores is specified in Table 1. Three-year OS and PFS estimated in each risk group according to the R-IPI and NCCN-IPI in all cases and alternative NCCN-IPI and m(NCCN)-IPI in the group of patients >60 years are presented in Table 1.

**Conclusions:** In this series, both R-IPI and NCCN-IPI demonstrated their usefulness to assess the outcome of patients with DLBCL treated with R-chemotherapy. In the subgroup of patients older than 60 years, neither alternative NCCN-IPI nor m(NCCN)-IPI showed better risk stratification than the R-IPI score. In our experience, NCCN-IPI is a useful predictor of outcome in DLBCL treated with R-chemotherapy, and its utility should be investigated in large studies including different populations of different ages.

### 364
### SIL INDEX IS A SIMPLE AND OBJECTIVE PROGNOSTIC INDICATOR IN DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** The International Prognostic Index (IPI) and revised IPI were widely accepted as prognostic indicators for diffuse large B-cell lymphoma (DLBCL). However, the performance status (PS) factor is sometimes ambiguous or subjective. Therefore, we developed a new prognostic index, SIL, that includes only three objective prognostic factors: clinical stage (S), soluble interleukin 2 receptor level >2500 U/mL (I), and elevated lactate dehydrogenase level (L) (*Cancer Sci.* 2012). We aimed to compare the SIL index with other risk factors that comprise the IPI and to evaluate its utility in the risk stratification of patients with DLBCL.

**Abs 363 - Table 1.**

**PFS and OS according to the NCCN-IPI and R-IPI in all patients**

| NCCN-IPI | | | | R-IPI | | | |
|---|---|---|---|---|---|---|---|
| **Risk group** | **Patients (*n*)** | **3-year PFS (%)** | **3-year OS (%)** | **Risk group** | **Patients (*n*)** | **3-year PFS (%)** | **3-year OS (%)** |
| Low | 10 | 78 | 100 | Low | 14 | 82 | 100 |
| Low-intermediate | 31 | 67 | 63 | Intermediate | 43 | 68 | 68 |
| High-Intermediate | 38 | 46 | 55 | | | | |
| High | 7 | 33 | 25+ | High | 31 | 25 | 33 |

**PFS and OS according to the NCCN-IPI and R-IPI in patients older than 60 years**

| NCCN-IPI | | | | R-IPI | | | |
|---|---|---|---|---|---|---|---|
| **Risk group** | **Patients (*n*)** | **3-year PFS (%)** | **3-year OS (%)** | **Risk group** | **Patients (*n*)** | **3-year PFS (%)** | **3-year OS (%)** |
| Low | 2 | 100 | 100 | Low | 4 | 100 | 100 |
| Low-intermediate | 16 | 79 | 51 | Intermediate | 29 | 67 | 53 |
| High-intermediate | 32 | 45 | 46 | | | | |
| High | 7 | 33 | 25 | High | 26 | 32 | 42 |

**PFS and OS according to the alternative NCCN-IPI and modified NCCN-IPI in patients older than 60 years**

| Modified NCCN-IPI | | | | Alternative NCCN-IPI | | | |
|---|---|---|---|---|---|---|---|
| **Risk group** | **Patients (*n*)** | **3-year PFS (%)** | **3-year OS (%)** | **Risk group** | **Patients (*n*)** | **3-year PFS (%)** | **3-year OS (%)** |
| Low | — | — | — | Low | 7 | 83 | 100 |
| Low-intermediate | 4 | 75 | 100 | Intermediate | 39 | 39 | 44 |
| High-intermediate | 35 | 59 | 63 | | | | |
| High | 10 | 27 | 20 | High | 13 | 19 | 35 |

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**Methods:** Between 2003 and 2012, we registered and treated 781 consecutive patients with DLBCL. All the included patients were scheduled to undergo primary therapy with six cycles of full-dose R-CHOP. Patients in whom the initial therapy dose was reduced by >20% were excluded. Finally, 572 of 781 patients were retrospectively analyzed. Patients with partial remission (PR) after the initial four cycles underwent eight R-CHOP cycles in total. If deemed necessary by the attending physician, additional local irradiation was performed in patients with PR or complete remission.

**Results:** The median age at diagnosis was 63 years (range, 18–89 years). Sixty-one patients (11%) received radiation therapy as primary treatment. The median observation time for survivors was 55 months (range, 1–131 months). For the 572 patients, the 5-year progression-free survival (PFS) and 5-year overall survival (OS) rates were 70% and 81%, respectively. According to the SIL index, 367 patients (64.2%) and 205 patients (35.8%) were classified as having standard (SIL index: 0 or 1) and high (SIL index: 2 or 3) risks, respectively. Five-year PFS rates in the standard-risk and high-risk groups were 79% and 53%, respectively ($p < 0.0001$). Five-year OS rates in the standard-risk and high-risk groups were 90% and 66%, respectively ($p < 0.0001$). Cox regression analysis of the SIL index, age (>60 years), PS (2–4), extranodal involvement sites (>1), and sex showed that the SIL index [$p < 0.0001$; hazard ratio (HR): 2.38] and PS ($p = 0.005$; HR: 1.73) were independent risk factors for PFS. Similarly, the SIL index ($p < 0.0001$; HR: 2.62) and PS ($p = 0.006$; HR: 1.89) were independent risk factors for OS. When the patients were divided into two groups by age (≤60 and >60 years), the SIL index was a good prognostic indicator for PFS and OS in both groups. When they were divided by the number of extranodal involvement sites (0–1 and >1) and sex, the SIL index was still a good prognostic indicator for PFS and OS in both groups. Lastly, when they were divided by PS (0–1 and 2–4), the SIL index was effective in the good-PS group. In the poor-PS group, however, the SIL index showed a statistically significant difference in the OS, but not in the PFS.

**Conclusion:** The SIL index is a simple and objective prognostic indicator for DLBCL patients treated with R-CHOP.

### 365
### PROGNOSTIC SIGNIFICANCE OF BODY MASS INDEX IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB-CHOP OR SIMILAR COMBINATIONS

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**Introduction:** Recent data suggested that increased BMI correlate with a favorable outcome in White American males with DLBCL. Two smaller studies (183 and 537 patients) provided controversial results in unselected DLBCL patients. Thus, the prognostic value of BMI in DLBCL needs further investigation in unselected patients treated with R-CHOP; potential findings also need to be interpreted.

**Methods:** Six hundred forty-nine patients with DLBCL (median age 64, range 16–94), treated with R-CHOP or similar regimens, were evaluated. PFS was determined considering toxic deaths as events in contrast to deaths from other causes in the first CR.

**Results:** The median BMI was 26.6 (IQR 23.8–29.7), 155 (24%) patients were obese (BMI ≥ 30), and 258 (40%) were overweight (BMI 25–29.9). Increased BMI correlated with increased age, while decreased BMI with PS ≥ 2, ≥2 E-sites, and B-symptoms. Seven-year PFS of patients with BMI ≥30, 25–29.9, and <25 was 77%, 71%, and 68%, respectively ($p = 0.07$ for BMI ≥30 vs <30). The prognostic impact of BMI in subgroups of patients defined by demographic characteristics and B symptoms is shown in the table: BMI had no prognostic impact on patients <60 years. On the contrary, males >60 years with BMI ≥ 30 and females >60 years with BMI ≥ 25 had more favorable outcomes than those with lower BMI. Similarly, BMI ≥ 30 was a favorable prognostic factor only for patients without B symptoms. In multivariate analysis, BMI was simultaneously evaluated with the five IPI factors, gender, and B symptoms: in the whole patient population, BMI had no independent prognostic impact, while BMI ≥ 30 was the strongest independent prognostic factor for PFS in males >60 years (HR 3.4, 95% CI 1.2–9.5, $p = 0.02$). Similarly, in females >60 years, BMI ≥ 25 was an independent prognostic factor along with LDH and Stage III/IV (HR 1.9, 1.04–3.3, $p = 0.04$). In a preliminary analysis of 277 patients, DI of chemotherapy did not differ between patients with BMI ≥30 or <30, while DI of rituximab was slightly (but statistically significantly lower) in obese patients ($p = 0.01$).

**Conclusions:** The data presented here suggest that the effect of BMI on prognosis is restricted to elderly DLBCL patients and, possibly, with different cutoffs (30 vs. 25 kg/m$^2$) for males and females. This could not be attributed either to the unfavorable prognosis of patients with weight loss, since the observation was restricted to patients without B symptoms, or to differences in DI of immunochemotherapy according to BMI. A possible correlation with pharmacokinetic parameters needs further verification.

**Abs 365 - Table 1.**

| BMI | | | BMI | | |
|---|---|---|---|---|---|
| **Males, >60** | **Patients/failures** | **7-year PFS** | **Males, ≤60** | **Patients/failures** | **7-year PFS** |
| ≥30 | 39/5 | 86% | ≥30 | 39/8 | 70% |
| 25–29.9 | 91/37 | 55%, $p = 0.004$ | 25–29.9 | 57/7 | 86%, $p = 0.07$ |
| <25 | 66/17 | 65% | <25 | 62/17 | 69% |
| <30 ($p$ vs ≥30) | 157/54 | 59%, $p = 0.01$ | <30 ($p$ vs ≥30) | 119/24 | 78%, $p = 0.86$ |
| **Females, >60** | **Patients/failures** | **7-year PFS** | **Females, ≤60** | **Patients/failures** | **7-year PFS** |
| ≥30 | 53/13 | 72% | ≥30 | 21/3 | 84% |
| 25–29.9 | 68/16 | 72%, $p = 0.05$ | 25–29.9 | 36/6 | 83%, $p = 0.99$ |
| <25 | 51/22 | 53% | <25 | 50/8 | 83% |
| ≥25 ($p$ vs <25) | 121/29 | 72%, $p = 0.02$ | ≥25 ($p$ vs <25) | 57/9 | 84%, $p = 0.94$ |
| **B symptoms (−)** | **Patients/failures** | **7-year PFS** | **B-symptoms(+)** | **Patients/failures** | **7-year PFS** |
| ≥30 | 115/14 | 84% | ≥30 | 30/10 | 64% |
| 25–29.9 | 194/43 | 76%, $p = 0.09$ | 25–29.9 | 56/23 | 54%, $p = 0.78$ |
| <25 | 156/34 | 73% | <25 | 70/307 | 55% |
| <30 ($p$ vs ≥30) | 350/77 | 75%, $p = 0.03$ | <30 ($p$ vs ≥30) | 126/53 | 54%, $p = 0.50$ |

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## CLINICO-BIOLOGICAL CHARACTERISTICS AND OUTCOME OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA CARRYING HEPATITIS B OR C VIRUS TREATED WITH IMMUNOCHEMOTHERAPY

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**Introduction:** Diffuse large B-cell lymphoma (DLBCL) patients carrying hepatitis B virus (HBV) or hepatitis C virus (HCV) are a management challenge in the clinical setting, particularly since rituximab-based treatments became the gold standard. The aim of this study was to assess the HBV and HCV incidence and their clinical and prognostic impact in a series of DLBCL patients treated with immunochemotherapy.

**Methods:** Three hundred twenty-one patients (161 M/160 F; median age 66) were diagnosed with DLBCL in a single center between 2002 and 2013. Primary central nervous system lymphoma, primary mediastinal lymphoma, and plasmablastic lymphoma were excluded, as well as transformed cases from other lymphomas, post-transplant lymphoproliferative disorders and HIV+ patients. HCV+ and HBV+ were defined by the presence of IgG anti-HCV and HBV core antibodies, respectively. The main clinicobiological characteristics and outcome were analyzed according to the viral status.

**Results:** Two hundred sixty-five patients were virus negative, 32 HBV+ (10%), and 29 HCV+ (9%). Five of the latter had HBV/HCV co-infection. The main initial features and outcome are detailed in the table. Elevated basal bilirubin correlated with higher liver toxicity during treatment (85% vs. 40%, $p < 0.001$) and shorter overall survival (OS). After a median follow-up of 49 months (range 2–146), median OS was not reached for patients without hepatitis and was 55, 38, and 14 months for HBV+, HCV+, and HBV+/HCV+ patients, respectively ($p = 0.005$). HCV+ patients without liver cirrhosis showed an almost identical OS to that of hepatitis-negative patients. When the analysis was restricted to patients receiving curative intention regimens, 5-year OS for hepatitis negative, HBV+, HCV+, and HBV+/HCV+ was 69%, 62%, 63%, and 25%, respectively ($p$ = NS).

**Conclusion:** The presence of HBV or HCV in DLBCL patients entails a higher number of complications; however, liver impairment, not the hepatitis viral status, is the key feature in the outcome of the patients.

**Abs 366 - Table 1.**

| | No hepatitis ($n$ = 265) | HBV ($n$ = 32) | HCV ($n$ = 29) | HBV/HCV ($n$ = 5) |
|---|---|---|---|---|
| Age ≥ 60 years (%) | 60 | 66 | 83 | 80 |
| Advanced stage (%) | 57 | 53 | 66 | 60 |
| Int-high or high risk IPI (%) | 46 | 31 | 62 | 40 |
| Low serum albumin (%) | 18 | 31 | 44 | 50 |
| High LDH (%) | 49 | 47 | 72 | 100 |
| Curative intention treatment (%) | 91 | 69 | 76 | 80 |
| Liver toxicity grade ≥ 2 WHO (%) | 6 | 14 | 39 | 75 |
| CR (%) | 73 | 61 | 55 | 40 |
| 5-year OS (%) | 65 | 42 | 42 | 20 |

*$p < 0.05$;
**$p < 0.001$.

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## PROGNOSTIC SIGNIFICANCE OF EPSTEIN–BARR VIRUS DNA DETECTION IN PRETREATMENT SERUM FROM PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** It remains unknown whether the detection of Epstein–Barr virus (EBV) DNA in pretreatment serum has any clinical implications in patients with diffuse large B-cell lymphoma (DLBCL). We measured EBV DNA load in pretreatment serum from DLBCL patients and attempted to clarify its prognostic significance with consideration for EBV-encoded small RNA *in situ* hybridization (EBER) status in diagnostic specimens.

**Methods:** We selected patients diagnosed with DLBCL from October 2007 to March 2012 at our hospital who did not have prior lymphoma or underlying immunodeficiency such as human immunodeficiency virus infection and rheumatoid arthritis treated with methotrexate. Among 140 such patients, 127 patients whose pretreatment serum was available were included. Anthracycline-based chemotherapy in combination with rituximab was used as an initial therapy for 93% of the patients. The median follow-up of surviving patients was 44.4 months (range, 1.5–78.0 months). Serum EBV DNA load was measured with a real-time quantitative polymerase chain reaction assay.

**Results:** Serum EBV DNA was detected in 15 patients (12%), with the EBV DNA load ranging from 250 to 5 500 000 copies/mL. Patients with detectable EBV DNA in serum were older ($p = 0.005$) and tended to have a more advanced stage ($p = 0.053$) than those without detectable EBV DNA. No significant intergroup imbalances were observed in terms of performance status, LDH level, and the number of extranodal sites. The EBER status was known for 123 patients, 8 (7%) of whom had positive results. Serum EBV DNA was detected in 6 of 8 (75%) EBER-positive patients and in 9 of 115 (8%) EBER-negative patients ($p < 0.001$). Patients with detectable EBV DNA had significantly worse progression-free survival (PFS; 30% vs. 81% at 4 years; $p = 0.001$) and overall survival (OS; 57% vs. 87% at 4 years; $p < 0.001$) than those who did not. After adjusting for age, performance status, LDH, stage, and number of extranodal sites, EBV DNA detection in pretreatment serum remained significantly associated with inferior PFS [hazard ratio (HR), 3.53; 95% confidence interval (CI), 1.49–8.32; $p = 0.004$] and OS (HR, 3.06; 95% CI, 1.13–8.27; $p = 0.027$). EBER-positive patients also had significantly worse PFS and OS than EBER-negative patients ($p = 0.001$ and $p = 0.029$, respectively). Given this finding and a positive interaction between EBER status and serum EBV DNA detection, we assessed whether outcomes for EBER-negative patients could be differentiated using serum EBV DNA detection. We found that even among EBER-negative patients, serum EBV DNA detection was associated with inferior PFS and OS ($p < 0.001$ each).

**Conclusion:** EBV DNA detection in pretreatment serum may have adverse prognostic impact in patients with DLBCL.

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## RETROSPECTIVE EVALUATION OF THE BRITISH COMMITTEE FOR STANDARDS IN HEMATOLOGY INDICATIONS FOR CNS PROPHYLAXIS IN A SERIES OF 122 PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** Central nervous system (CNS) recurrence is an uncommon but lethal event in patients with diffuse large B-cell lymphoma (DLBCL). Both the identification of patient at risk and the best prophylaxis strategy are still a matter of debate. In 2013, the British Committee for Standards in Hematology (BCSH) recommended a CNS-directed therapy consisting of four doses of intrathecal methotrexate (IT-MTX) for patients with testicular, breast and epidural involvement and for those with raised LDH associated with more than one extranodal localization (*BJH*, 2013). However, other authors proposed different strategies: in a recently published mono-institutional series, Ferreri *et al*. proposed treatment with systemic high-dose MTX in all patients with testis, spine, skull, nasal sinuses, orbit, naso-pharynx, kidney, and breast localization as well as in patients with high LDH and advanced-stage disease (*BJH*, 2014).

**Methods:** We retrospectively analyzed all HIV-negative DLBCL patients consecutively admitted at our institution between 2008 and 2014 and treated with R-CHOP chemotherapy or similar regimens. Patients with CNS disease at diagnosis and patients with Burkitt or Burkitt-like, grey-zone, primary mediastinal, and leg-type lymphoma

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were excluded. Our criteria for CNS prophylaxis were similar to BCSH ones; however, some patients with bone marrow (BM), nasal sinuses, or nasopharynx involvement were also treated. Prophylaxis consisted of four doses of IT-MTX 12.5 mg plus cytarabine 50 mg delivered through lumbar puncture on Day 1 of chemotherapy.

**Results:** One-hundred and twenty-two patients, median age 66 years, were included; 26 received CNS prophylaxis. According to the BCSH guidelines, 16 patients should have been considered at high risk: nobody experienced CNS recurrence even though five patients had not received prophylaxis as per the treating physicians' decision. Among 106 patients at low risk, 15 received CNS-directed therapy with one relapse in a patient with raised LDH and BM involvement (LDH + BM). Ninety-one low-risk patients received no CNS prophylaxis: two of them experienced a CNS recurrence. Interestingly, both presented with LDH + BM. Considering only this latter 91 patients, risk of CNS relapse was 50% in four patients with LDH + BM and zero among 87 patients without this feature ($p = 0.001$); it was 8% in 26 patients with high LDH and advanced stage and zero in the remaining 65 patients ($p$ = n.s.).

**Conclusions:** In our experience, isolated BM involvement, but not advanced-stage disease, in DLBCL patients with high LDH levels was a rare event significantly associated with CNS recurrence. These patients should receive CNS prophylaxis. Moreover, intrathecal MTX + cytarabine proved very effective in the prevention of CNS relapse. Larger and prospective studies are warranted to define the best prophylaxis policy in patients with high-grade lymphoma.

### 369
### SPONTANEOUS REGRESSION OF LYMPHOMA IN HIV-POSITIVE PATIENTS WITH IMMUNE RECONSTITUTION FOLLOWING HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT: NINE PATIENT RETROSPECTIVE SERIES

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**Introduction:** HIV-related non-Hodgkin lymphoma (NHL) in the developed world has significantly declined since the introduction of highly active antiretroviral therapy (HAART). HIV infection leads to immune activation and chronic inflammation, which may contribute to lymphoma pathogenesis. Immunologically, there are parallels with other immunodeficiency states in which lymphoma arises.

Primary management of EBV-driven post-transplant lymphomas (PTLD) aims to restore immune function by reducing immunosuppression or administration of specific cytotoxic T lymphocytes. HAART reduces HIV replication, decreasing chronic inflammation and improving T-cell surveillance and cellular immune responses.

Severely immunosuppressed patients with a diagnosis of HIV and concurrent lymphoma could respond to immune reconstitution in a similar way to post-transplant lymphoma.

**Method:** Selected lymphoma specialists throughout the UK were contacted via email, providing anonymized information to populate an Excel spreadsheet.

**Results**: Nine HIV-positive patients were diagnosed with lymphoma. All patients had spontaneous regression of lymphoma following commencement or alteration of HAART without requiring chemotherapy.

**HAART at diagnosis of lymphoma:**

Two patients on HAART, CD4 counts 198 and 110.

One patient non-compliant with HAART

Four patients not on HAART

Two patients recently commenced on HAART

**CD4 count:**

Diagnosis: mean 182 cells/mm$^3$, median 163 cells/mm$^3$ (range 70–340)

Remission: mean 398 cells/mm$^3$, median 347 cells/mm$^3$ (range 147–830)

**Viral load:**

Diagnosis: median for seven of nine patients was 227 426 (range 54–3 110 000).

Remission: <250 in six of nine patients; one patient's viral load reduced from 57 018 to 4000.

**Conclusion:** This is the largest case series of HIV patients undergoing spontaneous remission of lymphoma, all of whom are alive. Commencement of HAART in patients with HIV and concurrent lymphoma is standard practice. All patients described show a reduction in viral load and evidence of immune reconstitution with lymphoma regression. This is analogous to some cases of PTLD. Chemotherapy management in advanced HIV is complicated, and there is some evidence that chemotherapy delays HIV control and immune reconstitution. In selected patients, it may be reasonable to delay chemotherapy pending evidence of immune reconstitution.

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### AIDS-RELATED NON-HODGKIN LYMPHOMA IN THE ERA OF HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT. THE EXPERIENCE OF TWO CUBAN INSTITUTIONS

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**Abs 369 - Table 1.**

| Patient no. | Sex | Age at dx | Lymphoma | HIV+ at lymphoma diagnosis | CD4 count at dx lymphoma (cells/μl) | CD4 count at remission | Viral load at diagnosis | Viral load at remission | Last f/u |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 40 | DLBCL | Positive (HAART non-compliant) | 125 | 539 | 3 110 000 | 135 | 6.2014 |
| 2 | M | 33 | Primary CNS lymphoma | Positive | 198 | 282 | 54 | <50 | 5.2013 |
| 3 | M | 46 | Peripheral T-cell lymphoma | Positive | 130 | U | 241 790 | U | 5.2013 |
| 4 | M | 30 | Hodgkin | Positive | 110 | 830 | U | <50 | 2.2014 |
| 5 | F | 21 | DLBCL | Positive | 163 | 245 | 57 018 | 4000 | 12.2014 |
| 6 | M | 39 | DLBCL | Positive | 340 | U | U | U | 7.2014 |
| 7 | M | 65 | DLBCL | Concurrent | 70 | 147 | 227 466 | 241 | 8.2014 |
| 8 | M | 56 | Peripheral T-cell lymphoma | Concurrent | 177 | 347 | 2 000 000 | <50 | 4.2014 |
| 9 | F | 29 | CD8+ cutaneous T-cell lymphoma | Positive (new diagnosis) | 329 | 402 | 193 | <40 | 2.2011 |

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**Introduction:** Non-Hodgkin lymphoma is the second AIDS-defining malignancy in order of frequency. As a consequence of the introduction of the highly active antiretroviral treatment (HAART), a decrease in the incidence rates of the AIDS-related non-Hodgkin lymphoma (ARL) has been observed. On the other hand, an improvement in the prognosis and treatment outcomes of these patients has been achieved. HAART was introduced in Cuba at the end of the 1990s. Since the early 2000s, the National Institute of Oncology and Radiobiology (INOR) and the Institute of Tropical Diseases 'Pedro Kouri' (IPK) have collaborated for the treatment of HIV/AIDS-related neoplasm. A multidisciplinary team integrated by an oncologist, infectologist, pathologist, radiation oncologist, and radiologist was created, and unified protocols for the treatment of patients were implemented.

**Methods**: A retrospective analysis was performed to evaluate the outcome in the management of patients with ARL diagnosed and treated at the INOR and in the IPK between January of 2000 and December of 2012. A description of the prognostic variables associated with survival and treatment outcome was made.

**Results:** A total of 132 patients were diagnosed from ARL in the studied period. The majority of them were male (87%), with a median age at diagnosis of 41 years. Eighty per cent of patients were diagnosed with advanced stage (Stages III and IV), and 69.7% had extranodal involvement. Sixty-five per cent had age-adjusted IPI of intermediate-high or high, 23.2% had performance status of 2–3, and 65% had elevated LDH levels. Diffused large B-cell lymphoma was most the common lymphoma subtype (86.3%). Most of the patients (80.7%) were treated with chemotherapy using CHOP-like regimens with a median of eight cycles. Median overall survival was 30 months (95% CI 11–58), and 5-year overall survival was 31.8%. In the multivariate analysis, a decrease in the overall survival was significantly associated with a CD4 cell count $<200$ cells/mm$^3$ at diagnosis ($p < 0.01$), aaIPI of high and intermediate-high ($p < 0.018$), elevated LDH levels ($p < 0.039$), and treatment before the introduction of the multidisciplinary team and standardized protocols for the treatment of patients ($p < 0.001$).

**Conclusions:** In our series, the treatment of ARL patients by multidisciplinary teams, with standardized protocols, improve the outcomes and overall survival as independent factors.

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### SEQUENTIAL PLATINUM-BASED REGIMEN IN PRIMARY TREATMENT IMPROVES OUTCOMES OF YOUNG PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** Platinum-based chemotherapy is a potent salvage approach for refractory or relapse patients with diffuse large B-cell lymphoma, which implies its capacity to eradicate residual lymphoma cells after first-line anthracycline-based chemotherapy. Therefore, it is hypothesized that upfront platinum-based regimen may improve survival of patients with diffuse large B-cell lymphoma. This study aimed to evaluate the efficacy and safety of a sequential platinum-based regimen following anthracycline-based chemotherapy in primary therapy of young patients with diffuse large B-cell lymphoma.

**Methods:** Three hundred and thirty-one patients with newly diagnosed disease were retrospectively enrolled in the study. After three to four cycles of anthracycline-based chemotherapy, all of these patients achieved complete or partial remission. Then, 202 patients continued to receive three to four cycles of anthracycline-based chemotherapy, and 129 patients changed treatment and received three to four cycles of platinum-based chemotherapy instead. In total, all of these patients completed six to eight cycles of chemotherapy during primary therapy. The distribution of patients was not at random, so more patients with partial remission were distributed to the sequential group than those with complete remission.

**Results:** The sequential group yielded a better complete remission than the continuation group (79.84% vs. 62.87%, $p = 0.001$) after the completion of primary therapy. Higher percentages of 5-year PFS and OS were observed in the sequential group (5-year PFS: 71.5% vs. 58.0%, $p = 0.007$; 5-year OS: 70.3% vs. 58.5%, $p = 0.016$). A multivariate analysis revealed therapeutic strategy as an independent prognostic factor to improve survival of patients. Subgroup analysis showed its predominance in patients with age-adjusted IPI 0–1 score. At the same time, sequential platinum-based chemotherapy did not cause significantly increased toxicities ($p > 0.05$) compared with the continuation group.

**Conclusion:** Sequential platinum-based chemotherapy following anthracycline-based chemotherapy as primary therapy can increase response rate and improve long-term survival without enhancing toxicities in young patients with diffuse large B-cell lymphoma.

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### UPFRONT INTENSIFICATION INCORPORATING DOSE-DENSE RITUXIMAB AND HIGH-DOSE MTX TO CHOP-14 IN YOUNG HIGH-RISK AGGRESSIVE B-CELL LYMPHOMA PATIENTS

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**Introduction:** Although treatment of high-grade B-cell NHL has improved in the last decade by incorporating rituximab to upfront CHOP therapy, outcome in young high-risk patients (aaIPI 2–3) remains poor, with a substantial percentage of patients achieving only a short EFS and OS. We designed an upfront treatment schedule containing six-cycle CHOP-14 as backbone, incorporating dose-dense rituximab as suggested by data from Pfreundschuh *et al*. and high-dose MTX as a CNS-directed approach.

**Methods:** Since 2012, all patients aged <60 years presenting with high-risk DLBCL and PMBL at the Freiburg University Medical Center were administered with six cycles of CHOP-14 with dose-dense rituximab (375 mg/m$^2$) on Days 0, 1, 4, 8, 15, 22, 29, 47, 61, and 75. HD MTX (3.0 g/m$^2$) was administered on Days 30 and 76 followed by standard CHOP. We retrospectively reviewed all patients treated according to the protocol in an intention-to-treat analysis, identifying 31 patients meeting the criteria. Response was evaluated using PET-CT according to revised criteria for response assessment by Cheson *et al*. (2014).

**Results:** In 31 patients, histology included DLBCL ($n = 19$, 61%) and PMBL ($n = 12$, 39%). Median age at diagnosis was 42 years (range 22–68 years), and 52% were female. Stages I/II and III/IV were found in 23% and 77%, respectively; five patients with Stage I had a bulky disease, one patient had a testicular lymphoma, and one patient had orbital involvement. B symptoms were found in 42%. LDH was elevated in all patients. An ECOG > 1 was found in 26%. Sixty-one per cent had two or more extranodal sites. According to IPI and aaIPI, 28 patients (90%) had an intermediate high and high risk score.

Median follow-up was 18.3 months (range 0.4–35.9). Twenty-nine (94%) patients completed the protocol. No treatment-related deaths were observed. Toxicities were as expected and manageable; one patient could not receive the sixth R-CHOP due to severe infection. One early death was observed 4 days after the first R-CHOP due to lymphoma infiltration of the heart and consecutive arrhythmia. All patients responded, with 19 patients achieving a CR (61%) and 11 patients a PR (36 %). Three PET-positive patients underwent second biopsy. Two were lymphoma negative and one positive. Patients not in CR were treated as follows: three patients underwent intensification with HD BEAM and ASCT, and five patients received radiotherapy. For all patients, estimated 2-year OS was 96.8%, and 2-year PFS was 92.9%. No CNS events occurred.

One patient with diagnosis of PMBL locally relapsed within the radiation field 10 months after treatment initiation. Salvage therapy included HD therapy and allogeneic SCT, achieving a PR. The patient died after a second relapse with an OS of 35.4 months.

**Conclusions:** This intensified protocol is feasible for high-risk DLBCL and PMBL patients <60 years, showing promising results regarding ORR, progression-free, and overall survival. In our cohort, no CNS relapse was yet observed. A longer follow-up is required to confirm our results. This protocol should be tested in prospective randomized trials.

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### 373
### CARBOPLATIN, ARACYTINE PLUS DEXAMETHASONE WITH OR WITHOUT RITUXIMAB (DHAC +/− R) IS A FEASIBLE AND EFFECTIVE TREATMENT FOR LYMPHOMA PATIENTS

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Salt platins are used in relapsed/refractory (R/R) lymphomas. DHAP regimen combines dexamethasone (Dex), cisplatin and high-dose cytarabine (AraC-HD). Cisplatin-induced nephrotoxicity is a major concern: 15% of tubular complication sometimes leads to renal insufficiency that jeopardizes autologous stem cell transplantation (ASCT). Oxaliplatin may be associated with neuropathy. Carboplatin (Carbo) is widely used in solid tumours and combined with ifosfamide (ICE regimen) in lymphomas. Carbo, AraC-HD and Dex (DHAC +/− R) regimen has never been evaluated. Taking into account the potential benefits of carboplatin, we report the toxicity and efficacy of DHAC +/− R in lymphoma patients.

From September 2008 to September 2014, all R/R lymphoma patients treated in our institution received DHAC +/− R. Mantle cell lymphoma (MCL) patients received upfront DHAC + R. Rituximab was administrated in CD20+ lymphoma entities. DHAC consisted of Carbo (target AUC of 5—Calvert formula), AraC-HD 2 g/m$^2$ two times a day and Dex, with 21 days between two courses. The number of cycles was adapted to patients and therapeutic programmes with or without ASCT.

One hundred eighty-two patients received DHAC. Diagnoses were diffuse large B-cell lymphoma (DLBCL, $n = 97$, 53%), follicular lymphoma (FL, $n = 24$, 13%), Hodgkin disease (HD, $n = 22$, 12%), MCL ($n = 22$, 12%), peripheral T-cell lymphoma (PTCL, $n = 6$, 3%), seven low-grade B lymphomas and three plasmablastic lymphomas. Twenty-two patients received upfront DHAC +/− R; 54 were in relapse ($n = 54$, 30%), 61 in partial response (PR) (34%), 19 in stable disease/refractory (SD) (11%) and 23 with progressive disease (PD; 13%). The median number of treatment lines before DHAC +/− R was one (range, 1–4).

At the first cycle of DHAC +/− R, median age was 53 years (range, 18–75). Forty-eight per cent of patients received DHAC +/− R with overnight stay and 38% in day-care hospital. A median of three cycles of DHAC +/− R was administrated. Median dosing of Carbo at Cycles 1–4, 5 and 6 was (mg/m$^2$) 350, 310 and 315, respectively. Median dosing of AraC-HD was 4 g/m$^2$ through all cycles. Seventy-eight per cent of patients completed the scheduled number of cycles. A major cause of arrest of DHAC +/− R was PD or SD (32 out of 41). Grade ≥ 3 haematological toxicities were reported, mainly thrombopenia ($n = 53$) and anaemia ($n = 41$). One hundred six patients (58%) required transfusion, with a median of two red blood cell units (2–14) and two platelets units (1–27). Eleven sepses were reported. One patient died of septic shock. No Grade ≥ 3 renal toxicities were reported. Responses after DHAC were 27% complete remission (CR), 42% PR, 11% SD and 20% PD. Sixty per cent of the patients at diagnosis reached CR, while 72% of patients with relapsed lymphoma reached PR. The median follow-up was 24 months (range, 2–71), median OS was not reached (estimated 2-year OS was 74%) and median PFS was 46 months. For DLBCL patients with insufficient response before ASCT, 2-year PFS and OS were 64% and 85%, respectively. Among the 133 patients scheduled for ASCT, 96 were in response after DHAC +/− R, and they all underwent ASCT.

Herein, we show that DHAC +/− R is a safe chemotherapy regimen associated with good clinical response in all lymphoma entities and does not jeopardize ASCT when PR is reached.

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### DOSE ESCALATION TRIAL OF LENALIDOMIDE ADDED TO R-DHAP AS SALVAGE TREATMENT IN RELAPSED AND REFRACTORY AGGRESSIVE B-NHL

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Primary treatment results of aggressive B-NHL improved substantially after introduction of chemoimmunotherapy. However, current salvage treatment of primary progressive and relapsed disease is often disappointing with remission rates of <50%. Lenalidomide (L) has been shown to exert a single-drug activity in this situation. We therefore investigated the combination of L and a standard salvage regimen (R-DHAP: rituximab, dexamethasone, cytarabine, cisplatinum/carboplatinum) in a Phase I/II dose escalation study. Younger patients (18–70 years) with primary progressive or relapsed aggressive B-NHL were included in the study. R-DHAP at standard doses was combined with L. Three 21-day cycles of L at four dose levels (DL) were planned (DL1: 5 mg, Days 1–7, Cycles 2 and 3; DL2: 5 mg, Days 1–7, Cycles 1–3; DL3: 15 mg, Days 1–7, Cycles 1–3; DL4: 15 mg, Days 6–7). All patients were to receive IV heparin for prophylaxis of thromboembolic events. Mobilization of hematopoietic stem cells was attempted after Cycle 2 in patients scheduled for autologous stem cell transplantation. Primary endpoint was the maximum tolerated dose (MTD) of L in combination with R-DHAP. Death due to any cause, prolonged leukopenia, and thrombopenia (>28 days) as well as any organ toxicity Grade 4 with the exception of infection were considered dose limiting. Escalation to the next DL occurred after complete analysis of ≥18 treatment cycles failed to show prohibitive toxicities. From 11/2010 to 10/2011, 33 patients were included (28 DLBCL, 2 DLBCL/Burkitt-like, 1 DLBCL/FL Grade III, and 2 FL Grade III). Median age was 52.9 years (range: 24–70). Seventy-seven cycles combining L with R-DHAP were given. Ten patients did not receive all three cycles of therapy due to progressive disease (six patients), hematological toxicity (three patients), and withdrawal of informed consent (one patient). Ten patients experienced a severe adverse event: two cases of renal failure, two cases of arterial embolism of the lung, two cases of prolonged thrombocytopenia, and four infections. At DL1–DL3, no dose-limiting toxicity occurred. With 17 treatment cycles at DL4, two patients experienced prolonged cytopenia and two other patients failed to mobilize stem cells. Therefore, treatment at DL4 was stopped, and seven additional patients were treated at DL3: no further dose-limiting toxicities were observed. Seventeen of 31 evaluable patients (55%) achieved remission (12 CR/CRu and 5 PR). After a median observation time of 16 months, OS in the per protocol population is 77.3% (95% CI 44.3–92.2%) and PFS is 66.5% (95% CI 36.9–84.6%). The combination of L with R-DHAP was feasible and safe up to DL3 (15 mg of L, Days 1–7 with a maximum of three treatment courses). In contrast, administration of L during the time of recovering hematopoiesis (DL4) resulted in prolonged thrombocytopenia and failure to mobilize stem cells. The combination of L at DL3 and R-DHAP at the MTD gave promising results and will be evaluated in the Phase II part of this study (DSHNHL-R6).

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### OUTCOME OF RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA AFTER SECOND SALVAGE THERAPY: MD ANDERSON EXPERIENCE

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**Background:** The outcome of patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) who failed first-line platinum-containing salvage chemotherapy and underwent a second salvage therapy is poorly characterized. We conducted a retrospective study to evaluate these outcomes in patients treated at the MD Anderson Cancer Center with frontline rituximab chemotherapy.

**Methods:** We analyzed the outcome of all 191 patients treated at MD Anderson with relapsed/refractory DLBCL ($n = 172$) or transformed follicular lymphoma ($n = 19$) who were refractory to or relapsed after both initial rituximab–CHOP or CHOP-like (R-EPOCH or R-HyperCVAD) and first-line rituximab–platinum-containing salvage chemotherapy and subsequently received a second salvage

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therapy between August 2001 and October 2014. Patients with a history of CNS lymphoma were excluded.

**Results:** The median age of patients was 56 (range: 20–80 years). Of these patients, 61.7% were refractory to initial R-CHOP/CHOP-like therapy, and 17% relapsed after autologous stem cell transplantation (SCT). Second salvage therapies included rituximab-containing chemotherapies such as HyperCVAD (17%), ICE (15%), DHAP (14%), ESHAP (12%), Gem-Ox (9%), methotrexate–cytarabine (4%), other chemotherapies (14%), and therapies on clinical trials (15%). Overall response rate was 22% (42/191), and complete response rate was 11.5% (22/191) with the second salvage therapy. Median progression-free survival (PFS) was 2.8 months (95% CI: 2.4–3.3 months), and median overall survival (OS) was 8 months (95% CI: 6.6–9.6 months). There were no significant differences detected in response rates, PFS, or OS between the various therapies administered, with limited-sized cohorts. After the second salvage therapy, 31 (16.2%) patients underwent ASCT and 19 patients (10%) underwent allogeneic SCT. For patients who underwent autologous or allogeneic SCT after the second salvage therapy, median PFS and OS were 10.7 vs. 7.8 and 28.7 vs. 24.9 months, respectively.

**Conclusion:** Patients with relapsed/refractory DLBCL rarely respond to a second salvage therapy in the rituximab era. Although a small subset of responsive patients may achieve modest survival benefits with SCT, overall outcomes are dismal. Patients who do not respond to the first salvage rituximab chemotherapy should be considered for clinical trials with novel agents.

### 376
### USE OF COMPLEMENTARY AND ALTERNATIVE THERAPIES IN PATIENTS WITH LYMPHOMA—A VISION ABOUT WHAT HAPPENS IN THE NORTHEAST OF BRAZIL

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**Introduction:** In northeast Brazil, it is very common to use traditional medicinal herbs to treat most diseases in the population. In Brazil, the Ministry of Health develops a policy that encourages the use of alternative and complementary practices by family doctors and also by health workers. No published survey has specifically addressed the beliefs, knowledge, and usage of complementary and alternative medicine (CAM) in patients with lymphoma.

**Methods:** In this pilot project, 60 subjects were selected from a population of 240 long-term lymphoma survivors and responded to a questionnaire. The median time from lymphoma diagnosis to completion of the questionnaire was 8 months (range 1–12).

**Results:** Overall, 88% of the lymphoma patients reported that they have used CAM, a rate higher than the estimated usage rate reported for the general population The most commonly used modalities were prayers (88%), teas (68%), medicinal herbal (56%), and faith healers (39%). Less than 10% used meditation, and 7% relaxation. In terms of common herbal usage, 56% had used aloe vera and 37% had used *Peumus boldus*. While none of the patients reported that CAM usage was directed specifically towards treating their lymphoma, 8% of patients reported that CAM could cure cancer, and 54% reported that CAM could increase their feeling of control over their health.

**Conclusions:** This pilot study suggests that patients with lymphoma appear to use CAM at a rate higher than the general population. The use of potential agents of risk by the survivors and the lack of access to potentially beneficial modalities highlight the need for further study of CAM in this population.

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### RITUXIMAB PLUS NON-PEGYLATED LIPOSOMAL DOXORUBICIN IS FEASIBLE, SAFE AND ACTIVE IN PATIENTS WITH AGGRESSIVE NON-HODGKIN LYMPHOMA AGED 80 YEARS OR OLDER

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**Introduction:** The Italian population aged 80 years or older accounts for nearly four million people, as reported by the Italian Institute of Statistics, and the incidence of NHL in this age class is about 50 times higher than in patients aged 20 years. Old patients with NHL usually have a poor prognosis. Owing to treatment-related toxicities and frequent comorbidities, there is no standard therapy especially for patients aged ≥80 years. Our purpose is to evaluate the feasibility of a tailored immunochemotherapy for treatment of patients ≥80 years with aggressive NHL.

**Methods:** Given that non-pegylated liposomal doxorubicin (NPLD) has less cardiotoxicity and equal anticancer activity compared to conventional doxorubicin, we conducted a pilot study in a small group of patients ≥80 years with aggressive NHL to verify if the combination R-NPLD is safe and active. Treatment plan included R 375 mg/m$^2$ IV and NPLD 50 mg/m$^2$ IV administered on Day 1, plus prednisone 75 mg p.o. daily on Days 1–5, every 21 days for six courses. The study did not provide primary prophylaxis of febrile neutropenia.

**Results:** Twenty-one patients were consecutively enrolled from August 2010 to July 2014. At baseline, there were 13 males and 8 females, with a median age of 85 years (range 80–88). Histology was 19 DLBCL and 2 FL IIIB. Stage I and II disease was found in 12 patients and III and IV in nine. Extranodal and bulky diseases were recorded in 10 and 5 patients, respectively. High PS (≥2) was found in 12 patients and high IPI (3–5) in 11. The comorbidities ranged from 0 to 5 (median 2). Main comorbidities were cardiovascular disease and diabetes. After a median of six courses (range 3–6), 13 patients achieved a PET/CT− CR and 8 had stable or progressive disease (PD). Today, 13 patients are alive while 8 died of PD (7) or concurrent lung cancer (1), with a median follow-up of 21 months for alive patients (range 7–54). Among patients in CR, only two relapsed so far. Hematologic toxicity was very low (anemia G3 in two patients and no episodes of febrile neutropenia). Extrahematologic toxicity G2–G4 was nearly absent, with the exception of alopecia. After treatment, the median LVEF was 55% (range 48–70).

**Conclusions:** Our data show that the combination R-NPLD is well tolerated, safe, and active in patients ≥80 years. Because rituximab and doxorubicin remain the cornerstone of upfront therapy for aggressive NHL, R-NPLD may be a good opportunity for treating very old patients with or without concurrent cardiovascular disease.

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### ADDITION OF RADIOTHERAPY OR SURGERY OF EXTRANODAL LESIONS IS LESS EFFECTIVE THAN CHEMOTHERAPY ALONE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** Extranodal involvement has been considered as an unfavorable prognostic factor in patients with diffuse large B-cell lymphoma (DLBCL). Chemotherapy with or without rituximab is the cornerstone in the management of DLBCL with extranodal lesions. Local treatment to extranodal lesions, such as radiotherapy or surgery, has been expected to improve patient's outcome. This study aimed to explore the significance of adding local treatment to extranodal lesions in DLBCL with extranodal lesions.

**Methods:** One hundred fifty cases were consecutively chosen in our center with an age range of 16–59, who were newly diagnosed as DLBCL with one or more extranodal lesions from February 2005 to July 2012. All of them received chemotherapy of six to eight cycles in primary therapy, with 78 cases being added radiotherapy or surgery to extranodal lesions. The median time of follow-up was 68.5 months (24–133 months).

**Results:** ① Almost all of sites in the body were involved, and 15 different extranodal sites were categorized for analysis in the study. Gastrointestinal lesion was the most common, occupying 42.67% (64/150). The other extranodal lesions included 9.33% (14/150) in bones, 8.00% (12/150) in pancreas, 7.33% (11/150) in thyroid, 7.33% (11/150) in breast, 6.67% (10/150) in liver and in chest, 6.00%

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(9/150) in kidney/adrenal gland and in bone marrow, 4.00% (6/150) in the head, 3.33% (5/150) in the brain and in female genitalia, 2.67% (4/150) in salivary glands, and 1.33% (2/150) in skin/soft tissue and in testis. There were 124 cases with one lesion, accounting for 82.67% (124/150), and 26 cases with more than one lesion, accounting for 17.33% (26/150). ② There were median PFS of 71.439 ± 4.141 months and OS of 74.183 ± 3.889 months. Patients with more than one extranodal lesion had poorer prognosis than those with single extranodal lesion (median PFS: 30.784 ± 5.789 vs. 77.102 ± 4.415 months, $p = 0.000$; median OS: 35.846 ± 5.237 vs. 79.205 ± 4.169 months, $p = 0.000$). Of 124 cases with single extranodal lesion, those with involvement in the gastrointestinal tract, thyroid, salivary glands, and testis presented good prognosis, whereas those with involvement in the bones, breast, bone marrow, head, chest, female genitalia, liver, pancreas, skin/soft tissue, kidney/adrenal gland, and brain displayed poor prognosis. The discriminative prognosis was not associated with age-adjusted IPI. ③ Local treatment to extranodal lesion did not improve survival of patients in both good-prognosis and poor-prognosis groups. More dangerously, it affects survival of patients with more than one extranodal lesion.

**Conclusion:** The addition of radiotherapy or surgery to extranodal lesions plays a less important role than chemotherapy alone in young patients with DLBCL.

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**LONG-TERM RESULTS AND SIGNIFICANCE OF ANTIVIRAL THERAPY IN TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA ASSOCIATED WITH HEPATITIS C (DLBCL+C)**

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**Materials:** Ninety-four patients with diffuse large B-cell lymphoma and markers of hepatitis C (DLBCL+C) and 102 patients with diffuse large B-cell lymphoma without markers of hepatitis C (DLBCL−C) were included in the study. The age of patients with DLBCL+C ranged from 21 to 76 years (median 47), and that of the control group from 23 to 81 years (median 55; $p = 0.02$). The ratio of men to women in the two groups was equal. Stages III and IV were found in 89% of patients in DLBCL+C and 52% in DLBCL−C ($p = 0.00002$). Extranodal lesions were found in 72% of patients with DLBCL+C and in 46% of patients with DLBCL−C ($p = 0.006$). In the group of patients with DLBCL+C, GCB was 55% and non-GCB was 45%; in the control group with DLBCL−C, GCB was 36% and non-GCB was 64% of patients ($p = 0.001$). In the group of patients with DLBCL+C, antibodies to hepatitis C were positive in all patients, and positive PCR HCV was 78% (74 patients). Median viral load at the start of chemotherapy was $2 \times 10^4$ copies/mL. Fifteen patients had a viral load of more than $1 \times 10^6$ copies/mL.

**Methods:** All patients received CHOP/R-CHOP chemotherapy. Patients with DLBCL+C received chemotherapy antiviral therapy (AVT) with interferon alpha and ribavirin: 31 patients with GCB and 18 patients with non-GCB.

**Results:** After treatment in patients with DLBCL+C and DLBCL−C, complete remission was achieved in 60% and 63%, respectively. Disease-free survival (DFS) was 25 months in patients with DLBCL+C and 61 months in the control group. Median overall survival (OS) was 40 months in the DLBCL+C group and 71 months in the control group ($p = 0.0003$). Median DFS was 40 months in patients with GCB DLBCL+C and 60 months in patients with GCB DLBCL−C ($p = 0.003$). Median OS was 45 months in the GCB DLBCL+C group and 70 months in the control group ($p = 0.002$). In 31 DLBCL+C GCB patients, median OS was 68 months in those treated with AVT and 25 months in those without AVT. Median DFS was 10 months in patients with non-GCB DLBCL+C and 60 months in the non-GCB DLBCL−C control group ($p = 0.00001$). Overall survival was 18 months in the group of patients with non-GCB DLBCL+C and 70 months in patients with non-GCB DLBCL-C ($p = 0.00001$). AVT in patients with non-GCB DLBCL+C did not affect OS (18 months).

**Conclusion:** DLBCL+C is a separate group with characteristic clinical and morphological features. Despite the lack of differences in the effectiveness of the therapy, long-term results of therapy was significantly worse in patients DLBCL+C. Antiviral therapy is necessary in patients with GCB DLBCL+C.

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**THE COMBINATION OF IFOSFAMIDE, CARBOPLATIN, AND ETOPOSIDE ± RITUXIMAB IS A SAFE AND EFFECTIVE SALVAGE THERAPY FOR ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA**

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**Background:** Elderly patients are under-represented in clinical trials, especially in studies evaluating salvage combinations for relapsed or refractory diffuse large B-cell lymphoma (DLBCL). Yet, the risk of developing non-Hodgkin lymphoma increases with age, with approximately 50% of the patients diagnosed above the age of 65. This leads to uncertainty regarding the risks and benefits of these treatments and can have a substantial impact on treatment decisions.

**Methods:** A retrospective single-center study evaluating the efficacy and tolerability of ifosfamide, carboplatin, and etoposide ± rituximab (ICE ± R) regimen for the treatment of elderly patients (>70 years old) with relapsed or refractory DLBCL was conducted.

**Results:** A total of 32 patients (21 women and 11 men) with DLBCL who were older than 70 years were treated in our institute with the ICE ± R regimen, from October 2003 until June 2014. Median age of the entire cohort was 75.6 years (range 70.6–87.1). Most patients had an Ann Arbor Stage IV disease (56%, $n = 18$), an intermediate-high to high sIPI (63%, $n = 20$), a low Carlson comorbidity index (0–1 in 81%, $n = 26$), and an excellent ECOG score (0–1 in all cases). In 27 patients (84%), chemotherapy dosage was reduced, with a median dose reduction of 25% (range 0–50%). ICE ± R was administered as an inpatient therapy to 31 of 32 patients, and all received G-CSF primary prophylaxis. The overall response rate was 53.1% with a complete response rate of 40.6%. After a median follow-up of 12 months, the median progression-free survival (PFS) and overall survival (OS) were 3.9 and 17.0 months, respectively. Patients who responded to ICE ± R (including patients who proceeded to autologous stem cell transplantation) achieved a median PFS of 47.2 months and OS of 78.9 months. Previous response to first-line therapy appeared to be the strongest predictor of outcome (response to treatment, PFS and OS) in second-line treatment. Following treatment with ICE ± R, an attempt to harvest peripheral blood stem cells was performed in eight patients. Harvesting was successful in seven patients (>$2.5 \times 10^6$ of CD34 cells per kilogram) of which six (19%) proceeded to autologous stem cell transplantation (ASCT). Patients ineligible for ASCT who responded to ICE ± R ($n = 11$) were treated with extended cycles of ICE ± R (a median of four cycles per patient) and achieved a median PFS of 18.9 months and OS of 21.7 months. ICE ± R was generally well tolerated, and major toxicities were mostly hematological.

**Conclusions:** ICE ± R is a safe regimen and achieves high response rates in elderly patients with relapsed/refractory DLBCL. Response to first-line therapy is the strongest predictor of response to ICE ± R. Patients with chemosensitive disease, who are not transplant eligible, should be considered for extended treatment with this regimen.

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**PHASE 1B STUDY OF ALTERNATING RITUXIMAB INOTUZUMAB OZOGAMICIN (R-CMC544) AND R-GEMOX IN ELDERLY PATIENTS WITH RELAPSE/REFRACTORY DLBCL**

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**Introduction:** This study is a multicenter, Phase Ib open-label, single-arm trial evaluating the efficacy and safety of R-CMC544 alternated with gemcitabine–oxaliplatin plus rituximab (R-GEMOX) in patients with CD20-positive and CD22-positive DLBCL in relapse after/refractory to first-line or second-line treatment, who are not candidates for autologous transplantation.

**Methods:** The study was intended to alternate full doses of both regimens, starting with R-CMC544. The main dose-limiting toxicities (DLT) were defined as Grade 4 neutropenia or thrombocytopenia and Grade 3 non-hematologic toxicity lasting 7 days or any Grade 4 non-hematological toxicity with particular attention to liver

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toxicity (AST/ALT, bilirubin Grade 2, but not gamma-GT). In case of DLT, cohorts of three to six subjects should be evaluated at de-escalating doses of CMC544 in combination with set doses of R-GEMOX in order to obtain the MTD of CMC544 in this regimen. All patients were to receive two 56-day induction cycles of alternating R-CMC544 [given on Day 1 starting at dose level 1.8 mg/m$^2$ (Level 0) and de-escalation defined as 1.3 mg/m$^2$ (Level 1) and 0.8 mg/m$^2$ (Level 2)] and R-GEMOX (100 mg/m$^2$ oxaliplatin and 1000 mg/m$^2$ gemcitabine given on Days 29 and 43). Patients who obtained CR or PR were to go on a consolidation of another two identical 56-day cycles.

**Patients**: Eleven patients entered the study (five male and six female, age 71.2 years, 62–80). IPI was >2 at inclusion in 8/11 patients. Five had received 1 and six 2 lines of previous treatment; in six patients, the last line was less than 6 months before inclusion. Five were of GC phenotype, and six of non-GC by Hans IHC; three patients had double-hit lymphoma. Median follow-up is 11.1 months.

**Safety and Recommended Dose:** Eleven patients were enrolled in this study in two cohorts: one early progression, one early premature withdrawal by investigator decision and one DLT occurred in the initial three patients, extending this cohort to five patients (three evaluable with two DLT). In a second cohort, three patients were again extended to six because of three early PD, with three evaluable and one DLT. Of the total 11 patients, 6 were evaluable and 3 experienced DLT (Grade 4 thrombocytopenia in 2 and neutropenia in 1). One patient had a Grade 3 elevation in gamma-GT, which was not considered a DLT.

**Efficacy and Patient Disposition**: Ten of 11 patients withdrew prematurely from the study: 5 to progression, 4 to toxicity (1 DLT), and 1 by investigator decision. At the end of treatment, two patients showed CR/Cru/PR with an ORR of 18.2% (95% CI 2.3–51.8). Median PFS is 3.7 months. Five patients died with a median OS of 7.6 months.

**Conclusion:** Elderly patients with relapse refractory DLBCL continue to have a dismal prognosis. The alternating approach with R-CMC544 and R-GEMOX was possible from a toxicity viewpoint but, in this very poor patient population, had to be discontinued from development because of futility.

### 382
### A MULTICENTRIC RETROSPECTIVE ANALYSIS ON CLINICAL OUTCOMES FOR ELDERLY PATIENTS WITH STAGE I AND II DIFFUSE LARGE B-CELL LYMPHOMA

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**Purpose:** The aim of this multicentric retrospective analysis is to describe the patterns of disease, the patterns of care, prognostic impact of treatment regimen, comorbidities and toxicities in older localized DLBCL patients (≥65 years), with special focus on impact of involved field radiation therapy (IFRT) after immunochemotherapy (R-CT).

**Methods and Materials:** We retrospectively analysed the data and outcomes of 182 patients elderly patients treated between 2002 and 2012. All patients were assessed for the Charlson comorbidity index score and age-adjusted International Prognostic Index (aaIPI).

**Results:** Clinical characteristics of these patients were as follows: median age of 76 years (range, 66–92), 40% with Stage I and 60% with Stage II, 42% with bulky tumours and 8% with B symptoms. Eighty-two patients (45%) were treated with R-CT alone and 100 (55%) with R-CT followed by IFRT with a median dose of 36 Gy (range, 25–40 Gy). No statistically significant difference was observed between the two cohorts in relation to the major prognostic factors. The most common toxic effects were represented by haematological toxicity in both groups. In general, IFRT was well tolerated with minor acute toxicity. Response assessment of all treatment regimens showed a complete remission (RC) in 132 (72%) patients; in the group treated with R-CT alone, 73 out 82 (89%) patients reached an RC. In the group of combined therapy, only 75% of patients showed an RC after R-CT. In this group of patients, the percentage of RC increased at 95% (95 out 100 patients) after IFRT. There is a trend for improved RC with the addition of RT to R-CT ($p = 0.0518$). Median follow-up for all patients was 59.8 months. At time of analysis, 142 (78%) patients were alive. Five-year OS of all patients was 73% (95% CI 0.65–80%) with a median OS of 111 months; 5-year OS rates in R-chemotherapy and R-chemotherapy plus IFRT patients were 74% (95% CI 0.62–82) and 72% (95% CI 59–81), respectively, without any statistical difference between the two groups ($p = 0.476$). Median PFS for R-CT group was 52.5 months compared to 54 months in patients treated with R-CT plus IFRT, and 2-year PFS was 94.2% (95% CI 0.83–1) and 95% (95% CI 0.876–1), respectively. Univariate analysis showed that the age < 80 years, normal LDH value and aaIPI = 0 were favourable prognosticators of OS. In the multivariate analysis, only the age > 80 was significantly associated with OS ($p = 0.017$).

**Conclusion:** The results of our study suggest that both R-chemotherapy alone and R-chemotherapy plus RT may offer satisfactory disease control in elderly patients affected with Stage I and II DLBCL. With the addition of RT, the response rate is slightly higher, but there is no improvements in PFS and OS.

### 383
### PATIENT SATISFACTION WITH SUBCUTANEOUS VS INTRAVENOUS RITUXIMAB COMBINED WITH CHOP FOR UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS FROM THE PHASE IIIB MABEASE STUDY

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**Introduction:** Subcutaneous rituximab ($R^{SC}$) offers improved patient convenience and healthcare resource savings versus intravenous R ($R^{IV}$), with similar efficacy and safety seen in the SABRINA study in follicular lymphoma (FL). $R^{SC}$ is approved in Europe and other countries for FL and diffuse large B-cell lymphoma (DLBCL). In the PREFMAB study, 83% of patients with FL or DLBCL preferred $R^{SC}$ to $R^{IV}$. We compared satisfaction with $R^{SC}$ versus $R^{IV}$ plus CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) exclusively in DLBCL.

**Methods:** The open-label MABEASE study (NCT01649856) investigated the efficacy and safety of $R^{SC}$ versus $R^{IV}$ plus CHOP in untreated DLBCL. Patients were randomized 2:1 to receive $R^{SC}$ [$R^{IV}$ 375 mg/m$^2$, Cycle (C)1; $R^{SC}$ 1400 mg fixed dose, C2–C8] or $R^{IV}$ ($R^{IV}$ 375 mg/m$^2$, C1–C8), plus six or eight cycles of CHOP every 14 or 21 days. Patient-reported outcomes were assessed at C3 and C7 using the Cancer Treatment Satisfaction Questionnaire (CTSQ) and the Rituximab Administration Satisfaction Questionnaire (RASQ).

**Results:** In total, 576 patients were randomized ($R^{SC}$ $n = 381$; $R^{IV}$ $n = 195$; intent-to-treat population); baseline characteristics were balanced between arms. A total of 572 patients ($R^{SC}$ $n = 378$; $R^{IV}$ $n = 194$) received one or more dose of R. Nine $R^{SC}$ patients discontinued after C1, due to AE ($n = 5$), withdrawn consent ($n = 2$), PD and protocol violation (both $n = 1$); as both arms received $R^{IV}$ during C1, these patients were included in the $R^{IV}$ arm for safety analyses ($R^{SC}$ $n = 369$; $R^{IV}$ $n = 203$). Of 428 completed RASQs at C7 ($R^{SC}$ $n = 284$; $R^{IV}$ $n = 144$), median scores (scale 0–100%, 100 is best) were improved for $R^{SC}$ versus $R^{IV}$ for 'impact on activities of daily living' (83% vs 58%), 'convenience' (83% vs 67%) and 'satisfaction' (88% vs 75%). C7 CTSQ scores were similar between arms for all domains. At C7, more

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$R^{SC}$ than $R^{IV}$ patients found the R administration time 'just right' (79% vs 58%); fewer found it 'too long' (3% vs 39%). Most patients felt that R administration did not affect the amount of time available for them to talk to a nurse/doctor about their illness ($R^{SC}$ 81%; $R^{IV}$ 82%). For R-CHOP at C7, more $R^{SC}$ than $R^{IV}$ patients spent ≤4 h in a chair/bed (93% vs 53%) and ≤4 h total in hospital (41% vs 24%). Median R administration time (C2–C8) was shorter for $R^{SC}$ (6 min) than $R^{IV}$ (range 2.6–3.0 h). Complete response (CR)/unconfirmed CR rates were 52% ($R^{SC}$) and 51% ($R^{IV}$). AE rates were 92% ($R^{SC}$) and 91% ($R^{IV}$); no new safety signals were observed. In total, 63 patients (11%) died ($R^{SC}$ 10%; $R^{IV}$ 13%). AEs led to death in 6% ($R^{SC}$) and 7% ($R^{IV}$) of patients, most commonly due to infections (2%/arm).

**Conclusions:** In patients with DLBCL, RASQ scores for 'impact on activities of daily living', 'convenience' and 'satisfaction' were improved with $R^{SC}$ versus $R^{IV}$. The shorter administration time for $R^{SC}$ versus $R^{IV}$ did not affect patients' ability to discuss their illness with a nurse/doctor. R administration time, patient chair/bed time and patient total hospital time were shorter for $R^{SC}$ versus $R^{IV}$. Similar efficacy and safety outcomes were observed.

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**SHORT-COURSE R-CHOP FOLLOWED BY $^{90}$Y-IBRITUMOMAB TIUXETAN IN PREVIOUSLY UNTREATED HIGH-RISK ELDERLY DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: 7-YEAR LONG-TERM RESULTS**

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**Introduction:** High-risk elderly patients with diffuse large B-cell lymphoma (DLBCL) generally display a poor prognosis and markedly suffer from a chemotherapy-related toxic effect when undergoing standard regimens. Sequential combined strategies made up of a short course of chemoimmunotherapy followed by radioimmunotherapy (RIT) are aimed at enhancing the global efficacy of the treatment itself, along with a decreased exposure to excessive amounts of cytotoxic drugs. Long-term follow-up analyses are required to confirm the efficacy and safety of such approaches in this particular setting of DLBCL patients.

**Methods:** From December 2006 to October 2008, 55 high-risk, previously untreated DLBCL patients, aged 61–83 years, were treated in seven Italian institutions within a non-randomized multicenter Phase II trial of four cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP21) followed by a single administration of $^{90}$Y ibritumomab tiuxetan 6–10 weeks later (Zinzani *et al*., *Clin Cancer Res*, 2010). Response to treatment was evaluated after the four cycles of R-CHOP21 and after RIT through computed tomography (CT) and positron emission tomography (PET) scan. Thereafter, CT and PET scans were repeated every 6 months during the first 2 years and then annually for the next 5 years.

**Results:** The overall response rate to the entire regimen was 80.0%, including 40 of 55 (72.7%) patients achieving a complete response (CR) and 4 (7.2%) a partial response (PR). At the time of writing, 21 out of the 40 patients who obtained a CR (52.5%) are still in continuous CR, whereas 22 of 55 patients (40.0%) experienced a disease progression (PD). With a median follow-up of 7 years, the disease-free survival was 42.6%, with a progression-free survival of 36.1%. The overall survival at 7.9 years was 38.9%. Death occurred in 27 patients and was mostly related to lymphoma progression. Two patients developed a secondary hematologic malignancy, namely, a myelodysplastic syndrome and an acute myeloid leukemia, 3 and 4 years after the diagnosis of lymphoma, respectively.

**Conclusions:** Our data confirm the feasibility and safety of four cycles of R-CHOP21 followed by RIT consolidation treatment and highlight the long-term efficacy of this treatment approach. These results are comparable with those reported using six cycles of R-CHOP21. Last but not least, this combination allows dispensing of less chemotherapy, reducing short-term and long-term toxicity in such a group of elderly patients.

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**LENALIDOMIDE IN COMBINATION WITH R-ESHAP IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: A PHASE 1B STUDY FROM THE SPANISH GROUP GELTAMO**

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**Introduction:** Diffuse large B-cell lymphoma (DLBCL) patients failing rituximab-containing first-line therapy have a poor outcome with the current salvage regimens. Based on our preclinical data, we conducted a Phase Ib trial to determine the maximum tolerated dose (MTD) of lenalidomide in combination with R-ESHAP (LR-ESHAP) in patients with relapsed or refractory DLBCL eligible for autologous stem cell transplantation (ASCT). Efficacy data were collected as a secondary objective.

**Methods:** *In vitro* evaluation of the combination of lenalidomide with ESHAP was performed by MTT in a B-cell line obtained from a DLBCL patient carrying t(14;18) (SUDHL-6). During the clinical trial, subjects received three cycles of lenalidomide at escalating doses (5, 10, or 15 mg) given on Days 1–14 of every 21-day cycle, in combination with R-ESHAP at standard doses (rituximab 375 mg/m$^2$ Day 1, etoposide 40 mg/m$^2$ Days 1–4, cisplatine 25 mg/m$^2$ Days 1–4; cytarabine 2000 mg/m$^2$ Day 5, and methylprednisolone 500 mg Days 1–5). Responding patients received BEAM followed by ASCT.

**Results:** The addition of lenalidomide to the ESHAP regimen potentiated its activity *in vitro*, with combination indexes up to the synergistic range, providing a good rationale for setting up the clinical trial. During the escalating phase, one patient had a dose-limiting toxicity in the 15 mg cohort (Grade 3 angioedema), and two out of four patients treated in this cohort had a mobilization failure. The MTD was established at 10 mg of lenalidomide, and this cohort was expanded to further explore the safety. A total of 19 patients (3, 12, and 4 in the 5, 10, and 15 mg cohorts, respectively) were evaluable. Median age was 58 (23–70) years. The majority of patients (84%) had primary refractory disease or early relapse. The main toxicity of LR-ESHAP was hematologic. Eighteen patients (95%) completed the planned three cycles of treatment, although lenalidomide was permanently interrupted in five patients due to toxicity. The incidence of febrile neutropenia and Grade 3 infections was 12.5% and 11% of cycles, respectively. No Grade 4 non-hematologic toxicities were observed. All toxicities resolved appropriately, with no treatment-related deaths. The complete remission and overall response rates to LR-ESHAP were 47.4% and 78.9%, respectively. Fourteen patients (74%) underwent ASCT according to protocol. At the time of this analysis, nine patients had disease progression, and seven of them have died from lymphoma. With a median follow-up of 24.6 (range 17.4–38.2) months, the estimated 2-year progression-free survival and overall survival were 44% and 63%, respectively.

**Conclusions:** The addition of lenalidomide to the R-ESHAP salvage regimen is safe, feasible, and associated with encouraging response rates and survival outcomes in patients with relapsed or refractory DLBCL. A Phase 2 study is ongoing. This trial was registered at www.clinicaltrials.gov as NCT02340936.

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**HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION FOR INTRAVASCULAR LARGE B-CELL LYMPHOMA**

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**Introduction:** Clinical outcomes in patients with intravascular large B-cell lymphoma (IVLBCL) have been improved in the rituximab era. However, there is limited information on the efficacy of high-dose chemotherapy (HDC) followed by autologous peripheral blood stem cell transplantation (auto-PBSCT) against IVLBCL.

**Methods:** Data from the Japan Society for Hematopoietic Cell Transplantation database were retrospectively analyzed.

**Results:** A total of 91 patients with IVLBCL received HDC/auto-PBSCT between October 2003 and December 2011 after the approval of rituximab use in Japan. The median age at diagnosis was 58 years (range, 32–72). The median months from diagnosis to auto-PBSCT was 7.7 months. Among 91 patients, 69 (76%) were alive with a median follow-up period of 42.4 months (range, 0.8–103.3). The 3-year overall survival (OS) and progression-free survival (PFS) rates were 79.0% (95% CI, 68.3–86.5%) and 71.9% (95% CI, 61.0–80.3%), respectively. The cumulative incidence of relapse and transplant mortality at 3 years was 25.8% (95% CI, 17.0–35.6). We further evaluated outcomes of 67 patients (74%) who received up-front HDC/auto-PBSCT in sensitive disease (CR: $n = 64$). The 3-year OS and PFS were 84.6% (95% CI, 72.4–91.8%) and 77.6% (95% CI, 64.9–86.1%), respectively. All 16 patients who received conditioning regimens consisting of cytarabine (Ara-C) such as ranimustine, etoposide, Ara-C, and melphalan or high-dose Ara-C were alive without relapse and had better survival (OS: $p = 0.045$, PFS: $p = 0.017$) in comparison to the other regimens such as ranimustine, carboplatin, etoposide, and cyclophosphamide or melphalan, etoposide, cyclophosphamide, and dexamethasone.

**Conclusions:** HDC/auto-PBSCT might represent a useful treatment option for IVLBCL in the rituximab era. Further studies focusing on the role of Ara-C and the efficacy of HDC/auto-PBSCT as a consolidation in patients with IVLBCL are warranted.

### 387
### STEM CELL MOBILIZATION WITH GEMCITABINE, DEXAMETHASONE AND CISPLATIN IN RELAPSED/REFRACTORY AGGRESSIVE LYMPHOMA

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**Background:** Gemcitabine, dexamethasone and cisplatin (GDP) results in similar response rates, transplantation rate and less toxicity and is cost effective compared to dexamethasone, cytarabine and cisplatin (DHAP) as salvage therapy for aggressive lymphomas. Experience with this regimen for stem cell (PBSC) mobilization is limited. We evaluated PBSC kinetics and mobilization efficiency of GDP compared to our previous mobilization regimen in patients referred for autologous stem cell transplantation (ASCT).

**Methods:** We compared 47 consecutive patients with lymphoma mobilized with GDP (gemcitabine 1000 mg/m$^2$ IV Days 1 and 8, dexamethasone 40 mg orally Days 1–4, cisplatin 75 mg/m$^2$ IV Day 1 and G-CSF 10 μg/kg/day starting Day 9) to 47 patients randomly selected from our ASCT database mobilized with CE (cyclophosphamide 2 g/m$^2$ Day 1, etoposide 200 mg/m$^2$ Days 1–3 and G-CSF 10 μg/kg/day starting Day 6) treated at the Princess Margaret Cancer Centre from 2007 to 2015. High-dose chemotherapy was as follows: etoposide 60 mg/kg IV Day 4, melphalan 180 mg/m$^2$ IV Day 3; PBSC infusion Day 0. PBSC collection target was a CD34+ cell number $>5.0 \times 10^6$/kg. Data on patient characteristics, salvage regimen, PBSC collection, number of aphereses and neutrophil (ANC) and platelet engraftment were retrieved from a prospective ASCT database (Table 1).

**Results:** Peak peripheral blood CD34+ cell number occurred on Day 15 after GDP (median 21/μL; interquartile range 8.5–69) and on Day 13 after CE (median 61/μL, interquartile range 22.5–133). Patients treated with CE had significantly fewer aphereses compared to those treated with GDP ($p = 0.03$); there was no difference in the final stem cell number infused ($p = 0.27$) or ANC engraftment ($p = 0.5$) between the two groups. Patients mobilized with GDP had significantly shorter time to platelet engraftment ($p = 0.002$). One patient in the GDP group and four patients in the CE group did not mobilize and required rescue plerixafor.

**Conclusions:** GDP appears to be an effective regimen for PBSC mobilization for patients with relapsed or refractory lymphoma prior to ASCT, although twice as many apheresis sessions were required to achieve target CD34 collection. Early ANC recovery is similar with the two regimens, with possibly shorter time to platelet recovery in GDP-treated patients.

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### SAFETY AND EFFICACY OF RANIMUSTINE, ETOPOSIDE, CYTARABINE AND MELPHALAN REGIMEN FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MALIGNANT LYMPHOMA IN JAPAN

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**Introduction:** Carmustine (BCNU), etoposide, cytarabine and melphalan ± rituximab [(R-)BEAM] regimen followed by autologous stem cell transplantation (ASCT) has been the standard of care for relapse/refractory lymphoma in Western countries. BCNU is, however, unapproved in Japan. We developed, therefore, the

**Abs 387 - Table 1.**

| | Age, years (median, range) | HL/NHL | Salvage chemotherapy | Apheresis procedures[a] | CD34+ cell number infused[a] $N = 45$ | Engraftment day[a] | |
|---|---|---|---|---|---|---|---|
| | | | | | | ANC $N = 33$ | Platelets $N = 29$ |
| GDP ($N = 47$) | 41 (20–68) | 15/32 | GDP = 47 | 2 ± 0.8 | 7 ± 4.6 | 11 ± 0.8 | 11 ± 1.85 |
| CE ($N = 47$) | 51 (22–67) | 14/33 | GDP = 23<br>DHAP = 18<br>ESHAP = 2<br>Mini-BEAM = 2<br>Other = 2 | 1 ± 0.8 ($p = 0.03$) | 6.76 ± 4.1 ($p = 0.27$) | 11 ± 0.9 ($p = 0.5$) | 13 ± 3.6 ($p = 0.002$) |

HL Hodgkin lymphoma, NHL non-Hodgkin lymphoma.

[a]Median numbers and standard deviation.

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(R-)MEAM regimen in our hospital; ranimustine (MCNU), which was a hydrosoluble nitrosourea developed in Japan, was used in place of BCNU. Here, we report the safety and efficacy of the (R-)MEAM regimen.

**Method:** We retrospectively analyzed 90 patients with malignant lymphoma [44 diffuse large B-cell lymphoma (DLBCL) including 2 intravascular large B-cell lymphoma, 1 primary central nervous system lymphoma, and 1 primary mediastinal large B-cell lymphoma, 23 follicular lymphoma (FL), 6 peripheral T-cell lymphoma, 6 mantle cell lymphoma, 3 Hodgkin lymphoma, and 8 other subtypes] who received (R-)MEAM regimen as conditioning of ASCT from July 2001 to December 2014. MEAM is comprised of ranimustine 300 mg/m$^2$ on Day −6, etoposide 100 mg/m$^2$ twice daily on Days −5 to −2, cytarabine 100 mg/m$^2$ twice daily on Days −5 to −2, and melphalan 140 mg/m$^2$ on Day −1. Rituximab 375 mg/m$^2$ was infused on Day −7 as indicated.

**Result:** Median age at ASCT was 56 (range 16 to 69). Of 44 patients with DLBCL, 15 patients had high-intermediate or high (poor prognostic) International Prognosis Index (IPI), and of 23 patients with FL, 3 patients had poor prognostic IPI. Engraftment was confirmed in 89 patients (98.9%) at a median of 11 days, and complete remission (CR) or unconfirmed CR (CRu) was observed in 70 patients (77.8%). At a median follow-up of 32.1 months (range 0.7–143.5 months), 3-year overall survival was 66.0% for DLBCL and 82.0% for FL, and 3-year progression-free survival was 49.5% for DLBCL and 67.7% for FL. Of the 84 assessable patients, the most common treatment-related toxicities were nausea, diarrhea, and infection. Nausea, Grades 3 and 4, was observed in 13 patients (15.5%), and diarrhea, Grades 3 and 4, was observed in 42 patients (50.0%). Infection, Grades 3 and 4, including febrile neutropenia was observed in 79 patients (91.8%). No regimen-related death was observed within 100 days after ASCT. One case of myelodysplastic syndrome, one DLBCL, and one lung cancer were observed as secondary malignancy (3.3%). Relapse or refractory after ASCT was observed in 43 patients (47.8%). Of 32 patients who died after ASCT, 27 patients (84.3%) died of disease progression.

**Conclusion:** In terms of conditioning regimen for ASCT for malignant lymphoma, (R-)MEAM can be an effective and safe alternative for (R-)BEAM in Japan.

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### RETROSPECTIVE COMPARISON OF PERIPHERAL BLOOD STEM CELL MOBILIZATION IN LYMPHOMA WITH EITHER PEGFILGRASTIM (PEG-GCSF) OR FILGRASTIM (GCSF) FOLLOWING CHEMOTHERAPY

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**Introduction:** Peg-GCSF has been recently introduced as an alternative to GCSF for the mobilization of peripheral blood stem cells in lymphoma patients. Literature regarding its optimal use in this area however remains sparse.

**Methods:** We present a retrospective analysis of our single-centre experience in 65 lymphoma patients who underwent stem cell mobilization over a 7-year period (2007–2013). Outcomes were compared between two sequential cohorts of patients, assigned based on the time of enrolment, to mobilization with either GCSF ($n = 32$) or two doses of Peg-GCSF ($n = 33$), following ifosfamide/carboplatin/etoposide (+rituximab; ICE/R) chemotherapy. Data on baseline patient, disease and treatment-related characteristics as well as on outcome measures of harvest and engraftment were collected from chart reviews.

**Results:** Patients in both groups were similar in terms of baseline as well as disease and treatment-related characteristics (see Table 1). Four patients failed initial mobilization (CD34 cells collected $< 2 \times 10^6$/kg; GCSF group, $n = 1$; Peglasta, $n = 3$), necessitating either bone marrow harvest ($n = 2$) or plerixafor use ($n = 2$). There were no statistical differences in the GCSF group compared to the Peg-GCSF group in terms of CD34 dose collected ($5.2 \times 10^6$ vs $4.7 \times 10^6$/kg, $p > 0.5$) or in the number of aphereses needed to achieve targeted collection goals (median 2 vs 2 days, $p > 0.5$). However, the GCSF group was associated with a faster median time to collection compared to the Peg-GCSF group (13 vs 14 days, $p = 0.01$). Peg-GCSF use was associated with a reduction in the median number of growth factor injections needed (2 vs 18, $p < 0.05$). Post-transplantation neutrophil recovery (median 10 vs 10 days, $p = 0.05$) and platelet recovery (15 vs 20 days, $p > 0.05$) were similar between patients in the GCSF and Peg-GCSF groups.

**Conclusions:** Our study demonstrates that the use of two doses of Peg-GCSF is a valid alternative to that of GCSF to mobilize peripheral blood stem cells in lymphoma patients with comparable outcomes in both groups. Future studies evaluating the optimal dose, timing of Peg-GCSF and long-term outcome in larger cohorts of patients are warranted.

**Abs 389 - Table 1.** Comparison of baseline characteristics between the GCSF and Peg-GCSF groups

| | GCSF Group | Peg-GCSF Group |
|---|---|---|
| | (*n* = 32) | (*n* = 33) |
| Median age at leucopheresis | 50 | 52 |
| Range | (17–66) | (22–72) |
| Gender | | |
| Male, *n* (%) | 23 (72) | 22 (66) |
| Female, *n* (%) | 9 (28) | 11 (34) |
| Lymphoma subtype | | |
| DLBCL, *n* (%) | 14 (43) | 20 (61) |
| T-NHL, *n* (%) | 4 (13) | 5 (15) |
| Hodgkin, *n* (%) | 7 (22) | 6 (18) |
| Other B NHLs, *n* (%) | 7 (22) | 2 (6) |
| BM involvement | | |
| No, *n* (%) | 21 (66) | 15 (45) |
| Yes, *n* (%) | 11 (34) | 18 (55) |
| Prior chemotherapy regimens (median) | 2 | 2 |
| Range | (1–4) | (1–3) |

Legend: GCSF, granulocyte colony-stimulating factor; Peg-GCSF, pegylated granulocyte colony-stimulating factor; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; BM, bone marrow.

### 390
### AUTOLOGOUS TRANSPLANT WITH BEEAM (BENDAMUSTINE, ETOPOSIDE, ARA-C AND MELPHALAN) FOR NON-HODGKIN LYMPHOMA IN COMPARISON TO THE STANDARD BEAM: A MATCHED-CONTROL ANALYSIS

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**Introduction:** Recent data about BeEAM as a conditioning regimen have been published with promising results. The aim of our study was to compare feasibility and toxicity in non-Hodgkin lymphoma patients in first complete remission (CR) when compared to the standard BEAM.

**Method:** This series includes patients diagnosed with diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) recruited at a single institution from 01/2013 to 11/2014. DLBCL patients were pretreated with six cycles of R-CHOP while MCL patients received four cycles of R-DHAC (rituximab 375 mg/m$^2$ on Day 1, carboplatin AUC 5 on Day 1, Ara-C 4 g/m$^2$ on Day 2 and dexamethasone 40 mg on Days 1–4). Pulmonary tests and cardiac evaluation were performed before transplant. Bendamustine was delivered at a dose of 200 mg/m$^2$ on Days 1 and 2, in lieu of carmustine of the standard BEAM. No supportive GCSF was administered when at least $2 \times 10^6$ CD34/kg were infused. Blood cell units (BCU) and platelet transfusions were infused to maintain a hemoglobin level of >8 g/l and platelet count of $>10 \times 10^9$. Antimicrobial prophylaxis consisted in oral fungizone. Broad-spectrum antibiotics were delivered when fever developed. Patients receiving BeEAM were matched for age, sex, diagnosis, tumour stage, previous chemotherapy and pre-transplant assessment to patients consolidated with BEAM.

**Results:** Twenty-nine patients (18 DLBCL and 11 MCL) received BeEAM and were compared to 39 patients (32 DLBCL and 7 MCL) who received the standard

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BEAM. The matched controlled analysis showed no statistical difference for patient's characteristic. In both groups, a median number of $4 \times 10^6$ CD34 cells/kg were infused, and all patients fully engrafted. Time to achieve an absolute neutrophil count $>1 \times 10^9$ was shorter in the BeEAM group (10 vs 13 days) ($p = 0.001$). BeEAM patients significantly required more BCU ($p = 0.004$) and platelet transfusion ($p = 0.013$). The rate of documented infections was 58%. No difference was noted regarding bacteraemia, septic shock and pneumonia. Admission in intensive care unit was necessary for seven and five patients, respectively ($p = 0.2$). Grade III or IV cardiotoxicity was observed in 7% of all patients with no difference between the two groups (0.07) and consisted of atrial fibrillation. The significant toxicity for patients receiving BeEAM consisted of Grade III or IV renal failure (17% vs 7%) ($p = 0.001$) with 13% developing acute renal failure on Day 1. Renal function recovery was observed for all patients. No pulmonary toxicity was observed. With a median follow-up of 7 months (range: 1–20), 65 patients are alive and in CR (BeEAM: 28, BEAM: 37).

**Conclusion:** BeEAM is feasible, but when compared to BEAM, it shows additional toxicity mainly represented by Grade III or IV renal failure. This observation leads to precise exploration of renal function before transplant. Medications with renal toxicity should be used carefully, and adequate supportive hydration should also be considered.

### 391
### VITAMIN D DEFICIENCY IN LYMPHOMA PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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**Introduction:** Vitamin D deficiency is associated with risk of some cancers, but also an inverse relationship seems to be present between serum levels of vitamin D and risk of morbidity and mortality. Recent studies have demonstrated a positive relationship between vitamin D deficiency and increased mortality of cancer patients. In patients with newly diagnosed non-Hodgkin lymphoma, it was observed that 25-hydroxy vitamin D deficiency is associated with reduced overall survival and event-free survival. Maintaining a sufficient amount of vitamin D can be a promising approach for the prevention and/or treatment of disease without imposing significant financial costs or adverse effects. From these data, it is essential to assess the prevalence of vitamin D deficiency in hospitalized cancer patients.

**Objectives:** This study aimed to determine the prevalence of vitamin D deficiency in lymphoma patients undergoing HSCT.

**Material and Methods:** In this observational, cross-sectional study, we analyzed 72 patients, ≥18 years, undergoing HSCT from May 2012 to January 2014 in the Hematology-Oncology and Bone Marrow Transplantation Center at the Albert Einstein Hospital in São Paulo, Brazil, during the first 5 days of hospitalization. This sample had 12 lymphoma patients. We used in our study the VDD defined and recommended by the Institute of Medicine as 25(OH)D ≤ 20 ng/ml, VD insufficiency of 21–29 ng/ml, and VD normal ≥ 30 ng/m.

**Results:** Patients with lymphoma have the highest rate of VDD (41.7%); however, 100% had serum levels of vitamin D ≤20.

**Conclusion:** The lymphoma patients had high rates of vitamin D deficiency. Prevention and treatment of vitamin D deficiency in HSCT patients are easy and cheap, and it can improve the transplant outcome and reduce complications.

### 392
### LONG-TERM FOLLOW-UP OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMA: OUTCOMES AND SECOND MALIGNANCIES

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**Introduction:** Autologous stem cell transplantation (ASCT) has long been used for high-risk non-Hodgkin (NHL) and Hodgkin lymphoma (HL). However, it is associated with risk of secondary malignancy. The objective of the present study was to evaluate long-term outcome and second malignancies for 172 patients with lymphoma treated with ASCT between 1995 and 2012 at MMA, Serbia, Belgrade.

**Patients and Methods:** We retrospectively analysed 83 patients with HL and 89 patients with aggressive NHL. The median number of pretransplant regimens was two (range 1–5), and 45% of the patients received radiotherapy. At the time of ASCT, the resistant disease had 22% of patients. In all patients, stem cell source was peripheral blood. Conditioning regimens were BEAM in 81% patients and CVB in other patients. Follow-up was till December 2013.

**Results:** Median age was 30 years (range 17–63), and gender was male in 54%. Overall survival and progression-free survival at 6 years from ASCT were 56.8% and 53.4%, respectively, for HL and 79% and 66.6%, respectively, for NHL. A second malignancy occurred in eight patients., representing 4.7% of the patient population. All of them received both radiotherapy and chemotherapy. At a median of 29 (range 21–69) months, four cases of sAML have been observed. Also, four patients have developed a solid tumour with a median of 68 months (range 49–106). After a median follow-up of 64 months (range 24–209), 52 (30%) patients had died: 45 patients of lymphoma, 5 patients of a second cancer (sAML in 4 patients and brain tumour in 1 patient), 1 patient of organ failure and 1 patient of infection. The 5- and 10-year cumulative incidence of developing a second malignancy was 4.3% and 7.1%.

**Conclusion:** Relapse of HL and NHL was the leading cause of death. Despite satisfactory 6-year survival rates for lymphoma patients treated with ASCT, our results confirm that the risk of secondary malignancies remains considerable.

### 393
### DA-EPOCH REGIMEN WITH OR WITHOUT RITUXIMAB DOES NOT IMPAIR PERIPHERAL BLOOD STEM CELL YIELD FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION

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**Abs 391 - Table 1.** Vitamin D deficiency by Hematological Disease

| Serum levels of VD (ng/ml) | Hematological disease | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Leukemia | | Lymphomas | | Myeloma | | Other | |
| | *n* | % | *n* | % | *n* | % | *n* | % |
| ≥30: normal | 5 | 16.7 | 0 | 0.0 | 2 | 11.1 | 4 | 36.4 |
| 11–29: insufficiency | 21 | 70.0 | 7 | 58.3 | 14 | 77.8 | 6 | 54.5 |
| <10: deficiency | 4 | 13.3 | 5 | 41.7 | 2 | 11.1 | 1 | 9.1 |
| Total | 30 | 100.0 | 12 | 100.0 | 18 | 100.0 | 11 | 100.0 |

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**Introduction:** Autologous hematopoietic stem cell transplantation (auto-HSCT) has been used routinely and widely for lymphoma patients. We investigated the peripheral blood stem cell (PBSC) collection efficacy for patients who received DA-EPOCH regimen with or without rituximab.

**Patients and Methods:** A total of 51 patients with lymphoma were enrolled in this study, including 34 males and 17 females, with a median age of 45 years (16–62 years), and they had Hodgkin lymphoma (HL), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mucosal-associated lymphoid tissue (MALT) lymphoma, NK/T-cell lymphoma, and T-cell lymphoma. DA-EPOCH regimen chemotherapy was employed for at least four to six cycles before stem cell mobilization and high-dose etoposide (1.6 g/m$^2$) plus G-CSF were administered as the chemomobilization regimen.

**Results:** A total of 48 patients (94.11%) succeeded in the collection of PBSC, including three patients who experienced second chemomobilizations. There were 28 patients (58.33%) who got ample cells by only one collection procedure among the patients succeeding in collections. The median harvest MNC and CD34$^+$ cells were $6.00 \times 10^8$ and $3.88 \times 10^6$ cells/kg, respectively. Medians of $4.23 \times 10^8$ MNC cells/kg and $3.2 \times 10^6$ CD34$^+$ cells/kg were harvested in the first apheresis day, with a median ratio of CD34$^+$ cells in MNC of 0.87%. No serious adverse effect during PBSC harvest was observed. In the univariate analysis, age was an important factor for CD34$^+$ cell yield and CD34$^+$ cell proportion. The median age of patients with a ratio of CD34$^+$ cell yield in MNC lower than 1% by the first collection was significantly higher than that of patients with a CD34$^+$ cell ratio equal to or greater than 1% ($47 \pm 12$ vs. $38 \pm 12$ years; $p = 0.018$). Regarding both MNC yield and CD34$^+$ cell yield, no significance was found between the group that used rituximab and the group that did not ($p > 0.05$). The mean numbers of days to neutrophil $>0.5 \times 10^9$/L and platelets $>20 \times 10^9$/L were $10.41 \pm 1.49$ and $12.15 \pm 1.85$, respectively. No one died of any transplantation-related mortality.

**Conclusions:** In summary, our study indicated that the DA-EPOCH regimen with or without rituximab in the present study is a feasible first-line therapy for auto-HSCT candidates with lymphoma. Age and LDH were independent significant parameters for mobilization and collection, compared with gender, staging, disease status, and chemotherapy regimens, which did not influence the collection outcome significantly.

## 394
## IS AUTOLOGOUS STEM CELL TRANSPLANTATION SUCCESSFUL IN ELDERLY FIT PATIENTS (≥60 YEARS) WITH NON-HODGKIN LYMPHOMA?

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**Introduction:** Significant therapeutic advances have recently been made in the care of older patients with lymphoma. Although there are many fit elderly patients who are eligible for high-dose therapy (HDT) with autologous stem cell transplantation (ASCT) rescue, the evidence is inadequate. Therefore, we aimed to analyze the efficacy and safety of HDT with ASCT rescue in our elderly lymphoma patients.

**Patients and Methods:** Between January 2003 and October 2014, from 265 ASC rescue lymphoma patients, 17 elderly patients (≥60 years) (13 M; 4 F) admitted to the Ankara University Department of Hematology were included in the study. Their diagnosis were as follows: 10 diffuse large B-cell (3 active disease, relapsed), 6 mantle cell, and 1 follicular lymphoma. We retrospectively evaluated the early toxicity and outcome of the HDT.

**Results:** Patient characteristics are shown in Table 1. All of the patients were Stage III or IV at diagnosis, and 3/17 (18%) patients had active disease prior to transplantation. The conditioning regimens patients received prior to transplantation were as follows: 2/17 (12%) ICE, 2/17 (12%) R-BEAM, and 13/17 (76%) BEAM. None of the patient had mobilization failure; the median CD34 was similar between geriatric and non-geriatric groups ($6.13 \times 10^6$ vs $6.32 \times 10^6$/kg; $p > 0.05$). Oral mucositis was observed in 13/17 (76.4%) patients; 11 of them had Grade 4 mucositis and required total parenteral nutrition. All of the patients had diarrhea, and 8/17 (47%) were Grade 3 and 4 with median duration of 8 days (5–20 days). Renal toxicity occurred in 4/17 (24%) patients while hepatic toxicity in 3/17 (18%) patients. In 7/17 (41%) patients, infection was detected; one of them was invasive fungal infection. The median period to neutrophil recovery was 12.4 days (8–26 days), and platelet recovery was 14.9 days (9–32 days), which is similar to the non-geriatric group [neutrophil recovery 11.4 (range 10–32) days and platelet recovery 13.8 (range 9–48); $p > 0.05$]. Early transplant-related mortality (TRM) was seen in only one patient. Death was seen in a total of six patients, three of whom died after relapse. The median overall survival after transplantation of the group was 21.2 months (0.4–59.8 months).

**Conclusion:** HDT with ASCT rescue was demonstrated to be a safe option in the treatment of non-Hodgkin lymphoma in patients with advanced age in our cohort.

**Abs 394 - Table 1.** Patient characteristics

| | |
|---|---|
| Median age | 64 years (60–74 years) |
| Median time from diagnosis to transplantation | 49.1 months (9–188.7 months) |
| Median CD34 count | $6.13 \times 10^6$/kg |
| Disease status prior to transplantation | 3/17 active disease |
| Median period to neutrophil recovery | 12.4 days (8–26 days) |
| Median period to platelet recovery | 14.9 days (9–32 days) |
| Sorror Comorbidity Index (CI) > 1 | 11/17 (70.5%) |
| Relapse after transplantation | 5/17 (29%) |

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## ALLOGENEIC STEM CELL TRANSPLANTATION FOR HODGKIN AND NON-HODGKIN LYMPHOMA

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**Introduction:** Although long-term survival rates can be achieved with chemoradiotherapy approaches in lymphoma, one-third of the patients can relapse during follow-up. Allogeneic hematopoietic stem cell transplantation (HCT) remains to be the only curative treatment approach for relapsed/refractory lymphoma patients. Here, we present our relapsed/refractory Hodgkin and non-Hodgkin lymphoma patients who required allogeneic HCT.

**Materials and Methods:** We retrospectively analyzed 46 relapsed/refractory lymphoma patients (25 NHL and 21 HL) who underwent allo-HCT rescue treatment at the Ankara University Department of Hematology BMT Unit. A chi-squared test was used for comparison between two groups with regard to engraftment kinetics, post-transplant complications, and post-transplant responses. $p < 0.05$ was considered statistically significant.

**Results:** The median age of patients was $40.2 \pm 14.2$ in the NHL group and 28.8 $\pm$ 7.9 in the HL group. Peripheral blood was the preferred stem cell source in both NHL and HL patients (24/25 and 17/21). Sixteen of 25 NHL patients received a myeloablative conditioning regimen, whereas reduced-intensity conditioning was the preferred conditioning regimen in the HL group. Also, transplantation from an unrelated donor was also significantly higher in the HL than in the NHL group. Fifteen patients had primary refractory disease in both groups. Median follow-up period from transplantation was 26 months (range 4–98) in 46 patients. Complete remission was observed in 5/21 (23%) patients in the NHL group and 9/21 (42%) patients in the HL group. Engraftment could not be achieved in five NHL patients and three HL patients due to death in the aplasia period. Median time for neutrophil engraftment was 14 days, and platelet engraftment was 15 days for HL, whereas NHL neutrophil and platelet engraftment medians were both 16 days. Acute graft-versus-host disease was detected in 13/25 (52%) patients in the NHL group and 10/21 (47%) patients in the HL group. One-year overall survival in the NHL group was detected as 40% in the NHL group and 55% in the HL group.

**Conclusion:** Patients with HL received reduced-intensity conditioning regimen more frequently, and transplantation was mostly from unrelated donors. In our retrospective study, the allogeneic stem cell transplantation outcomes were found to be similar with the previous reports.

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**FIRST-LINE USE OF RITUXIMAB CORRELATES WITH INCREASED OVERALL SURVIVAL IN LATE POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: RETROSPECTIVE, SINGLE-CENTER STUDY**

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**Introduction:** Solid organ transplant recipients have increased risk for post-transplant lymphoproliferative disease (PTLD). Most PTLD express surface CD20, allowing the inclusion of rituximab in chemotherapy schemes. Data about rituximab benefit specifically on PTLD are scarcely available. We designed a retrospective study to evaluate the impact of rituximab on PTLD response and survival, in a single-center cohort.

**Methods:** This study reviewed PTLD cases between 1984 and 2012, including heart, kidney, liver, and lung transplant recipients. Patients with leukemic PTLD, T-cell phenotype, or post-mortem diagnosis were excluded from the analysis. PTLD time to diagnosis was evaluated in the function of the transplanted organ and the immune suppression. Survival analysis was performed by comparing EBV infection status, International Prognostic Index, Ann Arbor stage, the use of rituximab, and the achievement of response.

Time to PTLD analysis was performed with the Cox regression model and survival analysis using the Kaplan–Meier method with Breslow post hoc test. Response was compared using Fisher's exact test.

**Results:** Twenty-four PTLD patients were diagnosed, among 1335 transplanted patients in total. Median age was 64 years (range 29–75), median time to diagnosis was 59 months (range 4–182). PTLD type was predominantly monomorphic in 75% of cases, mostly with diffuse large B-cell histology. Overall response rate (ORR) was 62% (66% in the rituximab group vs. 50% in the non-rituximab group; $p = 0.66$). R-CHOP or sequential R-RCHOP regimens were the most frequently used (71% of patients treated with rituximab).

Median overall survival was 64 months (95% CI 31–96); with a significant increase in survival in patients treated with rituximab ($p = 0.01$; 95% CI rituximab 58–80; 95% CI no rituximab 0–31). Response after rituximab ($p = 0.03$) and early Ann Arbor Stage I and II ($p = 0.02$) were independent prognostic factors for survival.

Cyclosporin A use was associated with increased time to diagnosis (HR 3.11; 95% CI 1.2–7.8; $p = 0.01$), whereas azathioprine and mycophenolate had no impact on time to diagnosis. Transplanted organ was not associated with changes in time to PTLD.

**Conclusions:** Rituximab as part of first-line therapy in PTLD is associated with increased survival in our single-center series; we propose that it should be considered as a first-line therapy, adjunctive to chemotherapy in late-PTLD patients.

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**AKTIL: A PHASE II TRIAL OF MK-2206, AN AKT INHIBITOR FOR RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA**

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**Introduction:** The PI3K/AKT/mTOR pathway is considered an interesting therapeutic target for diffuse large B-cell lymphoma (DLBCL). However, clinical trials with mTOR inhibitors have demonstrated low objective response rate (ORR, ≈35%) and short progression-free survival (PFS, ≈3 months) in DLBCL patients. Considering that mTOR inhibitors may relieve a negative feedback loop allowing AKT to exert its survival effects, AKT inhibitor may generate a higher response rate in DLBCL patients.

**Methods:** This multicenter, two-stage, single arm, Phase II study assessed the efficacy and safety of MK-2206 (200 mg/week, p.o.), an allosteric inhibitor of AKT, in refractory/relapsed DLBCL. Eligible patients had histologically confirmed DLBCL, treated with at least two prior lines of systemic therapy. The primary endpoint was 4-month ORR as per Cheson 2007 criteria. Secondary objectives included ORR as per Cheson 1999, PFS, overall survival (OS), duration of response, and safety. Considering that MK-2206 would be uninteresting if 4-month ORR is ≤30% and promising if it is ≥50% and using Simon's optimal two-stage design (Type I error rate of 10% and power of 90%), a sample size of 46 evaluable patients was required (including 22 for the first stage). Pathological samples and radiological exams were centrally reviewed. Archival tumour samples were collected for analysis of PTEN/PIK3CA/AKT mutational status, and plasma samples were collected at serial time points to evaluate the phosphorylation of AKT signaling molecules. ClinicalTrials.gov identifier: NCT01466868.

**Results**: From April 2012 to June 2013, 22 DLBCL patients [median age: 63.1 years (min–max: 43.7–85.1), Ann Arbor Stage IV: 68.4%] were enrolled, and 19 received at least one dose of MK-2206. Median treatment duration was 37 days (min–max: 1–225). At the end of the first stage, no OR was seen (Cheson 2007 or 1999), and two patients had stable disease. The criterion for proceeding to the second stage (≥8/22 OR) was not met, and the study was terminated. Median PFS was 1.7 months (95% CI, 0.8–1.8), and median OS was 9.6 months (95% CI, 2.8–18.8); 18 patients (81.8%) were deceased at the time of the analysis (84.6% related to progressive disease). The most common (>15%) related AE included hyperglycemia, thrombocytopenia, neutropenia, asthenia, blood phosphorus decrease, nausea, diarrhea, cutaneous eruption, rash, leukopenia, lymphopenia, and fatigue. Ten patients (52.6%) experienced Grade ≥3-related AE [including mainly (>10%) neutropenia, rash, thrombopenia, and phosphorus decrease]. Related SAEs were reported for six patients (31.6%) including two SUSARs (one case of overdosage and one case of uremic hemolytic syndrome). Mutational status and phosphorylation of AKT signaling components will be presented at the meeting.

**Conclusion:** MK-2206 was overall well tolerated in heavily pretreated DLBCL patients, but clinical activity was minimal with only 2/22 SD after 4 months of treatment.

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**EFFICACY AND SAFETY OF RITUXIMAB BIOSIMILAR IN B-CELL LYMPHOMA PATIENTS TREATED WITH CHEMO IMMUNOTHERAPY: SINGLE-CENTRE RETROSPECTIVE ANALYSIS**

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**Background:** Rituximab along with multi-agent chemotherapy (R-CHOP, R-CVP, BR, R-BFM and R-Hyper-CVAD) has been the standard of care for various types of B-cell lymphoma. The addition of rituximab in various protocols has proven beneficial in terms of improved overall response and prolonged progression-free survival and overall survival. A biosimilar molecule (Reditux®) was developed by Dr Reddy's Laboratories, India, and was licensed for clinical use in India in 2007. With the availability of rituximab biosimilar (Reditux®) at an affordable cost in India, most of the patients are now getting the benefit of treatment with rituximab-based therapy.

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**Methods:** This retrospective study was done to analyse the efficacy and safety of rituximab (Reditux®). We reviewed all patients of low-grade as well as high-grade B-cell lymphoma who consulted at the Haemocare Centre, Vadodara, India, between 2008 and 2014 and received chemo-immunotherapy with rituximab (Reditux®).

Fifty-three patients were analysed for this study—37 males and 16 females. The median age was 49.21 years (range: 16–85 years). The distribution of disease was high-grade lymphoma ($n = 29$) and low-grade lymphoma ($n = 24$). High-grade lymphoma included DLBCL ($n = 22$), GIST with DLBCL ($n = 1$), primary mediastinal lymphoma ($n = 1$), mantle cell lymphoma ($n = 3$) and acute lymphoblastic lymphoma ($n = 2$). Low-grade lymphoma included CLL ($n = 8$), SLL ($n = 2$), FL ($n = 5$), CLPD ($n = 7$) and Waldenstrom macroglobulinemia ($n = 2$). Three patients (two mantle cell lymphoma and one CLPD) were excluded from the study, and the remaining 50 patients were analysed. The protocol used in high-grade lymphoma was six cycles of R-CHOP. The protocol used in acute lymphoblastic lymphoma was R-BFM or R-Hyper-CVAD. The protocol used in low-grade lymphoma was six cycles of RCVP or BR or RFND.

**Results:** The overall response was 86%, with high-grade NHL showing a response of 85% and low-grade NHL showing a response of 87%. At a median follow-up of 30 months, PFS for high-grade and low-grade lymphoma was 70%. OS of high-grade NHL was 74% and that of low-grade NHL was 83%. Low intermediate IPI had PFS and OS of 78% and 82% and those with high and high intermediate IPI had PFS and OS of 64% and 73%, respectively. Common toxicities observed were neutropenia (Grades III and IV) in 11% patients followed by urticaria in 9% patients, fever with chills in 5% patients, reversible bronchospasm in 2% patients and progressive multifocal leukoencephalopathy in 2% patients. A total of 12 patients (24%) died, of which 6 (12%) succumbed to febrile neutropenia with septicaemia, 2 patients (4%) had disease progression and 4 patients (8%) had mortality unrelated to lymphoma or drug toxicity.

**Conclusions:** Rituximab biosimilar (Reditux®) is an efficacious and safe drug for patients of various B-cell lymphomas in terms of safety profile, overall response rate, progression-free survival and overall survival. With the reduction in cost of rituximab biosimilar (Reditux®), majority of patients with B-cell lymphoma in India are now able to avail rituximab-based therapy.

**399**
**LONG TERM RESULTS OF A PHASE I/II TRIAL OF DASATINIB FOR RELAPSED NON-HODGKIN LYMPHOMA: CORRELATION WITH MOLECULAR ANALYSIS**

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**Introduction**: Dasatinib is a potent inhibitor of multiple tyrosine-kinase families including BCR-ABL, Src, c-kit and PDGFR kinases. This agent also has additional effects on the Lyk, Lin, and the NFkB pathways. Many of these pathways are important in the regulation of lymphoma cell growth.

**Methods**: A single center phase I/II study of single agent Dasatinib was done for relapsed/refractory NHL. A total of 33 patients were entered on the trial with histologies of follicular, small lymphocytic, diffuse large B-cell, mantle cell, marginal zone, NK/T-cell lymphoma, lymphoplasmacytic, and peripheral T-cell lymphoma. The maximum tolerated dose was 150 mg daily. The patients continued on the drug if they were responding or until there was a toxicity that precluded the Dasatinib from being taken. Twenty-seven patients with available bio-specimens were sequenced with the Foundation One Heme platform, which interrogates the coding regions of 405 genes, selected introns of 31 genes involved with gene rearrangements and RNA sequencing of 265 genes known to be involved in hematologic malignancies. Significant non-synonymous variants (SNV) were identified as mutations from the COSMIC database, amplifications of established oncogenes, or homozygous deletions and/or clear loss-of-function mutations of known tumour suppressors. Variants of unknown significance (VUS) were identified as mutations not in COSMIC database, amplifications of unknown significance, or homozygous deletions in known non-tumour suppressors.

**Results**: Of the 33 patients enrolled on the study, there were 2 complete remissions, 4 partial remissions for an overall response of 18%. The two patients who had a CR continue in CR and on Dasatinib now at 83+ and 79+ months. These two exceptional responders both had PTCL and had failed multiple other therapies.

In 27 samples, 22 samples had sufficient DNA/RNA for targeted sequencing. The average depth of coverage in 22 samples was >490× (range 249–641).

A total of 429 genomic alterations were identified with 106 SNV and 323 VUS. In the 106 SNV, alterations included 88 short variants (substitutions and in-dels), 3 copy number variations, 8 homozygous deletions, and 7 rearrangements. In the 323 VUS, 312 short variants, 6 copy number alterations, 1 homozygous deletion, and 4 rearrangements were observed. The median number of SNV in responder ($N = 2$) and non-responder ($N = 20$) were 2 and 5 respectively. The median number of VUS in responders and non-responder were 10 and 13 respectively. Additional analysis is underway to characterize the genomic profile of the two exceptional responders versus the non-responders.

**400**
**THE COMBINATION OF PIXANTRONE, ETOPOSIDE, BENDAMUSTINE AND, IN CD20+ TUMOURS, RITUXIMAB IS BOTH FEASIBLE AND EFFECTIVE IN RELAPSED AGGRESSIVE LYMPHOMAS**

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**Introduction:** Aggressive non-Hodgkin lymphoma (aNHL) relapsing after high-dose therapy or, in not transplant-eligible patients, after first-line chemotherapy, represents an unmet clinical need. Therefore, we aimed at evaluating a salvage combination regimen based on pixantrone, an aza-anthracenedione recently approved in Europe for patients with multiply relapsed or refractory (R/R) aNHL. Etoposide and bendamustine were chosen as companion compounds due to available feasibility data in combination with pixantrone, and a well-documented efficacy in salvage regimens in R/R aNHL. Rituximab was added, if the relapse tumour biopsy was CD20+. Four patients with CD20+ tumours did not receive the antibody due to recent previous refractoriness to rituximab-containing regimens.

**Methods:** The adopted schedule consisted of pixantrone 50 mg/m$^2$ i.v. Day 1 + 8, etoposide 100 mg i.v. Day 1, bendamustine 90 mg i.v. Day 1 with or without the addition of rituximab 375 mg/m$^2$ i.v. Day 1 (PREBEN/PEBEN). If feasible, each cycle was given at 3-weekly intervals for a maximum of six cycles. All patients were assessed for chemosensitivity with PET/CT, if possible after Cycle 1 or 2. G-CSF support was administered according to local guidelines.

**Results:** A total of 14 heavily pre-treated patients (9 males and 5 females, age range 49–81 years; mean N of previous regimens: 3, range 1–7) with RR aNHL were treated according to the PREBEN/PEBEN schedule. Ten had diffuse large B-cell (DLBCL), 2 transformed indolent, 1 post-transplant (of DLBCL type) and 1 peripheral T-cell lymphoma (PTCL). All patients had intermediate or high risk IPI prior to salvage start. Eight patients responded (overall response rate: 57%), 5 of these (36%) had a metabolic complete response and 3 (21%) a partial one. Response durations ranged between 4 and 17+ months. The treatment schedule was feasible and most patients received it on an out-patient basis. The most common Grade 3–4 toxicity was of hematological type (mainly neutropenia and thrombocytopenia). Grade 3–4 infections were seen in 40% of the patients and were all manageable.

**Conclusions:** In this elderly high-risk population of R/R aNHL, the PREBEN/PEBEN salvage schedule was feasible (out-patient regimen) and in individual patients it elicited substantial and durable responses early in the course of therapy. Efforts are ongoing to biologically characterize the patients, who are more likely to benefit from this regimen. A Phase 1 (dose-finding)/2 study in R/R aNHL will be launched in Q2 2015.

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**Abs 400 - Table 1.**

| Patient features | | | | | | PREBEN/PEBEN | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Dx** | **Age** | **Sex** | **CS** | **N prior Rx** | **Prior ASCT** | **N courses** | **Gr 3-4 tox** | **Best response** | **DoR (mo)** |
| DLBCL (ABC) | 69 | M | IV | 3 | N | 6 | thrombocytopenia | CR | 6 |
| DLBCL (ABC) | 49 | M | IV | 3 | Y | 2 | – | PD | – |
| DLBCL (ABC) | 52 | M | IV | 3 | N | 6 | neutropenic fever | CR | 9+ |
| DLBCL (ABC) | 64 | M | IV | I | N | 2 | neutropenic fever | PR | 4 |
| DLBCL (GCB) | 69 | F | IV | 2 | Y | 2 | – | CR | 6 |
| DLBCL (GCB) | 81 | M | IV | 4 | N | 3 | neutropenic fever | CR | 5+ |
| PTLD (DLBCL) | 65 | M | IV | 2 | N | 2 | neutropenic fever | PD | – |
| tFL | 62 | M | III | 6 | Y | 2 | neutropenia | PD | – |
| tSLL | 71 | F | IV | 7 | Y | 2 | neutropenic fever | PR | 5+ |
| PTCL-NOS | 57 | F | IV | I | N | 6 | diarhoea | PR | 17+ |
| DLBCL (GCB) | 69 | F | III | 3 | N | 6 | neutropenia | CR | 5 |
| DLBCL (GCB) | 69 | F | IV | 3 | N | 2 | – | PD | – |
| DLBCL (GCB) | 63 | M | II | 2 | N | 2 | – | PD | – |
| DLBCL (GCB) | 62 | F | II | 2 | N | 2 | thrombocytopenia | PD | – |

## EXTRANODAL LYMPHOMA

### 401
### A RETROSPECTIVE STUDY OF OCULAR ADNEXAL MUCOSA-ASSOCIATED LYMPHOID TISSUE (OA-MALT) LYMPHOMA IN JAPAN

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**Introduction:** MALT lymphoma is the third most common non-Hodgkin lymphoma (NHL), and develops in extranodal sites. OA-MALT lymphoma is a relatively rare NHL and the details of disease prognosis are poorly reported. Here we have analyzed a series of OA-MALT lymphoma in the southern area of Japan, and examined the diagnosis, the treatment, and the prognosis of this disease.

**Method:** From 1991 through 2014 in Ehime prefecture, Japan, cases of OA-MALT lymphoma were retrospectively analyzed.

**Results:** A total of fifty-seven cases of OA-MALT lymphoma (female/male; 26/31) with a median age of 63 years (range, 21–85), were analyzed over a median observation period of 6.8 years (dropout; 4 cases). Based on histopathological diagnosis, forty-five biopsy samples (84%) were diagnosed with additional IgH gene rearrangement using southern blot analysis and/or additional flowcytometric analysis of surface antigens using tumour cell suspensions. One patient was diagnosed as Stage III, and eight patients (14%) were diagnosed as Stage II (in both OA), and the others (84%) were diagnosed as Stage I (in single OA) according to the Ann-Arbor clinical staging system. All patients showed Grade 0 or 1 performance status. Laboratory data of only three cases (5%) showed increased levels of LDH, and ten cases (18%) showed increased levels of serum soluble interleukin-2 receptor at their diagnosis. As a treatment, surgical extraction was selected in the majority (fifty-six) of patients. After surgical treatment, radiotherapy or immunochemotherapy with rituximab treatment were administered in twenty-seven (47%) patients. Nine patients (16%) recurred, two patients (4%) died after their treatment, and forty-four patients (77%) presented a disease-free status. There were no significant differences in treatment outcomes between the patients treated with only surgical treatment and the patients treated with additional radiotherapy or immunochemotherapy after surgical extraction.

**Conclusions:** In our current retrospective study, the patients with OA-MALT lymphoma showed a 17% recurrence rate (over 5 years) after the initial treatment. OA-MALT lymphoma is an indolent disease, and patients have a better prognosis than those diagnosed with other NHLs; however, they still need disease-specific and risk-related strategic treatments after diagnosis.

### 402
### A STUDY OF CLINICAL OUTCOME AND SEQUELAE OF PRIMARY LYMPHOMA OF THE ORBITAL ADNEXA: A 20-YEARS SINGLE INSTITUTION EXPERIENCE

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**Purpose:** Orbital lymphomas are rare and usually present as early stage disease**.** We evaluated the clinical outcomes and toxicities of radiation therapy (RT) in primary orbital lymphoma (POL), and studied the effect of various prognostic factors on clinical outcome.

**Methods:** From 1990 to 2012, 81 consecutive patients with Stage IE/IIE (bilateral) primary orbital mucosa-associated lymphoma tissue (MALT) lymphoma or diffuse large B-cell lymphoma (DLBL), treated at our institution were evaluated. Sixty-six patients had MALT and 16 had DLBL. Eleven patients (14%) had bilateral involvement. Radiotherapy was delivered by either 6 MV photons, electrons or $^{60}$Co γ-rays. Radiation doses ranged from 39.6–45 Gy. Local control (LC), relapse free survival (RFS), overall survival (OS) and acute and late side effects were evaluated.

**Results:** The median follow-up was 44 months (IQR: 17–62 months). Sites involved were conjunctiva, lacrimal gland and soft-tissues in 25(31%), 12(15%) and 44(54%) patients, respectively. Megavoltage beam(s) was used in 93% and electrons in 7% of cases. The RT dose delivered was 39.6 Gy in 25 (31%) and 45 Gy in 56 patients (69%). Complete response was seen in 76 (93.8%) patients. The 10-year overall survival (OS), relapse-free survival (RFS), and local control (LC) rates for the entire cohort were 95.1%, 92.5%, and 79.4%, respectively. The RFS was significantly better for MALT than for DLBL (94% vs. 78.3%, respectively; $p = 0.004$; HR 0.2; 95% CI 0.017–0.67). MALT lymphomas presenting as superficial tumours (conjunctiva and Lacrimal apparatus) had better outcome. Most common acute reaction was redness (93%) and watering (91%) of eyes. Radiation-related late sequelae were documented in all patients. Cataracts were observed in 21 patients. The cumulative incidence of cataract at 5 years was 54%. All patients underwent lens-replacement surgery and had good visual acuity

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thereafter. Other late sequelae were dry eyes-23 (28.4%) patients and retinal detachment in 1 patient.

**Conclusion:** POL responds favorably to RT, with manageable late morbidities. Superficial tumours had better outcome as compared to deep tumours.

### 403
### TREATMENT OF CENTRAL NERVOUS SYSTEM LYMPHOMA OF ELDERLY PATIENTS BY ADAPTED HIGH-DOSE METHOTREXATE AND CYTARABINE: AN EFFECTIVE AND SAFE REGIMEN

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**Introduction:** There is no real consensus on CNSL treatment, Ferreri AJM *et al*. (*Lancet* 2009) showed that Mtx + AraC was superior to Mtx alone, leading to 46% of CR vs 18%, but doses used are too high and too toxic for elderly patients (Mtx 3.5 g/m$^2$ + AraC 2 g/m$^2$/12 h, 2 days). We propose an adapted treatment to these patients.

**Methods**: Patients of more than 60 years old, with a lymphoma localized in the brain (B), cerebrospinal fluid (CF) and/or vitreoretinal lymphoma (VRL), from December 2005 to November 2014, were treated in one center (Pitie-Salpetriere Hospital, Paris, France) by Mtx (3 g/m Day 1) and AraC (2 g/m$^2$ Day 1 for the first cure followed by 2 g/m$^2$/day for 2 days from Cures 2 to 6).

**Results**: 33 patients have been included, with 17 females and 16 males, median age 69 (60–87), on 27 patients with known OMS status at diagnosis, 6 had a status of more than 1. Localizations were B ($n = 16$), CF ($n = 3$), VRL ($n = 5$), B + CF ($n = 4$), B + VRL ($n = 3$), B + CF + VRL ($n = 1$), B + CF + systemic ($n = 1$, treated with R-CHOP + Mtx + AraC). Mean total received doses of Mtx and AraC were respectively 16 and 12 g/m$^2$. Fourteen patients had a toxicity of Grade 3 or 4: eight had cytopenia, five had renal insufficiencies and one patient had cytopenia, renal insufficiency and liver toxicity; there was no treatment-related death. The CR rate is 55% ($n = 18$), PR 6% ($n = 2$), with a median follow-up (mfu) of 16.5 months, the median PFS is 12 months and the median OS is not reach (1–78 months), 23 patients being still alive. Among the 18 patients in CR, 9 are still alive in CR with a mfu of 21 months.

**Conclusion**: This adapted treatment with high-dose Mtx and AraC seems to be particularly adapted to elderly patients with a limited toxicity and a high efficiency. Prospective multicenter studies re necessary to confirm these data.

### 404
### FIRST-LINE INTRA-ARTERIAL METHOTREXATE FOR ELDERLY PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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**Introduction:** Primary central nervous system lymphoma (PCNSL) is a rare extranodal non-Hodgkin lymphoma and at the time of diagnosis is confined to the brain, spinal cord or leptomeninges. The median age at diagnosis is approximately 60 years (years), and older age is a negative prognostic marker. Whole brain radiotherapy (WBRT) has been abandoned as primary therapy for PCNSL patients over 60 years because of increased risk of delayed neurotoxicity. High-dose (HD) methotrexate (MTX)-based chemotherapy regimens are feasible in the elderly and show efficacy, however most patients relapse. Herein we report outcomes using intra-arterial (IA) delivery of MTX-based chemotherapy in elderly PCNSL patients.

**Methods:** We obtained Institutional Review Board permission to retrospectively review efficacy and toxicity in PCNSL patients 65 years or older who were treated with first-line IA MTX-based chemotherapy with or without blood-brain barrier disruption (BBBD) and without first-line WBRT. Patients were treated at Oregon Health and Science University during 1982 to 2013, with follow-up through 2014.

**Results:** There were 55 patients (24 M/31 F), with 72% treated with IA MTX plus BBBD, and 28% IA MTX without BBBD. The median age was 70.6 years (min 64.2; max 82.7 years) and the median KPS was 60 (min 20; max 100). The median overall survival (OS) was 14.5 months; 38% were alive at 2 years and 26% were alive at 4 years from the date of first IA treatment. The response rate (complete [CR] plus partial [PR]) was 60% and the CR rate was 51%. Of note, 29 of the 55 patients (53%) were 70 years or older, with median OS of 15.6 months. Twelve of the 29 patients over 70 years (41%) were alive at 2 years, and 7 (24%) were alive at 4 years. Of the 7 patients over 70 years and alive at 4 years, 6 were treated with IA MTX plus BBBD. The most frequent Grade 3 or 4 toxicities were hematologic, infections, and deep vein thrombosis, analogous to toxicities previously reported in PCNSL patients treated with IA/BBBD (Angelov *et al*., *J Clin Oncol* 2009).

**Conclusions:** The CR rate (51%) in this elderly series ($n = 55$) with median KPS of 60 treated with IA MTX, is encouraging. Other investigators have reported encouraging CR rates after IV HD-MTX in elderly PCNSL series as well. However disease control remains a problem regardless of the route of MTX delivery. We will compare data using IA MTX reported above, with data from 208 patients using IV MTX, with median age 70.5 years (min 66; max 84 years) and median KPS 70 (min 20; max 100), from a study described in Roth *et al*. (*Neurology* 2012) and Thiel *et al*. (*Lancet Oncology* 2010). Results of the statistical comparison will be presented during the 13-ICML meeting. As a future direction, we hypothesize that maintenance CD20+ immunotherapy may provide a means to prolong response duration in elderly patients who attain CR, with less toxicity than more intensive chemotherapy regimens.

### 405
### OSTEOPONTIN IN CEREBROSPINAL FLUID AS DIAGNOSTIC BIOMARKER FOR CNS LYMPHOMA

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**Introduction:** Central nervous system (CNS) lymphoma is diagnostically challenging. The identification of reliable and easy to measure biomarkers in easily accessible patient material is desirable to facilitate diagnosis. *Secreted phosphoprotein 1 (SPP1, osteopontin)* is a strongly up-regulated gene in primary CNS lymphoma (PCNSL). Here, we evaluated the value of cerebrospinal fluid (CSF) osteopontin (OPN) as diagnostic biomarker for CNS lymphoma.

**Methods:** CSF samples were collected from 38 patients with PCNSL ($n = 29$) and secondary CNS involvement of systemic lymphoma (SCNSL; $n = 9$) at diagnosis, and compared to 8 patients with multiple sclerosis (MS) and glioblastoma each and 20 healthy controls. Serum samples were collected from 17 PCNSL patients and from all healthy controls. OPN concentrations in CSF and serum were determined using an enzyme-linked immunosorbent assay. For statistical analyses SPSS, version 22.0, was used. We performed non-parametric tests and receiver operating characteristic curves for determination of sensitivity and specificity.

**Results:** The median CSF OPN level in CNS lymphoma patients was 616.6 ng/mL (60.3–889.6 ng/mL) and was significantly higher than in patients with MS 162.5 ng/mL (28.8–370.5 ng/mL); $p < 0.005$, glioblastoma 38.9 ng/mL (21.9–115.0 ng/mL); $p < 0.005$ and healthy controls 328.5 ng/mL (142.0–531.0 ng/mL); $p < 0.005$. Sensitivity and specificity of CNS lymphoma compared to healthy controls at an OPN cut-off level of 419.2 ng/mL were 86.8% and 85.0%, respectively. Sensitivity and specificity of CNS lymphoma in comparison to MS at an OPN cut-off level of 361.9 ng/mL were 89.5% and 87.5%, respectively; as compared to glioblastoma at a cut-off level of 88.3 ng/mL 97.4% and 87.5%, respectively. In SCNSL patients, the median CSF OPN level was comparable to that of PCNSL patients: 619.7 ng/mL (60.3–889.6 ng/mL) vs. 591.7 ng/mL (153.1–817.7 ng/mL); $p = 0.539$. Median

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OPN serum levels were low and did not differ between PCNSL patients and healthy controls: 51.9 ng/ml vs 64.2 ng/ml; $p = 0.326$.

**Conclusions:** These results show higher CSF OPN levels in CNS lymphoma patients than in patients with MS or glioblastoma or healthy controls. If validated in prospective trials, OPN may represent a valuable and easy to measure diagnostic biomarker in CNS lymphoma.

**406**
**THE IDENTIFICATION AND DIAGNOSTIC VALUE OF A GROUP OF CEREBROSPINAL FLUID PROTEINS FOR CENTRAL NERVOUS SYSTEM DIFFUSE LARGE B-CELL LYMPHOMA**

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**Introduction:** The incidence of central nervous system (CNS) lymphoma has increased over the last decades. How to obtain diseased brain tissue and diagnosis were clinical challenges. The related research found that cerebrospinal fluid (CSF) protein concentration was significantly increased in the most patients with CNS lymphoma. The aim of this study was to find out potential diagnostic CSF protein biomarkers for CNS lymphoma.

**Methods:** We utilized one-dimensional SDS-polyacrylamide gel electrophoresis (1D SDS-PAGE) coupling with liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry (LC-ESI-Q-TOF MS) to identify CSF proteins which are differentially expressed in patients with or without CNS lymphoma. We compared CSF samples from ten patients with CNS diffuse large B-cell lymphoma (DLBCL) and ten controls without CNS DLBCL. Differentially expressed proteins were confirmed, located, and understood its clinical significance by immunohistochemistry, indirect immunofluorescent assay, and enzyme-linked immunosorbent assay (ELISA).

**Results:** The CSF total protein concentration increased in patients with CNS DLBCL. Approximately 166 CSF proteins were identified and 24 CSF proteins were found to be present at significantly different concentrations, both higher and lower, in patients with or without CNS DLBCL. In these CSF proteins, there are 12 proteins were obtained for the first time. These proteins include α1-antitrypsin, ceruloplasmin and heat shock protein and so on. Significantly different proteins, include hemopexin, apolipoprotein A1 and transferrin, were verified using immunohistochemistry in tissue specimens. Immunohistochemical analysis demonstrated these three proteins were strong expressed by tumour cells in CNS and nodal DLBCL. We detected these three proteins localization in the cytoplasm by indirect immunofluorescence. We used ELISA to confirm the relative expression of these three proteins in the CSF. CSF hemopexin, apolipoprotein A1 and transferrin concentration identified CNS lymphoma patients with the highest accuracy, and sensitivity was 80%, 83% and 70%, respectively, and specificity was 75%, 89% and 90%, respectively. By contrast, cytologic evaluation of the same CSF samples was only 25% sensitive.

**Conclusion:** Our results suggest that CSF protein concentration was increased in the majority of patients with CNS DLBCL. The CSF proteomic analysis demonstrates that there are differences between two groups of patients with or without CNS DLBCL. Hemopexin, apolipoprotein A1 and transferrin expressed higher concentrations in CNS DLBCL. The expression level of hemopexin, apolipoprotein A1 and transferrin in CSF is associated with CNS DLBCL involvement. The discovery of CSF protein will facilitate early and non-invasive diagnostic, and may become some potential therapeutic targets.

**407**
**PRIMARY CNS POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD-CNS) IS EBV-RELATED AND HAS GOOD RESPONSE TO RADIOTHERAPY AND ISS REDUCTION**

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**Introduction:** Post-transplant lymphoproliferative disorders are a heterogenous group of diseases occurring in the setting of post-transplant immunosuppression. Clinically, extranodal involvement is common, and it occurs in the CNS in approximately 7–15% of cases. Most data on PTLD-CNS are based on case series/reports, with an exception of a multicenter study of 84 patients. In this group, PTLD-CNS showed to be a poor prognosis, late-occurring EBV-associated disease. We retrospectively analyzed 16 cases of PTLD-CNS in Hospital do Rim, Sao Paulo, Brazil.

**Methods:** From 1998 to 2014, a total of 11 284 kidney transplants were performed at Hospital do Rim e Hipertensão/UNIFESP (Federal University of São Paulo). We retrospectively analyzed cases of PTLD with CNS involvement diagnosed over the past 17 years. Only confirmed cases of PTLD with available clinical and epidemiological data were included.

**Results:** From 1999 to 2014, a total of 20 patients were diagnosed with PTLD-CNS. Four patients were excluded from the analysis due to conflict data. Among 16 patients, the median age at time of diagnosis was 40, with a male : female ratio of 0.77:1. Fifty-six per cent of patients received ATG at the time of transplant. Regarding immunosuppression (ISS), all patients received Prednisone, 37.5% received tacrolimus, 43.7% received mycophenolate, and 56.2% received cyclosporine. The median time of transplant to PTLD was 93 months, with 25% patients with polymorphic PTLD and 75% with monomorphic PTLD (DLBCL). Regarding EBV positivity, 14/16 were EBV+, with 1 EBV- and 1 inconclusive. All patients had ISS reduction, 43% of patients received brain radiotherapy. Only 1 patient received high-dose methotrexate. Seventy-five per cent of patients responded to treatment. No death related to lymphoma progression was observed: four patients died, all of them due to infectious complications, and none of them during therapy.

**Conclusions:** In our population, CNS-PTLD was an EBV-related disease with good response to ISS reduction and radiotherapy, with most patients achieving responses. No death due to disease progression was observed. Late effects of brain radiation, however, should be taken in account when deciding the best therapy for this rare complication of transplant.

**408**
**PROGNOSTIC IMPACT OF PROLIFERATIVE INDEX KI-67 IN MARGINAL ZONE LYMPHOMA (MZL)**

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**Introduction:** Marginal zone lymphoma is a B-cell lymphoma characterized by an indolent course, which encompasses three subtypes, namely extranodal MZL or MALT lymphoma, nodal MZL and splenic MZL, with different clinical and biological characteristics. The present study examines the effects of Ki-67 on prognosis and its relationships with clinical parameters.

**Methods:** A retrospective analysis of 62 patients with MZL, observed between 2000 and 2013, was performed to determine prognostic factors. The patients were evaluated according to age, sex, stage, B symptoms, extranodal involvement, lactate dehydrogenase (LDH) levels. The Ki-67 proliferative index was assessed immunohistochemically. Patients' median age was 60 years (27–85). Thirty (48%) of them had a MALT type, 3 (5%) NMZL and 29 (47%) SMZL. Most had advanced-stage disease, bone marrow infiltration, and high β2μ values. Univariate analysis was performed to evaluate the correlation between Ki-67 and response rate, overall survival (OS) and progression-free survival (PFS).

**Results:** Ki-67 expression was increased (≥20%) in 21 patients (51%) with MALT lymphoma, 18 (44%) with SMZL, 2 (5%) with NMZL. Fifty-one patients (82%) received chemotherapy (CHOP-like protocol) with rituximab in 29 (46%); 11 (18%) patients were not treated. Among patients with Ki-67 < 20%, 30 (91%) reached complete remission (CR) and 2 (6%) partial remission (PR) after treatment, while 13 (88%) CR and 11 (74%) PR were observed in the Ki-67 > 20% subset ($p < 0.05$). Median follow-up was 53 vs 33 months in the low and high Ki-67 groups and 50 months OS was 100% vs 50%, respectively ($p < 0.05$). Median PFS was 43 vs 20 months in the two groups.

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**Conclusions:** Our data show that Ki-67 expression is predictive of treatment response, PFS and OS. It could represent a possible predictive factor of poor prognosis which would help to identify a high-risk subgroup of MZL.

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### A RETROSPECTIVE ANALYSIS ON SPLENIC MARGINAL ZONE LYMPHOMA: PROGNOSTIC FACTORS, ROLE OF WATCH AND WAIT AND OTHER THERAPEUTIC APPROACHES IN THE RITUXIMAB ERA

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**Introduction:** Splenic marginal zone lymphoma (SMZL) is an indolent lymphoma recognized as a distinct entity in the WHO classification. Arcaini's score (AS)—based on Hb < 12 g/dL, albumin < 3.5 g/dL, elevated LDH—could be useful for prognostication. Given that there are no standard criteria to initiate treatment, the watch-and-wait approach in asymptomatic patients is recommended. In symptomatic patients some data suggest that, apart from splenectomy, rituximab +/− chemotherapy is the best option. The aims of our study were to identify risk factors at diagnosis, to assess feasibility and progression rate of WW, to analyse the outcome of different therapies in the post-rituximab era.

**Methods:** We retrospectively examined clinical files from 83 patients with SMZL treated in our centre from 2000 to 2013. Asymptomatic patients were managed with WW policy. Splenectomy was performed in 21 patients with symptomatic spleen enlargement and limited bone marrow or nodal involvement. Patients with contraindication to splenectomy or more generalized disease were treated with chemo alone (12) and, after its introduction, with rituximab + chemo (8).

**Results:** Median age at diagnosis was 66 years. Male/female ratio was 1.1. HCV antibodies were positive in 3.7%. The 10-year overall Survival was 93% (CI: 84.7–100%). Notably, no patient died of disease progression. The 5- and 10-year progression-free survival (PFS) rates were 77% and 62%, respectively. In univariate analysis, negative predictors of worse PFS were splenomegaly (>18 cm) and bone marrow infiltration (>30%). Patients with a low AS had a better PFS than patients with intermediate and high risk ($p$-value = 0.01). Fifty asymptomatic patients underwent a W&W program. The median PFS of this population was 45 months; at 10 years, 17% of patients are still on W&W. Sixty-five patients, either at diagnosis or after a W&W period, were treated: those treated first line with splenectomy or R-chemo had similar results, while those treated only with chemo had inferior outcomes. However, when separately analysing patients with AS ≤ 1, splenectomy alone resulted in highly significant PFS advantage in comparison with the other treatment approaches.

**Conclusions:** This real-life study gives an insight into indolent SMZL, confirming a very good prognosis. We found a negative prognostic impact of splenomegaly and marrow infiltration, so they should be carefully evaluated at diagnosis. Contrary from previous reports, we did not observe high prevalence of HCV, especially considering a South-Italian population. Moreover, we confirm that AS model is a powerful prognostic index, defining 3 separate risk groups. W&W approach allows a median PFS of 45 months, longer than that reported in Follicular Lymphoma. Finally, our data confirm the inferiority of chemo against splenectomy and rituximab +/− chemotherapy in the whole population, while low-risk patients treated only with splenectomy fared very well, so it could become the suggested first approach even in the rituximab era. Prospective studies are needed to confirm these results.

### 410
### A PHASE II STUDY OF OXALIPLATIN-PREDNISONE TREATMENT FOR THE PATIENTS WITH RELAPSED OR REFRACTORY ADVANCED MARGINAL ZONE B-CELL LYMPHOMA

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**Introduction:** According to the previous large-scale analyses, marginal zone lymphoma (MZL) is usually a quite indolent malignancy. Over its long survival duration, MZL often involves frequent relapses. Overall, more than 50% of MZL patients experience a relapse within 10 years. However, the natural history of relapsed MZL, including its relapse pattern, the optimal salvage treatment modality, and response and survival rates, all have yet to be well established. Therefore, the search for effective forms of systemic therapy for relapsed or refractory advanced MZL is a critical issue. We conducted this multi-center, phase II trial to assess the efficacy and safety of oxaliplatin-prednisone (Ox-P) chemotherapy for patients with relapsed or refractory MZL.

**Methods:** Patients received oxaliplatin 130 mg/m$^2$ on Day 1 and prednisone 100 mg/day on Days 1–5 of each cycle. The treatment was repeated every 3 weeks and continued for six cycles until disease progression, withdrawal due to toxicity, or withdrawal of consent.

**Results:** Between February 2010 and July 2013, a total of 38 patients were enrolled (with informed consent) into this trial from 14 institutes in Korea. Among these patients, 4 patients dropped out without evaluation. The median age of the 34 (16 males, 18 females) evaluated patients was 53 (range 27–74) years. Twenty-seven patients (79.4%) evidenced involvement of extranodal sites. All patients received previous 1 (70.6%) or over 2 treatments for MZL. Eighteen patients (52.9%) had received prior rituximab combination chemotherapy. Total of 179 cycles of Ox-P chemotherapy (range 1–6 [median 6] cycles/person) were administrated. There were 7 CR (20.6%), 15 PR (44.1%) [overall response rate 64.7%; 95% confidence interval (CI), 48–82%], 8 SD (23.5%), and 4 PD (11.8%). Two patients stopped study because of prolonged cytopenia. Non-hematologic toxicities were mild and tolerable. However, no treatment-related death occurred in this study. The median progression-free survival (PFS) was 14.2 months (95% CI 2.1–26.3 months). Three-year overall survival (OS) rate was 77.7%. B symptom (+) patients had an independent influence on poor PFS.

**Conclusion:** Salvage Ox-P chemotherapy, in this dosage and at this schedule, evidenced moderate clinical activity in cases of relapsed or refractory advanced MZL.

### 411
### PRIMARY GASTRIC LYMPHOMA DETECTED BY SCREENING UPPER ENDOSCOPY IN HIGH PREVALENCE AREA OF HELICOBACTER PYLORI INFECTION

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**Background:** The role of screening endoscopy in primary gastric lymphoma (PGL) has not been investigated.

**Aim:** To evaluate the clinical characteristics and outcomes of PGLs detected by screening endoscopy in the high prevalence area of *Helicobacter pylori* infection.

**Methods:** This retrospective matched cohort study enrolled consecutive subjects who were diagnosed as PGL by endoscopic screening in Seoul National University Hospital Healthcare System Gangnam Center Between October 2003 and September 2013. The characteristics and outcome of screening-detected patients (screening group) were compared with age- and sex-matched subjects who were diagnosed with PGL in the outpatient clinic (outpatient group).

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**Results:** Of the 105 194 recipients of screening endoscopy, 52 (0.049%) were found to have PGL. The median age was 54.5 years (range, 22–80), and 65.4% were female. The proportion of PGL to gastric malignancy was 12.1% (52/429) overall, but >30% (25/73) in middle-aged (40–59) females. PGLs in the screening group were more likely to be low-grade mucosa-associated lymphoid tissue (MALT) lymphoma (98.1% vs 57.7%, $p < 0.001$) and treated with *H. pylori* eradication alone (90.0% vs 47.0%, $p < 0.001$) than those in the outpatient group. Moreover, the screening group showed better 5-year overall survival (100.0% vs. 89.5%, $p = 0.035$) and progression-free survival (94.9% vs. 82.9%, $p = 0.047$) than the outpatient group.

**Conclusions:** In Korea, the high prevalence area of *H. pylori* infection, PGL seems more prevalent than Western countries. Endoscopic screening may help to detect early stage *H. pylori*-positive MALT lymphoma. A high index of suspicion is needed, especially in middle-aged women.

### 412
### CUTANEOUS B-CELL LYMPHOMA: CLINICOPATHOLOGICAL AND THERAPEUTIC STRATEGY—A SINGLE-CENTER EXPERIENCE

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**Introduction**: Skin is the second most common primary extranodal presenting site in non-Hodgkin lymphoma. Approximately 20% of those represent cutaneous B-cell lymphoma (CBCL). We performed a retrospective study to determine the outcome of patients with CBCL diagnosed and treated in our department between 2000 and 2013.

**Methods:** CBCL were classified according to WHO 2008. In this study baseline characteristics, histopathology, response to initial therapy, event-free survival (EFS) -based on data of relapse, progression, death or last visit and overall survival (OS) were analysed.

**Results:** Forty one patients with CBCL were identified: 53.6% ($n = 22$) primary cutaneous follicular lymphoma (PCFCL), 34.1% ($n = 14$) primary cutaneous marginal zone B-cell lymphoma (PCMZL) and 12.2% ($n = 5$) primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL-leg-type). Median age was 60 years (21–87) and 23 patients (56.1%) were female. Lesions at diagnosis were predominantly solitary (70.7%) and nodular (56.1%) mainly presenting on head (41.5%) and trunk (34.1%). No demographic or clinical statistical differences were found among the 3 groups.

Initial therapeutic strategy was radiotherapy (RT) (56.0%), chemotherapy (QT) (26.8%), or QT followed by RT (4.9%), mainly in those with scattered and multiple lesions or PCLBCL-leg-type. A conservative approach with surgical excision only was done if surgical margins were not invaded (12.2%). Complete response were obtained in 92.7% patients, but 2 had no response (PCFCL and PCLBCL-leg-type). Median follow-up was 46 months (0–292). EFS at 3 years was 86.9% in PCFCL, 77.8% in PCMZL, and 75% in PCLBCL-leg-type. EFS at 5 years was 78.3% in PCFCL and 77.8% in PCMZL, not available in PCLBCL-leg type due to short follow-up. OS at 5 years was 95.0% in PCFCL, 85.7% in PCMZL and 75.0% in PCLBCL-leg-type. Progression of disease was the cause of death only in one patient with PCLBCL-leg-type; the cause of death was unrelated to disease in all other patients. OS was similar in both groups ($p = 0.32$).

**Conclusion:** Our study contributes to the epidemiological and clinicopathological characterization of CBCL. PCFCL and PCMZL are indolent neoplasms with an excellent prognosis; in contrast, PCLBCL is clinically more aggressive and potentially fatal.

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### PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE. CLINICAL FEATURES AND OUTCOME IN 48 PATIENTS. POLISH LYMPHOMA RESEARCH GROUP STUDY

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**Introduction:** Primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBL-LT) is a rare lymphoma subtype typically affecting elderly patients. Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) with or without radiotherapy is recommended as the first line of treatment for these patients, but efficacy data are limited. Here we describe clinical features and outcome of PCDLBL-LT patients mostly treated with R-CHOP over ten-year period.

**Methods:** We retrospectively collected data on PCDLBL-LT patients diagnosed between 2005–2014 at six oncology centers from the Polish Lymphoma Research Group.

**Results:** Forty-eight patients with a diagnosis of PCDLBCL-LT were identified, with 28 female and 20 male patients. Median age (range) was 72 (21–89), and 21 patients were 75 years or older. Skin lesions were located on the leg in 29 patients (60%), on the upper limb in 6 patients (13%), on other sites in 13 patients (27%), 22 patients presented bulky disease (48%). Risk category according to the modified NCCN-IPI was low or low-intermediate in 24 (50%) patients. Chemotherapy was the initial treatment in 46 (96%) including R-CHOP in 39 (81%) of patients. Two patients had surgery alone. Adjuvant radiotherapy was applied in 10 (9%) patients. In 46 patients evaluable for response after first-line therapy, overall response rate (ORR) was 91% and complete remission rate was 69%. After a median (range) follow-up of 16 (2–82) months, median progression-free survival (PFS) and overall survival (OS) for the entire group were 26 and 28 months, respectively. Three-year PFS and 3-year OS were 44% (95% CI [25%, 64%]) and 47% (95% CI [31%, 64%]). In patients who received rituximab, median OS was not reached, median PFS was 27 months, 3-year OS was 56% (95% CI [37%, 75%]), and 3-year PFS was 44% (95% CI [24%, 65%]). In patients with low/low-intermediate, and high-intermediate/high NCCN-IPI risk, 3 year OS was 74%, and 39% respectively ($p = 0.05$). After initial treatment, 23 (49%) patients subsequently progressed or relapsed, frequently in primary sites. Three patients had CNS relapse.

**Conclusions:** PCDLBL-LT has an aggressive course with high relapse rate and poor outcome. Rituximab in the first line of treatment might contribute to improved overall survival but not progression-free survival. Across all NCCN–IPI risk categories, overall survival of PCDLBCL-LT patients was inferior compared to patients with nodal disease.

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### FAVORABLE OUTCOME OF PRIMARY MEDIASTINAL DIFFUSE LARGE B-CELL LYMPHOMA: A PORTUGUESE SINGLE-CENTER EXPERIENCE

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**Introduction:** Primary mediastinal B-cell lymphoma (PMBCL) is a distinct clinicopathologic entity from diffuse large B-cell lymphoma and presents with a rapidly growing dominant mediastinal mass. The optimal first-line therapy for PMBCL is subject of ongoing debate with no accepted standard of care.

**Methods:** We searched retrospectively for patients aged 18 years or older with newly diagnosed PMBCL treated at our department between 1999 and 2014. Baseline patients, disease and treatment data were collected. Staging and response assessment of patients included PET and/or CT scan. Clinical outcome was followed until date of death or last encounter.

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**Results:** Thirty-three patients with PMBCL (21 female and 12 male) were identified. The median age was 35 years (18–79 years). Sixty-five per cent ($n = 22$) had limited-stage disease and 67% ($n = 23$) were bulky. The majority of patients (85%, $n = 28$) were treated with R-CHOP. Other regimens were R-HCVAD ($n = 3$), R-CHOEP ($n = 1$) and CHOP ($n = 1$). Overall and complete response rate were 94% and 85%, respectively. Twenty-three of the responding patients (74%) received radiotherapy and other 7 (22%) underwent autologous stem cell transplant. Progressive disease occurred in 2 (6%) patients. Febrile neutropenia was the most frequent acute adverse event. With a median follow-up of 45 months, three patients developed late toxicity: pulmonary disease ($n = 2$) and hypothyroidism ($n = 1$). The 5-year overall survival was 82%. Six patients died, five of which died within the first year after diagnosis. The limited sample size precluded prognostic-factor analysis.

**Conclusion:** These data demonstrate good response rates and survival outcomes consistent with results of different institutions in Europe and North America. Patients failing induction regimen showed a very poor prognosis. Larger studies are needed to identify high-risk patients who should be tested with innovative approaches.

**415**
**DO WE HAVE SUFFICIENT EVIDENCE TO DETERMINE THE STANDARD OF CARE IN PMBL?**

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**Introduction**: Progression-free (PFS) and overall survival (OS) rates for PMBL have risen to 84% and 92%, respectively, with the addition of rituximab to standard CHOP. Despite general acceptance of RCHOP as standard of care for Diffuse Large B-Cell lymphoma (DLBCL), many centers recommended alternative regimens for PMBL such as RMACOPB, RVACOPB, RCHOP-RICE and DA-EPOCH-R. The latter was adopted with great worldwide enthusiasm, despite its lack of proven superiority in randomized controlled trials (RCT). The benefits of DA-EPOCH-R include the omission of consolidation radiotherapy (RT), an attractive option in PMBL patients, given their demographic profile—mainly females in their third decade. We aimed to evaluate the PFS, the OS, the number of hospitalization days for treatment, and complications and the need for consolidation RT in a single center in the rituximab era, where since 2007, most of our patients were treated with the RCHOP-RICE regimen consisting of four courses of RCHOP followed by three courses of RICE.

**Methods:** We reviewed the files of all PMBL patients who received first-line treatment in the Hadassah Medical Center between 8/2002 and 10/2014, extracting clinical, laboratory and imaging data.

**Results:** Of the 47 patients, 24 were treated with RCHOP-RICE (80% since 2007), 12 with RMACOPB, 3 with RVACOPB, 6 with RCHOP and 2 with DA-EPOCH-R. Patients were mainly female, with Stage I–II disease and a high LDH level. Patient characteristics were comparable between the protocols (see table). In total, 21 (45%) of our patients received RT; only 3 patients (12%) were treated with RCHOP-RICE compared to 18 patients (78%) treated with other protocols ($p < 0.01$). A mean of $11 \pm 8$ hospitalization days/patient was needed to administer the RCHOP-RICE regimen, significantly more than that required for other treatments combined ($p < 0.01$), except DA-EPOCH-R where the mean hospitalization days to administer six courses was $37 \pm 2$/patient (2 patients). Treatment-related toxicities did not differ between the groups. Late toxicity included advanced breast cancer in one patient who received RMACOPB and radiotherapy. The median 5-year PFS and OS were 93% and 98% respectively, with no difference between treatment regimens.

**Conclusion**: The RCHOP-RICE regimen does not appear inferior to other regimens which omit RT in PMBL and demonstrated no significant late toxicities. Published Phase 2 data on DA-EPOCH-R (93% EFS and 95% OS) do not demonstrate an advantage compared to the simpler regimens described here. RCTs are required to establish the standard for efficacy, efficiency and safety of care in PMBL.

**Abs 415 - Table 1.**

| Characteristics | RCHOP-RICE | Others | All patients |
|---|---|---|---|
| No. of patients | 24 | 23 | 47 |
| Median age | 34 | 34 | 34 |
| Female *n*(%) | 16 (67) | 15 (65) | 31 (66) |
| Stage 1–2 *n*(%) | 17 (71) | 21 (91) | 38 (81) |
| Median tumor size (cm) | 10.2 | 10.5 | 10.2 |
| High LDH n(%) | 21 (87) | 17 (81) | 38 (84) |
| Effusion *n*(%) | 10 (42) | 9 (39) | 19 (40) |
| Median 5 year PFS | 90% | 95% | 93% |
| Median 5 year OS | 100% | 95% | 98% |

**416**
**PROGNOSTIC FACTORS IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA UNDER STANDARD CHEMOTHERAPY WITH R-CHOP WITH OR WITHOUT RADIOTHERAPY: AN ANALYSIS OF 213 PATIENTS**

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**Background:** PMLBCL is a rare entity; prognostic factors (PFs) have not been extensively studied and prognostic models specifically applicable to PMLBCL have not been developed. Prior to the rituximab era, the International Prognostic Index (IPI) appeared to retain its prognostic significance in several but not all studies. R-CHOP provides very good results in PMLBCL, minimizing failure rates. In the only relevant study, high IPI and serous effusions emerged as adverse prognostic factors in 123 Japanese patients treated with R-CHOP without RT (64 patients who received RT were excluded). Given that more intensive chemotherapy (R-da-EPOCH) might be better than R-CHOP, the applicability of various PFs needs to be urgently evaluated in the rituximab era in order to define subgroups of patients at high risk for treatment failure and death.

**Aims:** The identification of PFs for the outcome of patients with PMLBCL treated with RCHOP ± RT.

**Patients and Methods:** Two hundred thirteen patients with PMLBCL were treated in a multicenter setting with RCHOP ± RT (usually six to eight cycles). The following potential prognostic factors were evaluated: Age (median 31; range 17–82; >60 years only 4%), gender (female 64%), B-symptoms (31%), Stage III/IV (11%), infradiaphragmatic disease (7%), extranodal involvement (40%), pleuritis (34%), pericarditis (28%), performance status (PS) ≥2 (17%), LDH levels

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(81%), anemia (38%), leukocytosis $\geq 10 \times 10^9$/L (25%), ESR ≥30 mm/h (68%), albumin <4 g/dL (43%), bulky disease (≥10 cm; 59%), age-adjusted IPI (aaIPI; ≥2 in 21%).

**Results:** The median follow-up of currently alive patients was 50 months. Among 52 failures, 51 occurred within 17 months from diagnosis. The 3-year freedom from progression (FFP) was 75%. With 22 deaths recorded (excluding 2 unrelated deaths), the 5-year overall survival (OS) was 88%. The aaIPI identified a minority of patients (aaIPI ≥ 2; 21% of total) with a 5-year FFP of 64% vs. 79% for those with aaIPI 0–1 ($p = 0.04$) and 5-year OS of 76% vs. 92% ($p = 0.003$). Many of the examined variables had a significant ($p < 0.05$) or borderline ($p < 0.15$) association with both FFP and OS. In multivariate analysis of OS, extranodal involvement and bulky disease were independent PFs ($p = 0.02$ and $p = 0.03$). None, 1 or 2 of these factors were present in 29%, 42% and 30% of the patients. OS at 5 years was effectively predicted being 100%, 93% and 73% for patients with 0, 1 or 2 PFs respectively ($p = 0.0001$). The corresponding 5-year FFP rates were 88%, 79% and 59% ($p = 0.002$).

**Conclusions:** In the largest patient series reported so far, RCHOP ± RT provided satisfactory results in PMLBCL with long-term FFS of 75% and excellent OS of 88%. The aaIPI was moderately predictive of the outcome. The combination of extranodal involvement and bulky disease defined a subgroup comprising ~30% of patients with a ~40% risk of failure and >25% risk of death, who might be suitable for trials of treatment intensification.

### 417
### PRIMARY THYROID LYMPHOMA IN THE IMMUNOCHEMOTHERAPY ERA: NATURAL HISTORY AND RISK OF PRIOR MALIGNANCIES

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**Introduction:** Clinical features, natural history and outcomes of primary thyroid lymphoma are not well defined. This study reports single institution experience with primary thyroid lymphoma including history of prior malignancies.

**Methods:** Patients with primary thyroid lymphoma were identified on prospective, actively maintained Mayo Clinic Lymphoma Data Base. Pathology was centrally reviewed. The history of other malignancies was abstracted from the chart.

**Results:** Thirty-three patients were identified from 2000 to September 2014. The median age was 65 years (range 20–84). Eighteen patients were males and 15 females. Histologic subtypes included the following: diffuse large B-cell lymphoma (DLBCL) 21 (64%), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) 7 (21%), follicular lymphoma (Grade 1–3A) 3 (9%), DLBCL/MALT 1 (3%), and nodular sclerosis classical Hodgkin lymphoma 1 (3%). Nineteen patients were Stage IAE, 13 IIAE, and one IBE. The median follow-up was 44 months (range 2–174). One patient was found to have a synchronous papillary carcinoma of the thyroid. Seven patients had 8 prior malignancies (time prior) including oligodendroglioma (1.5 years), acute myelomonocytic leukemia (8 years), non-melanomatous skin cancer (10 years), breast carcinoma (17 years), malignant melanoma (19 years), uterine carcinoma (24 years) followed by oligodendroglioma (1.5 years), and colon cancer (26 years). The median time to diagnosis of primary thyroid lymphoma in patients with a prior malignancy was 17 years (range 1.5–26 years). All patients were treated. Treatment included surgical extirpation (13 total), chemotherapy, and radiation therapy (RT) alone or in consolidation. The treatment was histology dependent and included the following: 7 R-CHOP/RT, 4 CHOP/RT, 2 R-CHOP, 1 CHOP, 1 R-CVP/RT, 2 R-CVP, 1 CVP, 3 RT only, 2 surgery only, and 1 ABVD/RT. Three patients relapsed including two FL Grade 1–2 patients, one treated with surgery only with relapse 9 months later followed by complete remission (CR) following rituximab monotherapy and the other patient was treated with radiation therapy alone that transformed to DLBCL 8 years later subsequently treated with R-CHOP to CR in remission 4 years later. The third relapse was a DLBCL patient treated with R-CHOP to CR with relapse 3 years later in the thyroid with a biopsy proven MALT NHL subsequently treated with BR to a CR. The median overall survival was not reached was 85% at 5 years.

**Conclusions:** The outcomes for patients with primary thyroid lymphoma managed with multiple modalities in the immunochemotherapy era are excellent even in patients with history of prior or concomitant malignancies.

### 418
### A CLINICAL ANALYSIS OF 44 CASES OF PRIMARY PULMONARY LYMPHOMA—RETROSPECTIVE STUDY OF POLISH LYMPHOMA RESEARCH GROUP

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**Introduction**: Primary pulmonary lymphoma (PPL) is one of the rarest malignancy (0.4% of cases) affecting the lung. Lung involvement in B-cell lymphoma mainly occurs as a part of generalized disease. We aimed to evaluate the clinicopathologic characteristics and clinical outcome in patients with PPL.

**Material and Method:** We reviewed 44 patients treated in 8 Polish hemato-oncology centers between 2000 and 2014. There were 26 (59.1%) female patients and 18 (40.9%) male, aged from 26 to 82 years (median age 60.6). Histiotypes of PPL were as follows: 21 (47.7%) MALT, 18 (40.9%) DLBCL, 4 (9.09) MCL, 1 (2.2%) PTCL. The main clinical manifestations of disease were dyspnea (52.6% patients) and cough (39.5% patients), in 13% hemoptysis, in 18% patients recurrent infection but 4 patients were asymptomatic. Imaging examinations showed in 33 patients (86.8%) lung mass, in 4 patients additional patchy opacities and in 5 patients (13%) isolated patchy opacities. In 15 patients (34%), PPL had bilateral occurrence. In the MALT PPL group, 10 patients were treated with surgery combined with chemotherapy, 7 with chemotherapy alone and 4 patients with surgery alone. In the DLBCL PPL group, 2 patients were treated by surgery and subsequent chemotherapy, 1 patient by radiotherapy and chemotherapy and 15 patients by chemotherapy alone. In MCL PPL group 1 patient underwent radiotherapy alone and 3 patients were treated by chemotherapy alone.

**Results:** In the MALT PPL group, five patients relapsed and one died, and median PFS was 38.7 months (5–96) and OS 44.5 months (5–120). In the DLBCL PPL group, four patients relapsed and four patients died, and median PFS was 42.2 months (1–108) and OS 45.5 months (2–108)

**Conclusion:** In our analyzed series of PPL we observed high percentage of DLBCL subtype (40.9%). Most patients had non-specific symptoms of lymphoma, mimicking respiratory infection. In some patients mainly with MALT lymphoma imaging exam revealed infiltration as in pneumonia. Bilateral presentation of PPL in our series occur in similar frequency in MALT and DLBCL group. PPL has good prognosis in both histologic subtypes.

### 419
### PRIMARY TONSILLAR LYMPHOMA: PROGNOSTIC FACTOR AND TREATMENT OUTCOME

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**Introduction:** Most studies have reported the treatment result of tonsillar lymphoma as a group of the Waldeyer's ring. And the prognosis of tonsillar lymphoma was reported controversial. We evaluated the treatment outcome and prognostic factor of tonsillar lymphoma.

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**Methods:** We analyzed a total of 27 patients with Stage IE (25.9%), IIE (63.0%) and IIIE (11.1%) treated at our institution from 1996 to 2014. The 22 patients (81.5%) underwent combined chemo-radiotherapy, while five patients (18.5%) underwent radiation therapy only. Four patients of combined chemo-radiotherapy group were treated with salvage aim due to disease progression or recurrence after chemotherapy.

**Results:** Median age was 53 years (range, 25–75 years) and male to female ratio was 1.1:1. The majority of histology was diffuse large B-cell lymphoma (81.5%). Chemotherapy regimen was (R-)CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) and the median number of cycles was 6. Radiation was prescribed to tonsillar area with median 50.4 Gy (range, 30–55.8 Gy) with 2 Gy/fraction, and neck lymphatics with median 39.6 Gy (range, 25.2–50.4 Gy) with 2 Gy/fraction. After a median follow-up of 44.6 months (range, 7.1–243.3), 4-year overall survival (OS) was 76%, and progression-free survival (PFS) was 70.4%. Neck lymph node (LN) involvement (4-year OS: 88.9% vs 68%, $p = 0.028$) and RT response after 3 months (complete remission vs no complete remission; 4-year OS: 83.6% vs 50%, $p = 0.038$) were significant prognostic factors for OS. On multivariate analysis, the significant prognostic factors were not identified. Higher radiation dose to tonsillar area and neck lymphatics did not show the significant influence on OS or PFS.

**Conclusions:** A combined chemo-radiotherapy was effective for tonsillar lymphoma, and neck LN involvement and RT response after 3 months were prognostic factors for OS. Further study with a higher number of patients is required.

### 420
### LYMPHOMA OF BONE INVOLVEMENT: A SINGLE CENTER EXPERIENCE

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**Introduction:** Skeleton involvement in lymphoma is an uncommon form of extranodal lymphoma. As a result of this, many aspects of lymphoma involving bone are controversial: management strategies, response criteria and prognostic factors. We sought to determine the following in an analysis from a single center between 2006 and 2014: (1) clinical features and characteristics of patients with lymphoma with bone involvement; (2) 5-year progression-free survival (PFS) and overall survival (OS) of patients with lymphoma with bone involvement; and (3) whether prognostic factors (sex, site of tumour, age, IPI) were associated with 5-year survival.

**Methods:** A total of 835 patients with lymphoma were retrospectively evaluated. Among these, 51 patients had skeleton involvement in lymphoma. The patients who had a minimum follow-up of 2 months (median, 23.5 months; range, 2–101-.5 months) were further analyzed for the skeletal site of involvement, the radiotherapy for the skeletal site of involvement, and survival.

**Results:** The overall median age was 49 years (range, 10–74 years). The female-to-male ratio was 1:1.68. There were 9 (3 unifocal, 6 multifocal) patients with primary bone lymphoma (PBL). The vertebrae was the most frequent site involved. Diffuse large B-cell lymphoma was the most common histology in these patients accounting for 45.1%. Thirty-eight patients (74.5%) presented with Ann-Arbor Stage III/IV. Fifteen patients (29.4%) had radiotherapy for at least one skeletal site of involvement. Thirty patients (58.8%) had radiotherapy in systemic management. The 5-year PFS and OS for patients with bone involvement was 71.0% and 76.5%, respectively. We have not yet identified important unfavourable prognostic factors for neither PFS nor OS using multivariate analysis.

**Conclusions:** Radiotherapy was usually needed for consolidation treatment in lymphoma. The potential for long-term survival suggests that additional radiotherapy for the site of skeleton involvement has the best chance of long-term success.

### 421
### NON HODGKIN LYMPHOMA INVOLVING THE HEART: A POLISH LYMPHOMA RESEARCH GROUP MULTICENTER RETROSPECTIVE ANALYSIS

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**Introduction**: Primary cardiac lymphoma (PCL) is defined as isolated infiltration of myocardium and/or pericardium. It is a rare entity accounting for 1% of primary cardiac tumours and 0.5% of extranodal lymphomas. The term of secondary cardiac lymphoma (SCL) denotes the infiltration of the heart in the course of disseminated lymphoma. At autopsy, up to 20% of such patients have evidence of cardiac involvement. The aim of our study was to analyze clinical course, radiological, laboratory findings in patients with NHL involving the heart

**Methods**: Retrospective analysis of patients with NHL presenting cardiac involvement, who were treated in the years 2010–2015 in 7 hematology centers from the Polish Lymphoma Research Group.

**Results**: We included 13 patients:-F/M 5/8, with the median age 58 (range 27–85) years. Histopathology revealed the diagnosis of B-cell lymphomas in all patients with a prevalence of PMBCL. Only one patient met criteria of PCL, the rest was classified as SCL. Noteworthy, in one patient we diagnosed SCL after autopsy. Clinical manifestations were dyspnoea, oedema, chest pain, arrhythmia. In most cases, troponin I and pro-BNP remained in normal limits. ECG was non contributory. In echocardiography, CT and MRI the most frequent findings were pericardial effusion and the infiltration of the wall of heart. In eight patients, we found involvement of the myocardium, presenting as either tumour mass or intramural infiltration. Tumours were found in the following locations: RA in five patients, RV in four patients and LV in one patient. Pericardial effusion was confirmed in seven patients. Moreover, in four patients, there was also infiltration of vena cava superior, pulmonary artery and aorta. It is noteworthy that, in nine patients, we confirmed multiple localizations. At the beginning, three patients underwent surgery including pericardiocentesis and one patient subtotal resection of tumour with reconstruction of RA. The rest of the patients were initially treated with anthracycline-based immunochemotherapy with further chemotherapy (ChT) if necessary. In eight patients, we confirmed at least PR of heart involvement after the first-line CHT, independently of the response in other places. Interestingly, one patient received rituximab intrapericardially and intrapleuraly. Additionally, four patients were treated with radiotherapy. Until now, after the median follow-up of 23 (range 1–65) months, 11 patients have been alive with 7 patients in CR. Two patients died: one because of cardiac involvement and the other one from reasons unrelated to cardiac involvement.

**Conclusions**: Cardiac involvement by lymphoma is often characterized by nonspecific clinical symptoms, and the intensity of manifestation is usually inadequate to the degree of the infiltration, therefore the diagnosis of SCL is often delayed. Moreover, the count of diagnosed cardiac lymphomas seems to be underestimated. Cardiac involvement is usually moderately sensitive for immunochemotherapy and in most cases does not lead to death.

### 422
### TESTICULAR DIFFUSE LARGE B-CELL LYMPHOMAS IN SOUTHERN FINLAND—A RETROSPECTIVE STUDY OF 91 CASES

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**Introduction:** Testicular lymphomas, consisting mainly of aggressive diffuse large B-cell lymphomas (DLBCLs), are rare malignancies affecting mostly elderly men.

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Testicular affision is associated with increased risk of central nervous system (CNS) progression, and eligible patients commonly receive either CNS targeted or systemically administered methotrexate or AraC as prophylaxis. However, little is known about the clinical and biological risk factors of testicular DLBCLs.

**Methods:** We searched for pathology databases at three Southern Finland University Hospitals for testicular DLBCLs. The clinical data was collected, and a tissue microarray (TMA) from the diagnostic tumour samples was assembled. Lymphoma diagnosis was reviewed according to the current WHO classification, and putative biological risk factors analyzed by immunohistochemistry.

**Results:** Clinical data was available from 91 patients. Median age at diagnosis was 70 years (range 37–92 years). The disease was limited to the testis in 35 patients according to standard staging procedures. Both testes were affected at primary diagnosis in 10% of the patients. For the entire cohort, 5-year progression-free (PFS), disease-specific (DSS), and overall survival (OS) rates were 49%, 63%, and 54%, respectively. High IPI score (≥3) was seen in 29% of the patients and it correlated with adverse outcome (5-year DSS 18% vs 84%, $p < 0.001$). Seventy-five (78%) patients were treated with curative intent; 35 with CHOP-like regimen before the rituximab (R) era, and 40 with R-CHOP or R-CHOP-like chemotherapy. Nine patients were not fit for chemotherapy. Seven Stage I patients were followed, only one of whom was alive and in remission at the date of last follow-up (29 months). Rituximab improved outcome (5-year DSS 71% vs 55%, $p = 0.046$). CNS prophylaxis was given to 47% of the patients, which was associated with better prognosis (5-year DSS 78% vs 48%, $p = 0.004$). The effect was not dependent on the age of the patient or the use of rituximab in the treatment regimen. Contralateral testis was prophylactically treated by either radiotherapy or orchiectomy in 29% of the patients, resulting in a trend towards improved survival (5-year DSS 79% vs 58%, $p = 0.124$).

Thus far, we have reviewed the diagnosis of 49 tumours. According to the Hans algorithm, 34 of these were non-GCB subtype. Additionally, four tumours expressed MUM1 indicating the activated B-cell like phenotype. BCL-6 expression was rare. BCL-2 was expressed in 39 of the tumours with a trend towards poor prognosis (5-year DSS 63% vs. 90%, $p = 0.068$).

**Conclusions:** We present a relatively large cohort of testicular DLBCL patients. The results support the use of CNS prophylaxis and prophylactic treatment of contralateral testis. Preliminary results that suggest biological factors can provide additional prognostic value in testicular DLBCL.

### 423
### GASTROINTESTINAL TRACT POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER AFTER KIDNEY TRANSPLANT: A 17-YEAR BRAZILIAN SINGLE-CENTRE EXPERIENCE

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**Background:** Post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication following both solid organ and hematopoietic stem cell transplantation. This condition is characterized by the development of lymphoid or plasma cell neoplasms in the setting of post-transplant immunosuppression. Epstein–Barr virus is considered the most important causative factor. Gastrointestinal (GI) involvement of PTLD is a relatively common life-threatening condition, usually associated with aggressive presentation and high mortality rates.

**Methods:** From 1998 to 2014, 11 284 kidney transplants were performed at Hospital do Rim e Hipertensão/UNIFESP. We retrospectively analyzed cases of PTLD with GI tract involvement diagnosed over the past 17 years. Only confirmed cases of PTLD with available clinical and epidemiological data were included.

**Results:** PTLD was reported in 94 recipients. Two patients were excluded from analysis. GI tract involvement occurred in 39% (37 patients). The median age at diagnoses was 41 (range 4 to 64). The M:F ratio was 1.92:1. The median time from transplant to PTLD was 57 months. Twelve (32%) patients received induction therapy with antithymocyte globulin (ATG) (50%) or Basiliximab (50%) at the time of transplant. The immunosuppressive regimen the patients were receiving at the time of diagnosis of PTLD were: Prednisone (100%), azathioprine (88%), tacrolimus (62%), cyclosporine (37%), mycophenolate mofetil (8%) and 1 patient received FTY720. Regarding EBV, 28 patients (76%) were EBV positive, 4 patients (11%) were EBV negative and 5 patients (13%) were not tested or had inconclusive results. Monomorphic PTLD was present in 32 patients (86%), the majority (90%) diagnosed as diffuse large B-cell lymphoma. Polymorphic PTLD corresponded to only 4 cases (11%) and 1 patient could not be classified. Eight (22%) patients had multiple gastrointestinal tract involvement, 11 (31%) patients had only small intestine involvement, followed by 9 (24%) patients with stomach involvement, 8 (22%) with hepatic involvement and 1 patient had esophageal involvement. Seventeen (46%) had concomitant non GI-tract extranodal involvement. At diagnosis, all patients had their immunosuppression reduced or suspended. Twenty-eight (76%) patients received anti-CD20-based therapy with 21 (75%) patients receiving R-CHOP; 5 patients (13.5%) did not receive chemo/immunotherapy, 11 patients (30%) required surgery (gastrectomy or enterectomy) and in 4 (11%) cases surgery was the only treatment. Nineteen (51%) of our patients achieved CR. Seventeen patients (46%) died, most of them (10) from infection, three patients due to GI perforation, two patients from pulmonary embolism and two from undetermined causes. At total, 6 (16%) cases of GI perforation were observed.

**Conclusion:** In our study, GI tract was the most common involved site. EBV was positive in the majority of cases. Although 19 patients achieved CR, a high number of deaths occurred, mostly due to infection. PTLD should be considered in every transplant recipient presenting with abdominal symptoms.

## T-CELL

### 424
### EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA IN WEST OF SCOTLAND: HIGHLY EFFECTIVE CHEMORADIATION IN LOCALISED DISEASE

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**Introduction:** Extranodal natural killer/T-cell lymphoma is a rare type of NHL occurring most commonly in nasal cavity and sinuses. It accounts for only 1% of NHL in Europe and North America, and the limited clinical experience in most centres of this aggressive condition has led to a variety of treatment approaches rather than standardized protocols. We report the treatment and outcome for all such patients recorded in our cancer network over a ten year period.

**Methods:** The West of Scotland Haemato-oncology network covers a population of 2.6 million. We collected demographics and treatment data on all NK/T-cell NHL patients diagnosed from 2004 to 2014. Pathology was reviewed centrally by experienced haematopathologists. Site and stage of disease and use of radiotherapy with dose, fractionation and field details were recorded together with chemotherapy regimen, number of cycles and timing relative to radiation. Outcome was assessed as time from diagnosis to death or progression-free survival for those in continuing remission.

**Results:** There were 14 confirmed cases: 12 men, 2 women aged 29–90 years (median 53). Two patients presented with disseminated disease, two more had skin nodules on the legs and one had testicular disease. The remaining 9 had lymphoma arising in nasal cavity, antra, palate or oropharynx. Thirteen patients received chemotherapy: six CHOP, one SMILE, two ESHAP, three VIPD and one Gianni. Of note, six patients with localized Stage I/II disease were prescribed radiotherapy to 50 Gy or above with concurrent chemotherapy (cisplatin and in one also pegylated asparaginase) followed by consolidation ESHAP or VIPD. Of these, five remain in remission at 12–80 months from diagnosis (median 34 months). One of these six patients died of infection but in remission. The 90-year-old received palliative radiotherapy and survived for 15 months. One patient treated with only 40 Gy relapsed and died at 9 months. The 7 patients who were treated with chemotherapy alone for advanced-stage disease all died between 3 and 8 months from diagnosis.

**Conclusion:** In NK/T-cell lymphoma, there are little trial data to guide treatment protocols but there is increasing evidence that patients with early-stage disease can be cured. Chemotherapy produced poor outcomes in advanced-stage disease. Stage

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I/II patients treated with high-dose radiation with concurrent cisplatin/asparaginase and followed by VIPD or ESHAP had 100% disease-free survival.

### 425
### PHASE II STUDY OF CONCURRENT CHEMORADIOTHERAPY FOLLOWED BY PEG-ASP-GDP FOR LOCALIZED NASAL NK/T-CELL LYMPHOMA: A PROSPECTIVE SINGLE-CENTER CLINICAL TRIAL

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**Introduction:** Extranodal NK/T-cell lymphoma (NKTCL) is an Epstein–Barr virus (EBV)-associated lymphoid malignancy, which accounts for 3% to 10% of malignant lymphomas in East Asia. Two-thirds of patients have Stage IE or IIE in the nasal cavity and its adjacent site. The standard therapy for newly diagnosed localized nasal NKTCL remains to be established. Radiotherapy (RT) is an effective treatment, but RT alone is not sufficient, with 5-year overall survival ranging from 30% to 40%. Concurrent chemoradiotherapy (CRT) is expected to improve local and systemic disease control and has been reported by some prospective trials for NKTCL. The purpose of the trial is to explore a more effective and safe treatment for localized NKTCL.

**Methods:** Patients with a newly diagnosed Stage IE or IIE NKTCL with cervical node involvement and a performance status of 0 to 2 were eligible for enrollment. Treatment comprised concurrent radiotherapy (56 Gy in 27 fractions) and five courses of cisplatin (DDP, 25 mg/m$^2$, intravenous, weekly) followed by three courses of Peg-Asp-GDP. The drug dose and administration schedule were as follows: pegaspargase, 2500 IU/m$^2$ (cap 3750 IU) intramuscular on Day 4; gemcitabine, 850 mg/m$^2$ intravenous on Days 1 and 8; cisplatin, 20 mg/m$^2$ intravenous on Days 1–3; and dexamethasone, 40 mg/day intravenous on Days 1–4. Chemotherapy was planned to repeat every 3 weeks. All patients were treated with a photon beam of 6MV. Extended involved-field intensity-modulated radiation (IMRT) planning was used. The primary end point was a 2-year survival.

**Results:** To February 2015, a total 16 patients were enrolled in the trial. The median age of patients was 36 years (range: 19–68 years), and female-to-male ratio was 1:1.6. Ten patients had Ann Arbor Stage IE, 6 patients had Stage IIE, and 13 patients had B symptoms. All patients were assessable for response, 15 (93.7%) achieved complete response, with one progressive disease. The most common Grade 3 nonhematologic toxicity was mucositis related to radiation (3/16, 18.75%). Of 16 patients, six (37.5%) experienced Grade 3 neutropenia, and 1 of 16 patients (6.25%) experienced Grade 4 neutropenia. Of 16 patients, 2 (12.5%) experienced Grade 3 and 4 thrombocytopenia. No treatment-related deaths were observed.

**Conclusions:** The preliminary data suggest concurrent chemoradiotherapy with cisplatin followed by Peg-Asp-GDP is an effective and safe treatment for localized nasal NKTCL and warrants further investigation.

### 426
### CD56-NEGATIVE EXTRANODAL NK/T CELL LYMPHOMA SHOULD BE REGARDED AS A DISTINCT SUBTYPE WITH POOR PROGNOSIS

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**Introduction:** The majority cases of extranodal NK/T-cell lymphoma (ENKTL) have a natural killer cell origin, and a small minority has clonal T-cell receptor rearrangements and appears to be derived from cytotoxic T cells, which usually featured CD56-negative expression. Previous results about the clinical and prognostic significance concerning CD56 expression status are controversial due to small sample size and the heterogeneity nature of this disease.

**Methods:** The complete data of 288 patients with early stage upper aerodigestive tract ENKTL were retrospectively reviewed.

**Results:** One hundred and eighty-three patients (63.5%) had Stage I disease, and the primary tumour site of 204 patients (70.8%) was in nasal cavity. Sixty patients (20.8%) were categorized to the CD56-negative ENKTL group. There were more patients with a primary tumour site in the extranasal upper aerodigestive tract (43.3% vs. 25.4%, $p = 0.011$) and poor ECOG performance score (8.4% vs. 2.2%, $p = 0.036$) in the CD56-negative group. There were no significant correlations between CD56 expression status and gender, age, lactate dehydrogenase (LDH) level, Ann-Arbor stage, B symptoms and IPI score ($p > 0.05$). At a median follow-up time of 69 months, the 5-year and 10-year PFS rate were 52% and 41% respectively, and the 5-year and 10-year OS rate were 69% and 68% respectively. Patients with primary tumour site located in the nasal cavity or with CD56-positive expression had significantly superior PFS and OS ($p < 0.05$). In multivariate Cox regression model that included age, Ann Arbor stage, LDH level, primary tumour site, and CD56 expression status, all these five factors remained to be independent prognostic factors. In subgroup analysis according to primary tumour site location, CD56 expression status significantly correlated with survival outcomes in patients with primary nasal cavity involvement, however, it lost the prognostic value in patients with primary extranasal upper aerodigestive tract involvement ($p > 0.05$).

**Conclusions:** In this largest cohort of early-stage ENKTL ever reported, we found that CD56-negative ENKTL had significantly inferior survival outcomes, indicating CD56-negative ENKTL should be regarded as a distinct phenotype and optimal treatment strategies need to be evaluated further for this entity.

### 427
### EARLY NEGATIVE CIRCULATING EBV DNA IS A PREDICTION OF SURVIVAL IN EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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**Background:** Predictive tumour markers are essential for extranodal NK/T cell lymphoma, nasal type (ENKL), which pursues an aggressive clinical course with poorer prognosis. This prospective study was conducted to evaluate the dynamic monitoring value of circulating EBV DNA concentration for the prediction of ENKL.

**Methods:** From Jan 2006 to Aug 2012, plasma samples from 113 ENKL patients were collected before and/or every two cycles of chemotherapies for circulating EBV DNA measurement by a real-time PCR assay.

**Results:** The positive rate of circulating EBV DNA was 61.9% (70/113) with a median concentration of $1.21 \times 10^3$ copies/ml. After two cycles of chemotherapies, 45 patients were tested for circulating EBV-DNA, and 53.33% (24/45) patients became EBV-DNA-negative candidates. There were 87.5% (21/24) patients who obtained complete remission at the end of the treatment, compared with 42.86% (9/21) who are still in the EBV-DNA-positive groups ($p = 0.002$). There was a tendency that the patients whose circulating EBV-DNA became negative after two cycles would have better prognosis (5-year OS: 75% vs 46%, $p = 0.074$). The similar situation of CR rate and OS happened in the EBV-DNA detection of completion of total chemotherapies. The more advance of the stage, the higher concentration of EBV-DNA was found. When the positive group was divided into low-dose and high-dose ones depending on the cut-off value of $2 \times 10^4$ copies/ml, the CR rate was much lower and the 5-year OS was significantly better in the high-dose group than in the low-dose group and the negative group. Of 13 relapsed patients, 17 had $>1 \times 10^3$ copies/ml EBV-DNA at the time of recurrence, and the survival outcome was dismal for them compared to that of the other six patients with $\leq 1 \times 10^3$ copies/ml (5-year OS: 0% vs 80%, $p = 0.001$).

**Conclusions:** Circulating EBV-DNA level can predict the efficacy of treatment as a dynamic marker of ENKL. Patients with early positive detection of EBV-DNA after two cycles of chemotherapy may receive more aggressive treatments.

### 428
### EXTRANODAL NK/T CELL LYMPHOMA, NASAL TYPE: A REPORT OF 18 HUNGARIAN CASES

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**Introduction:** Extranodal natural killer NK/T cell lymphoma, nasal type (ENKTL) is a distinct clinicopathological entity according to classification of World Health Organization (WHO). ENKTL is described by vascular damage, destruction and necrosis predominantly in the nasal cavity and upper aerodigestive tract. ENKTL shows association with Epstein–Barr virus (EBV) infection and cytotoxic phenotype. It originates from NK cells, some cases show cytotoxic T-cell phenotype. It has a unique geographic distribution with lower incidence in Western countries. The prognosis is variable, early-stage, localized nasal disease is curable with radiotherapy and chemotherapy, but the optimal dose and combination of radiotherapy and chemotherapy are still undefined. The chemotherapy is often ineffective because of expressing the multidrug resistant (MDR) P-glycoprotein by tumour cells. The disseminated and extranasal disease is highly aggressive.

**Methods:** Authors retrospectively analysed records of 16 patients (nine male and seven female) diagnosed with primary ENKTL nasal type and two patients (one male and one female) diagnosed with extranasal disease in three Hungarian university hematological centres between 2000 and 2013.

**Results:** The median age of patients at diagnosis was 49, 5 year. Complete staging data were available for 16 patients: 9 Stage I/II and 7 Stage III/IV. Therapy was known for 15 patients, 1 patient died of severe pneumonia before starting chemotherapy. Eight patients were treated according to CHOP regimen (in 6 cases followed by radiotherapy), 2 patients with Hyper-CVAD, 1 patient received ProMACE-MOPP, 1 patient MegaCEOP and 1 patient SMILE. Two patients had palliative treatment with steroid and cyclophosphamide. Nine patients (60%) responded to initial treatment (six complete remission and three partial remission), four (26.6%) were nonresponders (NR), and two patients with extranasal disease (13.3%) had stable disease. Only in one patient was performed high-dose chemotherapy with autologous stem cell support after first complete response. Three patients died despite of second (Hyper-CVAD, VIM, BFM) and third -line chemotherapy (DHAP, ESHAP, VIPD).

**Conclusions:** ENKTL is an aggressive type of lymphomas and represents a rare disease in Western countries. There is few cases and extensive clinical trials which can improve efficiently the response to treatment. It is described that newly devised non-P-glycoprotein efflux medications help to improve survival but the clinical trial is in short supply. Multinational, multicenter trials should be to have definitive therapy for ENKTL.

### 429
### CLINICAL FEATURES AND OUTCOMES OF PATIENTS WITH EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE: A PROSPECTIVE MULTICENTER STUDY FROM THE THAI LYMPHOMA REGISTRY

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**Background**: Extranodal NK/T-cell lymphoma (ENKTL), nasal type is a rare subtype of lymphoma with a strong geographic predilection for Asian and South American populations. This study aims to describe the clinical features and treatment outcomes of Thai patients with ENKTL, nasal type.

**Patients and Methods**: A clinical review of patients with ENKTL, nasal type according to WHO 2008 classification obtained from The Thai Lymphoma Registry between 2002 and 2014 was performed.

**Results**: Among 4019 cases of lymphoma, there were 81 cases (2%) of ENKTL, nasal type. The median age was 45 years old (range, 21–91). The male : female ratio was 2.5:1. At diagnosis, approximately half of the patients had Stage I/II disease (55.6%), International Prognostic Index (IPI) score of ≤1 (51.9%), B symptoms (54.0%), and elevated serum LDH (54.0%). A majority of the patients (92.6%) presented with good ECOG performance status. The patients were treated with chemoradiotherapy in 34.6%, chemotherapy alone in 35.8%, and radiotherapy alone in 11.1%. Fifteen patients (18.5%) did not receive any treatment. The most common first-line induction chemotherapy regimen was CHOP (92.9%). Of the 66 evaluable patients, the overall complete remission (CR) rate and overall partial remission (PR) rate was 39.4% and 6.1%, respectively. The CR rate was achieved in 71.4%, 27.6%, and 22.2% of the cases treated with chemoradiotherapy, chemotherapy alone, and radiotherapy alone, respectively. With the median follow-up time of 9.7 months (range, 0.5–316.2), the median overall survival (OS) and progression-free survival (PFS) for the whole group was 10.8 months (95% CI: 0.2–21.5) and 8.2 months (95% CI: 3.0–13.5), respectively. The median OS of the patients treated with chemoradiotherapy was significantly higher than those treated with chemotherapy alone [5.8 years (95% CI: 1.2–10.4) vs. 0.4 year (95% CI: 0.1–0.7); $p = 0.002$] and radiotherapy alone [5.8 years (95% CI: 1.2–10.4) vs. 0.3 year (95% CI: 0.1–0.5); $p = 0.021$]. The median PFS of the patients treated with chemoradiotherapy was significantly higher than those treated with chemotherapy alone [17.1 months (95% CI: 8.7–25.5) vs. 4.1 months (95% CI: 2.2–5.9); $p = 0.014$] and radiotherapy alone [17.1 months (95% CI: 8.7–25.5) vs. 3.1 months (95% CI: 1.6–4.6); $p = 0.024$]. Furthermore, the IPI score was of prognostic significance to predict the median OS [IPI score = 0 (6.4 years) vs. IPI score ≥ 1 (0.4 year); $p = 0.016$] and 5-year OS [IPI score = 0 (56.9%, 95% CI: 30.8–76.3) vs. IPI score ≥ 1 (25.2%, 95% CI: 14.5–37.5); $p = 0.012$] in this cohort.

**Conclusion:** ENKTL, nasal type is rare among Thai population. At presentation, approximately half of the patients had early-stage diseases, B symptoms, elevated serum LDH, and low IPI score. Chemoradiotherapy was the most effective treatment. In this cohort, the IPI score significantly predicted the OS.

### 430
### A MODIFIED-INTERNATIONAL PROGNOSTIC INDEX INCLUDING PRETREATMENT HEMOGLOBIN LEVEL FOR EARLY STAGE EXTRANODAL NK/T CELL LYMPHOMA

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**Introduction:** Anemia is a common finding in cancer patients, even before patients have received radiotherapy or chemotherapy and even when there is no bone marrow infiltration. Recent studies have found that pretreatment anemia status is correlated with shorter survival in patients with several hematological malignancies. However, no literatures about the relationship between pretreatment hemoglobin level and survival outcomes in patients of extranodal NK/T cell lymphoma, nasal type (ENKTL) have ever been published.

**Methods:** We retrospectively investigated the prognostic role of pretreatment hemoglobin level in 352 patients with Stage I/II ENKTL.

**Results:** Of 352 patients, 67% had Stage I disease, and 79.8% had international prognostic index (IPI) scores between 0 to 1. There were no significant correlations between baseline hemoglobin level and age, ECOG performance status score, lactate dehydrogenase (LDH) level, and Ann-Arbor stage. The complete response rate in

**Abs 430 - Table 1.** Survival outcomes according to Risk Group based on the modified-IPI model

| Risk group | No. of adverse factors* | No. of patients (%) | 5-year PFS rate | 5-year OS rate |
|---|---|---|---|---|
| Group 1 | 0 | 76 (21.6) | 74.4% | 90.6% |
| Group 2 | 1–2 | 212 (60.2) | 47.0% | 61.8% |
| Group 3 | ≥3 | 64 (18.2) | 20.1% | 33.4% |

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patients with anemia was 64.7%, slightly lower than that in patients without anemia (75.6%, $p = 0.076$). The overall response rate in both groups were similar (84.1% and 88.7%, $p > 0.05$). At a median follow-up time of 83.8 months, the 5-year and 10-year PFS rate were 48.0% and 40.0%, respectively, and the 5-year and 10-year OS rate were 63.0% and 60.0%, respectively. Patients with pretreatment hemoglobin level < 120 g/L had significantly inferior PFS and OS than those with hemoglobin level ≥ 120 g/L ($p < 0.05$). In a multivariate Cox regression model, age, ECOG performance status, LDH level, Ann-Arbor stage, and pretreatment hemoglobin level were all independent prognostic factors for PFS and OS ($p < 0.05$). Using these five parameters, a modified-IPI model (mIPI) was constructed and three prognostic groups were classified: group 1, no adverse factors; group 2, one or two factor; group 3, three or more factors. This mIPI could categorize three groups with significantly different PFS and OS (both $p < 0.0001$). Using the cohort of patients who received asparaginase-based therapy as a validation set, this mIPI retained its prognostic value.

**Conclusions:** This large-cohort retrospective study confirmed the independent prognostic role of pretreatment hemoglobin level in early stage ENKTL, and a newly modified IPI including pretreatment hemoglobin level could be used to further optimize treatments for patients with Stage I/II ENKTL, even in the era of asparaginase.

Abbreviations: PFS, progression-free survival; OS, overall survival.

### 431
### A NOVEL AND SIGNIFICANT PREDICTOR IN EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA, NASAL TYPE: THE PROGNOSTIC NUTRITIONAL INDEX (PNI)

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**Introduction:** Extranodal NK/T-cell lymphoma, nasal type (ENKTL) is an aggressive disease with a poor prognosis. The prognostic nutritional index (PNI) is reported to be associated with survival in several types of tumours. The prognostic value of PNI in lymphoma remains unclear. The aim of the present study is to evaluate the prognostic significance of PNI in patients with ENKTL.

**Methods:** 157 patients with newly diagnosed ENKTL were retrospectively evaluated between August 2000 and October 2011 at the Sun Yat-sen University Cancer Center. Patients in whom the combined albumin (g/l) +5 × total lymphocyte count × $10^9$/l ≥45 were allocated a PNI score of 0. Patients in whom this total was <45 were allocated a score of 1. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan–Meier method. The significance of differences between survival curves was tested using the log-rank test. Significant variables in the univariate analysis were considered variables for the multivariate survival analysis, which was performed using Cox regression mode.

**Results:** Forty-nine patients (31.2%) had an abnormal PNI (PNI = 1).

After a median follow-up duration of 31.0 months, an estimated 5-year OS and PFS rate in 157 patients was 58.4% and 39.4%, respectively. Patients with pretreatment PNI score = 1 had lower complete remission rates ($p = 0.018$) and worse OS (5-year OS: 71.7% vs 21.8.0%, $p < 0.001$) and PFS ($p < 0.001$) compared with PNI score = 0 patients. Using the International Prognostic Index (IPI) and peripheral T-cell lymphoma (PIT) scoring systems, more than 70% of all cases were in the low-risk category (with no or one adverse factor), but these two prognostic models failed to differentiate between patients with different outcomes in the low-risk group. PNI could differentiate low-risk patients using IPI and PIT scoring (all $p < 0.05$).

The Korean Prognostic Index (KPI) model balanced distribution of patients into different risk groups better than the IPI and PIT models. However, the KPI model also failed to significantly differentiate between patients with different outcomes in low-risk and low intermediate-risk groups ($p = 0.859$). PNI also helped to differentiate between patients with different prognosis in low-risk and low intermediate-risk groups ($p = 0.000$). However, the KPI prognostic model failed to show significant prognostic value among patients with PNI = 0 ($p = 0.646$) or among the patients with PNI = 1 ($p = 0.115$).

**Conclusion:** PNI is an independent predictor of survival in ENKTL and is superior to IPI, PIT and KPI.

### 432
### OUTCOMES OF PERIPHERAL T-CELL LYMPHOMA IN THE CURRENT ERA: 10 YEAR EXPERIENCE AT THE ROYAL MARSDEN AND CHRISTIE HOSPITALS

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**Introduction:** Peripheral T-cell lymphoma (PTCL) is a rare and heterogeneous group of non-Hodgkin lymphomas (NHL) comprising ~10% of cases. Combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) is frequently used first line, except for ALK-positive anaplastic large-cell lymphoma; long-term outcomes are historically poor with 5-year overall survival (OS) rates of ~36% reported following CHOP. We retrospectively assessed outcomes following first-line chemotherapy +/− stem cell transplantation (SCT) for patients with PTCL treated at the Royal Marsden (RM) and Christie Hospitals (CH) over a 10-year period.

**Methods:** Patients aged ≥18 years with PTCL treated at RM and CH between 1 January 2002 and 31 January 2012 were identified from hospital databases and included if they had received one or more cycle of first-line chemotherapy +/− SCT. Cases of precursor T-cell malignancies, mycosis fungoides or adult T-cell leukaemia/lymphoma were excluded, as was cutaneous T-cell lymphoma not requiring combination chemotherapy. Clinical data were collated from electronic patient records. The diagnosis of PTCL was confirmed in all cases by an expert haematopathologist. The study was approved by our institutional review board. Response was assessed using the IWG 1999 response criteria. OS and progression-free survival (PFS) were calculated from diagnosis and analysed using the Kaplan–Meier method and Cox regression model.

**Results:** A total of 143 (RM $n = 69$, CH $n = 74$) PTCL patients were included with a median follow-up of 63.4 months. The median age at diagnosis was 59 years (range 18–89 years). First-line chemotherapy included CHOP ($n = 97$), gemcitabine ($n = 23$) or etoposide containing chemotherapy ($n = 7$), asparaginase ($n = 2$) and other ($n = 14$). For all patients the objective (ORR) and complete (CR) response rates on completion of chemotherapy were 81% and 41% respectively. When CHOP was compared with gemcitabine-based first-line treatment, the ORR was 80% in each group ($p = 0.963$), with CR rates of 46% and 35% ($p = 0.375$), respectively. For the entire cohort, the 5-year OS was 39.6% and 5-year PFS was 20.4%. The 5-year OS and PFS for CHOP versus gemcitabine-based induction were 41.2% and 34.3% ($p = 0.309$) and 25.5% and 14.5% ($p = 0.184$), respectively. Autologous SCT (autoSCT) in first remission (CR1) was performed in 15.3% of cases ($n = 22$) and was associated with superior 5-year OS (autoSCT 65.8% vs no autoSCT 35.4%; $p = 0.017$) and PFS (54.5% autoSCT vs no autoSCT 14.7%; $p < 0.001$) in this unmatched patient series.

**Conclusion:** Outcomes following first-line treatment for PTCL OS and PFS remain disappointing and more effective first-line treatments are urgently required. To this end, the ongoing UK NCRI randomized Phase II CHEMO-T study compares CHOP with a gemcitabine-containing and cisplatin-containing regimen (GEM-P) in the first-line treatment of PTCL. Autologous SCT in CR1 may offer a survival benefit for eligible patients.

### 433
### PERIPHERAL T-CELL LYMPHOMA-UNSPECIFIED: LONG-TERM OUTCOME OF ADRIAMYCIN AND ETOPOSIDE CONTAINING COMBINED CHEMOTHERAPY

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**Introduction:** Peripheral T cell lymphomas are a heterogeneous group of rare malignancies derived from mature T cells. The most frequent entities according to the WHO classification are peripheral T cell lymphoma not otherwise specified (PTCL-NOS), accounting for 4.25% of all non-Hodgkin lymphomas in China. Compared with aggressive B-cell non-Hodgkin lymphomas, Long-term survival of PTCL-NOS with standard CHOP (Cyclophosphamide, doxorubicin, vincristine, prednisone) or CHOP-like protocols is unsatisfactory, and a median 5-year overall survival (OS) of only 20–30%. At present, there is no standard first-line therapy. This prospective study evaluated the efficacy and safety of adriamycin and etoposide containing combined chemotherapy (CHOEPP) for PTCL-NOS.

**Methods:** This study examines the outcome of 39 patients with PTCL-NOS over a 10-year period at a single institution. Patients were eligible for enrollment between Jan 2004 and Dec 2013. Patients received combined chemotherapy CHOEPP (cyclophosphamide 750 mg/m$^2$ on Day 1, doxorubicin 50 mg/m$^2$ on Day 1, vincristine 1.4 mg/m$^2$ on Day 1, cisplatin 20 mg/m$^2$ on Days 8–12, etoposide 60 mg/m$^2$ on Days 8–10, and prednisone 60 mg on Days 1–5 and 8–12 of a 21-day cycle for up to eight cycles).

**Results:** In total of 39 patients with PTCL-NOS were enrolled, and 77% were newly diagnosed. These patients were 27 male and 12 female with a median age of 45 years (range 14–73 years). The majority (67%) presented with nodal disease, but extranodal disease was present in 33%. Twelve patients (31%) had B symptom, International Prognostic Index ≥2 (82%), and Stage III or IV disease (90%). Six patients (15%) had bone marrow involvement. All patients (100%) underwent chemotherapy, whereas only 10% underwent hematopoietic stem cell transplantation and 3% underwent radiation. Thirty-nine patients completed 191 cycles of chemotherapy, with a median cycle of 5 (range 1–8 cycles). The overall response rate was 92.3% (36/39) with 58.9% complete responses and 33.3% partial responses. OS at 5, 10-year of PTCL-NOS patients treated with CHOEPP was 18% and 12%, respectively. The most common adverse event was bone marrow suppression. Adverse events included 43.6% (17/39) Grade 3–4 neutropenia and 10.3% (4/39) Grade 3–4 thrombocytopenia. Adverse events in the digestive tract were mild and only two were Grade 2, nausea and vomiting. All patients had no treatment-related deaths.

**Conclusions:** Combined chemotherapy CHOEPP in PTCL-NOS was well tolerated, and the overall response rate was increased than CHOP alone, but the increasing the types and dose density of drugs did not improve the long-term survival. Further clinical studies are needed to determine optimal treatments.

## 434
## DOSE-SPARING REGIMEN OF ROMIDEPSIN IN CUTANEOUS T-CELL LYMPHOMA PATIENTS WITH DURABLE RESPONSE

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**Introduction:** Cutaneous T-cell lymphoma (CTCL) is a heterogeneous primary cutaneous malignancy that may have a chronic, relapsing course, with patients frequently requiring multiple, consecutive therapies for palliation of symptoms. Romidepsin is a histone deacetylase (HDAC) inhibitor that has shown activity in the treatment of T-cell lymphomas in pre-clinical studies and in two early-phase clinical trials. We observed that our patients could often maintain a good response for long periods while on a reduced schedule maintenance phase with treatments given less often than the recommended 3 weeks on, 1 week off cycles. We retrospectively reviewed our experience with romidepsin in CTCL to establish an ideal schedule to achieve optimum results with fewer adverse events and allow prolonged maintenance.

**Methods:** This is a single center retrospective chart review of CTCL patients treated at Northwestern University and that had received romidepsin. Clinical, pathological and hematological data was assessed.

**Results:** Forty-seven patients (22 females and 25 males) with a median age of 64 years (21–87) were identified. Twenty-four patients were affected with mycosis fungoides, 15 with Sézary syndrome, and 8 with other CTCL variants. The median follow-up from the time romidepsin was initiated was 10.2 months (1–42.8). Patients had received an average of four treatments (1–9) preceding romidepsin. Median treatment period with romidepsin was 3.77 months (0.2–30.6). Seventeen patients received concurrent treatments with romidepsin; including radiation therapy, systemic steroids and phototherapy. Twelve per cent of the patients withdrew romidepsin due to side effects, mostly fatigue. Fifteen patients received romidepsin for more than 6 months, with an average of 18.1 months (6.8–30.6). Among these patients, five were on a dose-sparing schedule tapering from biweekly to monthly doses (12–14 mg/m$^2$), and three were initiated on monthly doses once complete remission or partial response was achieved. The average period between starting romidepsin until dose-sparing schedule was 8.9 months (3.5–21). Four patients are still in complete remission, 2 achieved partial response and 1 is with stable disease after an average of 18.78 months. Eight patients had progressive disease and were switched to other treatments. Reduced-schedule was overall well tolerated with fewer side effects.

**Conclusions:** Patients with long-term response to romidepsin may benefit from dose-sparing regimens that allow keeping response while reducing toxicity with better tolerance to side effects. However, further studies are necessary to standardize the optimal dose-sparing regimen for these patients.

Limitations: This is a retrospective chart review with a small size sample.

## 435
## SEMI-SYSTEMATIC, QUALITATIVE REVIEW OF CURRENT AND FUTURE TREATMENT OPTIONS FOR RELAPSED/REFRACTORY (R/R) PERIPHERAL T-CELL LYMPHOMA

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**Introduction:** Most PTCLs, which arise from the clonal proliferation of mature postthymic T cells, tend to be aggressive and chemorefractory, with many patients (up to 70%) developing R/R disease following frontline induction/consolidation. To elucidate the treatment landscape for R/R PTCL, we conducted a literature review to explore the effectiveness of approved and investigational therapies in this setting.

**Methods:** A semi-systematic review was undertaken to identify studies reporting efficacy outcomes following conventional and investigational therapies in patients with R/R PTCL (defined according to the WHO 2008 classification). MEDLINE was searched for studies published up to October 2014, and bibliographies of recent systematic and treatment reviews (2011–2014) were searched manually. Conference proceedings from ASCO, ASH, ESMO, and EHA (2013–2014) were also examined. There were no restrictions regarding study design or treatment, although prospective trials were selected preferentially; frontline studies were excluded. Despite no formal analysis, data were extracted on study type, patients, diagnosis, prior treatments, and efficacy outcomes.

**Results:** Until recently, conventional systemic chemotherapies remained the only salvage options for patients with R/R PTCL, aside from stem cell transplantation. However, these regimens only improve median overall survival (OS) by ~1 month compared with palliation. Four novel drugs are approved in the USA for the treatment of R/R PTCL: pralatrexate, romidepsin, belinostat, and brentuximab vedotin [approved in systemic anaplastic large-cell lymphoma (sALCL) only]. Of these agents, the anti-CD30 antibody–drug conjugate brentuximab vedotin has exhibited the highest clinical activity [overall response rate (ORR) 86%, complete response (CR) rate 57%, median progression-free survival (PFS) 14.6 months, 3-year OS rate

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63% in a Phase 2 study in R/R sALCL]. ORRs with the other three approved agents range from 25% to 54% (CR rate 10–31%) in mixed R/R PTCL patient populations. Experimental agents that have been studied or are under investigation in R/R PTCL include alisertib, bendamustine, panobinostat, denileukin diftitox, alemtuzumab, lenalidomide, mogamulizumab, crizotinib, bortezomib, and plitidepsin. The most clinically advanced of these treatments are alisertib and mogamulizumab, which are currently being evaluated in Phase 3 trials in R/R PTCL and R/R CCR4+ adult T-cell leukemia/lymphoma (ATLL), respectively. Mogamulizumab has shown promising activity (ORR 50%, CR 31%, median PFS 5.2 months, median OS 13.7 months) with an acceptable safety profile in a Phase 2 study in R/R CCR4+ ATLL.

**Conclusions:** Over the past 10 years, there has been a transformation in the way that R/R PTCL is managed. With novel treatments, specific PTCL subtypes can be targeted. Further treatment options for R/R PTCL are likely to become available as large-scale trials report results.

**436**
**SEMI-SYSTEMATIC, QUALITATIVE REVIEW OF CURRENT AND FUTURE TREATMENT APPROACHES FOR RELAPSED/REFRACTORY PRIMARY CUTANEOUS T-CELL LYMPHOMA**

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**Introduction:** Primary CTCLs, such as mycosis fungoides and Sézary syndrome, are a rare subgroup of non-Hodgkin lymphomas derived from the malignant transformation of mature skin-homing/resident T cells. Initial treatment for CTCL typically involves a multimodal approach (surgery, radio/phototherapy, extracorporeal photopheresis, topical/systemic agents, hematopoietic transplantation). Unfortunately, many CTCL patients who receive frontline therapy will relapse and develop refractory disease. We undertook this literature review to explore the efficacy of treatment options for R/R CTCL.

**Methods:** A semi-systematic review was conducted to identify studies reporting on treatment outcomes in patients with R/R CTCL (WHO 2008 classification). MEDLINE was searched for studies published up to October 2014, and reference lists of recent review articles and meta-analyses (2011–2014) were investigated manually. Congress abstracts from ASCO, ASH, ESMO, and EHA (2013–2014) were also evaluated. Prospective trials were selected as the primary data sources; studies of previously untreated patients were excluded. Data were collected on study type, patients, diagnosis, treatment history, and efficacy.

**Results:** Treatment for R/R CTCL has historically relied on single-agent chemotherapies, as multi-agent therapy can lead to immunosuppression and poor tolerance. Chemotherapies associated with the highest overall response rates (ORRs) are gemcitabine (48–68%), pentostatin (14–71%), and pegylated liposomal doxorubicin (41–84%). Agents approved in the US for the treatment of R/R CTCL include the established oral retinoid bexarotene and the targeted histone deacetylase (HDAC) inhibitors romidepsin and vorinostat. Romidepsin and vorinostat have both been shown to produce responses in heavily pretreated CTCL patients (ORRs of 34% and 24–30%, respectively), while also alleviating bothersome symptoms, such as pruritus. Promising investigational therapies include the anti-CD30 antibody–drug conjugate brentuximab vedotin, the anti-CCR4 antibody mogamulizumab, and the fusion protein immunotoxin A–dmDT390-bisFv(UCHT1) (Resimmune®). Brentuximab vedotin, in particular, has shown high activity (ORR 71%, complete response rate 35%, median progression-free survival 20 months) in a Phase 2 trial in CD30+ R/R CTCL. Although at an early stage of clinical development, A-dmDT390-bisFv(UCHT1) has demonstrated the potential for cure in a subset of pretreated patients with early-stage CTCL and a Modified Severity Weighted Assessment Tool score of <50%.

**Conclusions:** Treatments for R/R CTCL are generally systemic in nature. Targeted agents, such as the HDAC inhibitors, are now important options when other approaches have failed, and various investigational therapies are also showing promise in this setting.

**437**
**MINIMAL RESIDUAL DISEASE BY FLOW CYTOMETRY PREDICTS POOR OUTCOME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PERIPHERAL T-CELL LYMPHOMA**

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**Introduction:** Peripheral T-cell lymphomas (PTCL) encompass a heterogeneous group of neoplasms accounting worldwide for 10 to 15% of non-Hodgkin lymphomas. Prognosis for PTCL patients is poor and consolidation in first remission with autologous stem cell transplantation (ASCT) is widely used, although most patients still relapse after the procedure. We hypothesized that pre-transplant minimal residual disease (MRD) in bone marrow (BM) detected by multi-parameter flow cytometry would identify patients with poorer outcome after ASCT.

**Methods:** We retrospectively analyzed the relapse and overall survival (OS) rates of 33 consecutive PTCL patients who underwent ASCT at our institution from April 2004 through July 2014. Bone marrow (BM) involvement was defined as the morphological identification by pathology review of an abnormal T-cell population in a BM biopsy. MRD was defined as the presence of an abnormal T-cell population by multi-parameter flow cytometry analysis in a bone marrow aspirate obtained pre-ASCT.

**Results**: A total of 33 patients were included: 12 patients (36%) with angioimmunoblastic T-cell lymphoma, 9 (27%) with anaplastic large-cell lymphoma (8 patients were ALK negative at diagnosis), 8 with not otherwise specified T-cell lymphomas (24%) and 4 with other PTCL sub-types (12%). Median age at transplant was 54 (range: 29–72). Nineteen patients (58%) received BEAM as conditioning regimen, while CYVPTBI was used in 7 (21%). Seven patients (21%) were treated with an alternative regimen. Seventeen patients (52%) underwent ASCT in first remission. Median follow-up after transplant was 3.77 years (range: 2–105 months). At diagnosis, BM involvement by morphology was reported in 8 patients (24%). At ASCT, flow cytometry data on BM aspirates were available in 29 patients (88%). Patients with MRD in BM pre-ASCT ($n = 7$) exhibited the following findings: an IPI score $\geq 2$ ($n = 7$), CNS involvement ($n = 1$), Deauville scores consistent with complete remission by PET/CT ($n = 4$). Three patients with history of BM involvement morphologically at initial diagnosis had pre-ASCT evidence of MRD in BM. Compared to MRD-negative patients, patients with detectable MRD at ASCT experienced a significantly higher 4-year relapse rates in univariate analysis (79% *versus* 33%, $p = 0.002$) and poorer OS (29% *versus* 86%, $p < 0.001$). High IPI at diagnosis (≥2), CNS involvement, ASCT beyond first complete remission and absence of complete remission by CT were also associated with poor post-transplant outcome.

**Conclusion:** Detectable MRD in BM determined by multi-parameter flow cytometry at the time of autologous transplantation correlates with higher relapse rates and poorer OS. Consequently, assessment of pre-ASCT MRD in BM helps stratify prognosis. PTCL patients with pre-SCT MRD should be considered for alternative therapeutic approaches including additional therapy with novel agents, allogeneic transplant or use of post-ASCT consolidation therapy.

## PLASMA CELL DISORDERS

**438**
**ANTI-EPSTEIN–BARR VIRUS NUCLEAR ANTIGEN ANTIBODY NEGATIVITY MAY BE A NEW INDICATOR OF POOR PROGNOSIS IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB-BASED REGIMENS**

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**Introduction:** Multiple myeloma (MM) remains incurable, although novel agents, including bortezomib (BOR), have shown high efficacy in patients with MM. The Epstein–Barr virus (EBV) is known to achieve latent infection in B-cells. To date, several reports regarding the correlation of prognosis with EBV infection in lymphoma patients have been published. However, no such reports exist for MM patients.

**Methods:** We examined the blood samples of MM patients before the treatment in our institute from 2010 to 2014. All patients received BOR-based regimens as first-line treatment. Specifically, transplant-eligible patients received four cycles of combined treatment referred to as VD (BOR: 1.3 mg/m$^2$ on Days 1, 4, 8, and 11/DEX orally administered at 40 mg on Days 1–4) or CyBorD (BOR: 1.3 mg/m$^2$ on Days 1, 4, 8, and 11/orally administered CY and DEX at 500 mg/m$^2$ and 40 mg, respectively, on Days 1, 8, and 15) and upfront autologous stem cell transplantation if partial or better response was achieved. Non-transplant-eligible patients received nine cycles of combined treatment referred to as VMP (BOR: 1.3 mg/m$^2$ on Days 1, 4, 8, and 11/orally administered MEL and PSL at 9 mg/m$^2$ and 40 mg, respectively, on Days 1–4). EBV-infectious status was tested using anti-EBV VCA IgM, anti-EBV VCA IgG, and anti-EBV nuclear antigen antibody (EBNA). We compared progression-free survival (PFS) and overall survival (OS) using EBNA and other known risk factors. Data was analyzed by log rank tests and Cox proportional hazard regression.

**Results:** EBNA was measured in 39 patients. There were no significant differences in the baseline characteristics of patients between the negative (titer $< 10$, $n = 15$) and positive (titer $\geq 10$, $n = 24$) groups with regard to median age, transplant eligibility, gender, ISS score, LDH, serum Ca2+, creatinine clearance, del17 and/or t(4;14), EBV VCA IgM, EBV VCA IgG, and response rate by first-line treatment. Median follow-up was 37.3 (9.9–48.4) months in the negative group and 26.7 (5.3–45.8) months in the positive group, respectively. Two-year PFS was 18.3% (95% CI: 3.0–44.2) in the negative group and 66.5% (38.2–84.1) in the positive group, respectively ($p = 0.01$). Two-year OS was 86.7% (95% CI: 56.4–96.5) in the negative group and 100.0% (95% CI: 100–100) in the positive group, respectively ($p = 0.84$). In a univariate analysis with EBNA and other known risk factors, EBNA $< 10$ [hazard ratio (HR): 3.1, $p = 0.02$] and ISS $> 2$ [HR: 37, $p = 0.003$] worsened PFS significantly. EBNA $< 10$ (HR: 3.7, $p = 0.03$) and ISS $> 2$ (HR: 58.6, $p = 0.002$) were also associated with significantly worsened PFS in a multivariate analysis with EBNA and other known risk factors.

**Conclusions:** EBNA $< 10$ and ISS $> 2$ were significant predictors of shorter PFS for MM patients when they were treated with BOR-based regimens. EBV VCA IgG was positive in most of the EBNA-negative patients. To the best of our knowledge, this is the first report regarding prognosis correlation with EBV infection in patients with MM.

**439**
**LOW BECLIN-1 EXPRESSION PREDICTS IMPROVED OVERALL SURVIVAL IN PATIENTS TREATED WITH IMMUNOMODULATORY DRUGS FOR MYELOMA. AN ANALYSIS FROM THE AUSTRIAN MYELOMA REGISTRY (AMR)**

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**Introduction:** Beclin-1 is a key regulator of autophagy and has been suggested to play a role in the development of drug resistance in Multiple Myeloma (MM). To further investigate the role of Beclin-1 as a potential biomarker, we analyzed bone marrow trephine biopsies with therapy-naïve MMs as well as residual/relapsed disease for the expression of Beclin-1 and correlated our results with clinical and pathological parameters including overall (OS) and progression-free survival (PFS) as well as response to different treatments.

**Methods:** Bone marrow trephine biopsies of 70 therapy naïve patients diagnosed with MM between 2000 and 2011 were stained by means of immunohistochemistry using a monoclonal antibody to Beclin-1 (clone EPR1733Y, Epitomics). Follow-up biopsies with residual/relapsed MM were also evaluated if available. Percentage of Beclin-1+ plasma cells was assessed compared to the extent of marrow infiltration by plasma cells highlighted with the plasma cell specific antibody HPC (clone vs38c, Dako). Statistical analysis was performed by applying Kaplan–Meier and Cox regression models using receiver operator characteristics (ROC) curves for determination of cutoffs of continuous variables.

**Results:** Beclin-1 expression did not influence OS and PFS in patients with MM. Patients treated with immunomodulatory drugs (IMiDs) had a significantly better OS and PFS than those treated with Bortezomib Mono/Dexamethasone or non-novel agents. Stratified for Beclin-1 expression patients with Beclin-1 expression below the cutoff of 55.0% for OS and 27.5% for PFS had a significantly improved OS and PFS when treated with IMiDs compared to those treated with Bortezomib Mono/Dexamethasone or non-novel agents ($p = 0.002$ and $p = 0.014$, respectively). No such difference was seen in patients with Beclin-1 expression above the cutoff for OS and PFS. Beclin-1 expression was significantly higher and more frequently detected in trephine biopsies with relapsed MM obtained $>6$ months after initial diagnosis than in therapy naïve MMs ($p = 0.003$), while in residual MMs $<6$ months after diagnosis and thus treatment most MMs did not express Beclin-1.

**Conclusions:** If validated prospectively testing for Beclin-1 expression might identify a Myeloma patient subset prone to profit above average from IMiD based therapies and enable a more rational allocation of anti-myeloma therapies. Furthermore inhibition of autophagy could be a promising druggable target to improve response to treatment, especially in the relapsed/refractory setting.

**440**
**RESPONSE AND EARLY RELAPSE ASSESSMENT IN MULTIPLE MYELOMA TREATED WITH BORTEZOMIB: ROLE OF HEAVY/LIGHT-CHAIN IMMUNOASSAY**

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**Background**: Multiple Myeloma (MM) is characterized by multiples relapses, for that reason the objective of MM therapy is to achieve the most deeper and sustained response; actual approaches included a consolidation and/or a maintenance period with a close monitoring to assess the relapses and identified the best moment to re-start the therapy. The routinely follow-up for minimal residual disease in MM is based in the M-protein quantification by serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX). The incorporation of serum-free light-chain assay (FLC), and the quantification of paired clonal and non-clonal immunoglobulins (HLC) in serum, offers the possibility to assess the response to therapy more accurately detecting early non-symptomatic (biological) relapses (EBR).

**Aims:** We aimed to analyze the usefulness of HLC and FLC during MM follow-up to detect EBR in patients who received bortezomib-based first-line therapy in MM patients in our center.

**Patient and Methods**: Since January 2008 we have incorporate in the protocol for M-components assessment the quantification of FLC, later the HLC, at baseline and in follow-up of MM. We have analyzed these parameters in all consecutive patients diagnosed as secretory MM who complete at least four cycles of Bortezomib-based upfront therapy between Jan 2008-Jun 2014 in our center.

**Results**: A total of 135 MM patients started bortezomib-based therapy. Median follow-up 25 months. Females: 40.7%, mean age 69.6 years (32–91). Subtype: IgG-Kappa: 38.2%, IgG-Lambda: 9.8%, IgA-Kappa: 17.9%, IgA-Lambda: 15.4%, IgDL: 1.6%, Bence-Jones-Kappa: 6.5%, Bence-Jones-Lambda: 8.9%, oligo-secretory: 1.6%. Durie-Salmon Stage: IA: 9.8%, IB: 1.6%, II-A: 27.6%, IIB: 8.9%, III-A: 21.1%, III-B: 21.1%. ISS: I: 25.2%, II: 32.5%, III: 23.6%. The 21.1% of patients were not included in the analysis of response by uncompleted treatment. Response at end of therapy: minimal response: 5.1%, PR: 44.3%, VGPR: 16.5%, CR: 14.4% SR: 7.2%, Failure: 12.5%. During follow-up 65.7% patients, who achieved at least PR had clinical relapsed/progressed, in 86.9% of them, a previous EBR were detected at a mean of 4.4 months before; methods who detected

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BER: FLCr (28.8%), HLCr (13.5%), FLC + SPE + IFX (9.6%), FLC + IFX (5.8%), FLC + HLC + SPE (28.8%), FLC + HLC + SPE + UPE (5.7%) Median PFS 20 months (12.8–27.1) Biological PFS: 18 months (12.1–23.8).

**Conclusion**: Both FLCr and HLCr are sensitive and precise tools to perform MRD assessment response in MM, in our cohort a 42.3% of EBR were detected ahead of other techniques. More investigations on the role of EBR for therapy initiation are necessary.

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**441**
**MULTIPLE MYELOMA IN ELDERLY AFRO-CARIBBEAN PATIENTS TREATED WITH MELPHALAN PREDNISONE AND THALIDOMIDE IN MARTINIQUE**

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**Introduction:** In African-Americans (AA), the incidence of multiple myeloma (MM) is twice as high as in Caucasians. Only few studies have focused on overall survival. A recent population-based study comparing white and AA subjects showed a better survival in AA patients over a entire study period ranging from 1973 to 2005. However, significant survival improvement after the current era of intensive treatment or new therapies was only seen among whites, with smaller, non-significant change seen among blacks. This study hypothesized that the disparities in improvement in outcome observed could be related to a lower response to new therapies in AA patients. In Martinique, a French overseas department, the vast majority of the population is of Afro-Caribbean origin.

**Methods:** We conducted a retrospective study on transplant-ineligible patient, newly diagnosed with MM who were treated in the Hematology Department of Fort de France in Martinique between 2007 and 2012. Our aim was to assess the characteristics, progression-free (PFS) and overall survival (OS) of this subgroup.

**Results:** During this period, the Martinique Association for Epidemiological Research on Cancer revealed an incidence of 8/100 000 inhabitants per year. Fifty four patients were not eligible for autologous stem cell transplantation and had been treated with melphalan, prednisone and thalidomide as part of frontline therapy. The patients had a median age of 80 years, with ISS Stage III documented in 48%. The overall response rate was 76%. With a median follow-up of 35 months, PFS and OS were 28.9 and 48.6 months, respectively.

**Conclusions:** Compared to metropolitan France, our results show a higher incidence and a similar outcome in this population, despite an advanced age and disease status at diagnosis. No data have been published focusing only on the outcome of AA or African-Caribbean elderly patients treated with combination therapies that include novel agents. Our study highlights findings which had so far not been described and do not support the hypothesis that AA patients had a lower response to new therapies.

**442**
**NELFINAVIR AND LENALIDOMIDE/DEXAMETHASONE IN PATIENTS WITH LENALIDOMIDE-REFRACTORY MULTIPLE MYELOMA—A PHASE I TRIAL—SAKK 39/10**

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**Introduction:** Based on preclinical results, we hypothesize that the addition of nelfinavir (NFV) to standard lenalidomide/dexamethasone (LD) treatment may be active in lenalidomide-refractory patients by inhibition of the PI3K/Akt pathway and modulation of proteasome function.

**Methods:** This multicenter, dose escalation phase I trial used a 3 + 3 design to identify the recommended dose (RD) of dose-escalated NFV in combination with standard dose LD therapy in patients with lenalidomide-resistant MM.

Patients were treated with a fixed dose of L 25 mg d1–d21 with weekly D 40 mg, combined with NFV at dose levels (DL) of 1250 mg (DL 1) and 1875 mg (DL 2) bid d1–d21. Courses were repeated every 4 weeks for four cycles. Patients had to have progressive MM that has failed during or within 60 days after termination of L-containing therapy. Dose-limiting toxicity (DLT) was defined as one of the following events occurring within the first cycle: therapy-related death, ≥6 missed therapy days of L and/or NFV or delay of >2 weeks of Cycle 2 due to trial drug-related toxicity, neutropenia $< 0.5 \times 10^9$/L (Grade 4) for ≥7 days, thrombocytopenia $< 20 \times 10^9$/L or ≥20–$50 \times 10^9$/L with major bleeding, or any Grade 3 or 4 non-hematological adverse event (AE) *possibly, probably, definitely* related to trial treatment (ClinicalTrials.gov number NCT01555281).

**Results:** Ten patients with advanced MM were enrolled, six in DL 1 and four in DL 2. Median age was 59 years (range 51–72). The following Grade ≥3 AEs related to trial treatment occurred during subsequent cycles: anemia (2), thrombocytopenia (2), infection, and (3) one each of fatigue, insomnia and a fall. No Grade 4 non-hematologic toxicities occurred. No DLT occurred at DL 1. Two DLTs occurred at DL 2 within the first cycle: one patient had Grade 3 diarrhea, and one patient experienced Grade 4 thrombocytopenia.

Of 10 patients, 7 completed four cycles of trial treatment. Of 10 patients, 2 experienced a DLT and therefore stopped the trial treatment during Cycle 1. One patient had progressive disease and stopped during Cycle 2. Pharmacodynamic analysis of PBMC (peripheral blood mononuclear cells) by activity based affinity labelling of active proteasomes revealed a reduction of proteasome activity during trial treatment, consistent with the preclinical activity of NFV against MM.

**Conclusion:** RD for phase II has been established at DL1 with NFV 1250 mg bid d1–d21 in combination with L 25 mg/day d1–d21 and D 40 mg weekly. The combination of these oral substances is well tolerated.

**Abs 442 - Table 1. Patient characteristics**

| | Total 10 patient |
|---|---|
| Performance status | |
| 0 | 4 |
| 1 | 3 |
| 2 | 3 |
| Comorbidity | 5 |
| ISS Stage | |
| I | 3 |
| II | 4 |
| III | 3 |
| Prior lines of therapy | |
| 2 | 2 |
| 3 | 4 |
| ≥4 | 4 |
| Prior autologous stem cell transplantation | 8 |

**443**
**DETECTING EARLY RELAPSE IN MULTIPLE MYELOMA AFTER ASCT: USEFULNESS OF IMMUNOASSAYS**

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**Background**: There are new tools for accurate follow-up and diagnosis assessment in Multiple Myeloma (MM) patients. While the Free Light chain immunoassay (FLC) (Bindingsite, Birmingham, UK) is part of the mandatory response assessment according to IMWG-criteria and recently one of the criteria for diagnosis, the role of

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the Heavy/Light Chain immunoassay (HLC), is still under investigation with promissory results. Relapses in MM patients are frequent and there is an especial interest in select therapy for consolidation/maintenance after Autologous Stem Cell Transplantation (ASCT) and to detect early relapse to optimize the therapy. We hypothesized that the combination of these techniques could permit to detect early biological (non-symptomatic) relapses (EBR) in these kind of patients.

**Aims**: To analyze the usefulness of HLC and FLC to detect EBR in MM after ASCT in our hospital.

**Patient and Methods**: A retrospective study was performed including all consecutive patients treated in our center, following these criteria: Diagnosed of secretory MM, upfront-therapy including ASCT between May 2011-August 2014, assessed with our protocol including FLC, HLC, serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX), previous to ASCT, after 12 weeks and every 3 months later with a minimum follow-up of 6 months after ASCT. EBR was defined as an increase of 25% on M-protein (any amount for patients on CR/SR) and/or an increase of ≥20 mg/dl for FLC, and/or 25% increase on involved HLC with abnormal ratio. For urine, an increase >500 mg/24 h of involved free-chain protein.

**Results**: Fifty-five patients were registered. Median follow-up 21 months. MF ratio: 29/26, mean age 59.5 years (33–71). Immunoglobulin subtype: IgG-Kappa: 41.8% (23), IgG-Lambda: 23.6% (13), IgA-Kappa: 16.4% (9), IgA-Lambda: 7.3% (4), Bence-Jones-Kappa: 3.6% (2), Bence-Jones-Lambda: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as conditioning regimen. Status pre-ASCT: minimal response: 12%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and string response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 13.0% CR, 30.4% VGPR and 39.1% PR. During follow-up, 27/50 (54.0%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 24 months (19.8–28.1). EBR were detected in 19/27 relapsed patients at median time 7 (2–19) months before symptomatic relapse. The EBR were detected by FLCr (31.6%), HLCr (21.0%), FLC + SPE (10.5%), FLC + IFX (5.2%), FLC + HLC + SPE (15.8%), FLC + HLC + SPE + UPE (15.8%).

**Conclusion**: Both FLC and HLC are useful tools to detect EBR in more than 50% of patients in our cohort ahead other techniques.

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### THE UTILITY OF MODERN RADIOTHERAPY IN THE TREATMENT OF PLEURAL PLASMACYTOMAS

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**Introduction:** Plasmacytomas involving the pleura are rare with some occurring as solitary disease or against a background of multiple myeloma. Survival rates for this group of patients are poor, mostly due to respiratory complications (survival range: 16 days to 10 months); this has been in part due to the technical challenges of treating this anatomical site with radiotherapy. In the literature, radiotherapy has been used in only one case but the patient expired before its completion. There have been significant advances in chemotherapy and radiotherapy since; modern radiotherapy techniques can overcome clinical situations which were previously deemed challenging. Although notable differences exist between them, comparison of all of these modern conformal radiotherapy techniques in pleural disease has not yet been reported.

**Methods:** We examined and compared the utility of different conformal radiotherapy techniques in a patient with a pleural plasmacytoma who was being treated with chemotherapy (for multiple myeloma) and radiotherapy (20 Gy). A dosimetric comparison was performed between the following techniques: an anterior-posterior plan, a 9 field intensity-modulated radiotherapy (9F-IMRT) plan, a volumetric modulated arc therapy (VMAT) plan and a helical tomotherapy (HT) plan, highlighting the relative merits of each technique.

**Results:** There was significant improvement in conformity and organ sparing when using the highly conformal techniques over the classic AP-PA technique (Table 1). All the dose constraints for organs at risk (OAR) were met with the advanced techniques. IMRT provided the lowest integral dose whilst HT provided the most uniform dose with optimal target coverage; VMAT delivered the treatment in the shortest time. The patient, who was treated with modern chemotherapy regimens and modern radiotherapy techniques had a good clinical outcome (currently alive at 58 months from diagnosis) and to our knowledge is the first case to have completed radiotherapy.

**Conclusion:** We have demonstrated that a combination approach with modern radiotherapy and chemotherapy techniques can yield favourable results in patients with pleural plasmacytomas highlighting this as a feasible and effective option for this difficult clinical problem. This is the first case to have completed a course of radiotherapy and the first to compare the relative merits of the different modern radiotherapy techniques available to treat pleural disease.

**Abs 444 - Table 1.** Dose-volume parameters of the four radiotherapy techniques described.Dose-volume parameters of the four radiotherapy techniques described

| | AP-PA | 9f-IMRT | VMAT | HT |
|---|---|---|---|---|
| **Planned target volume** | | | | |
| $D_{95\%}$ (Gy) | 18.58 | 19.62 | 19.39 | 20.02 |
| Homogeneity index | 3.06 | 1.60 | 1.76 | 1.08 |
| Coverage index | 0.44 | 0.83 | 0.77 | 0.95 |
| Healthy tissue irradiation index | 0.24 | 0.84 | 0.87 | 0.66 |
| Conformity index | 0.10 | 0.70 | 0.67 | 0.63 |
| **Organs at risk** | | | | |
| *Spinal cord maximum dose (Gy)* | 17.24 | 13.05 | 13.24 | 13.06 |
| *Heart* | | | | |
| Mean dose (Gy) | 13.60 | 10.39 | 9.55 | 7.73 |
| $V_{15Gy}$ (%) | 63.64 | 18.23 | 8.70 | 2.07 |
| $V_{3Gy}$ (%) | 76.59 | 93.07 | 100 | 73.43 |
| *Contralateral lung* | | | | |
| Mean dose (Gy) | 0.42 | 2.08 | 2.91 | 0.76 |
| $V_{5Gy}$ (%) | 0 | 1.03 | 0.97 | 0.10 |
| *Ipsilateral lung* | | | | |
| Mean dose (Gy) | 20.70 | 16.83 | 17.06 | 14.54 |
| *Both lungs* | | | | |
| Mean dose (Gy) | 8.40 | 7.86 | 8.46 | 6.16 |
| $V_{5Gy}$ (%) | 39.30 | 39.80 | 38.91 | 37.31 |
| $V_{20Gy}$ (%) | 32.88 | 4.35 | 5.94 | 7.38 |
| *Kidney, mean dose (Gy)* | 1.80 | 1.22 | 1.82 | 2.02 |
| **Treatment delivery time (min)** | 4 | 16 | 7 | 10 |
| **Planning time (h)** | 0.2 | 4 | 4 | 3 |

## TOXICITIES

### 445
### HIGH EFFICIENCY OF PRIMARY CARDIOPROTECTION IN LYMPHOMA PATIENTS WITH INCREASED RISK OF ANTHRACYCLINE CARDIOTOXICITY—A SINGLE-CENTRE EXPERIENCE

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**Introduction:** Advances in anti-lymphoma therapy and subsequent prolongation of overall survival, make doxorubicin cardiotoxicity an important clinical issue.

**Methods:** Doxorubicin cardiotoxicity and efficacy of primary cardioprotection was assessed retrospectively in a single center analysis of 197 lymphoma patients treated at diagnosis with (R)-CHOP. One hundred and sixty-two patients, including 62 with a high risk of cardiovascular disease (CVD), were treated without primary cardioprotection. Thirty-five patients, all at high CVD risk, were subjected to primary cardioprotection with ACE-I and/or beta-blockers at the maximal tolerated doses. Patients with hypertension, diabetes mellitus, systolic or diastolic dysfunction in echocardiography and/or cumulative doxorubicin dose exceeding 200 mg/m$^2$ received liposomal doxorubicin. High CVD risk was defined by past medical history

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(PMH) as at least one of the following: hypertension, ischemic heart disease, cerebrovascular accidents, diabetes mellitus, hypercholesterolemia, nicotinism, arrhythmia or abdominal obesity. Additional value of NT-proBNP levels and spatial QRS-T angle assessment, as independent indicators of individual sensitivity of cardiomyocytes to doxorubicin were also analyzed.

**Results:** At the average observation time of 2 years, in patients without primary cardioprotection CVD was the second commonest cause of death: 5.5% (9/162) in the whole group, 14.5% (9/62) in patients with high risk of CVD. It was significantly reduced (0/35) in patients with primary cardioprotection, despite more CVD risk factors present (average 3.06 vs 1.71, $p < 0.05$). Primary cardioprotection also decreased the number of patients with systolic dysfunction at the end of therapy (3.03% vs 53.3%; $p < 0.05$) and frequency of new CVD-related symptoms (2.86% vs 24.2%; $p < 0.05$). The accuracy of determining high CVD risk based on PMH may be further increased by assessing NT-proBNP and spatial QRS-T angle after the first chemotherapy cycle (values >400 pg/ml and >90% respectively).

**Conclusions:** The preliminary results are in favor of investigating the role of primary cardioprotection in specifically designed Phase III trial.

| | Patients at high risk of CVD, *n* (%) | |
|---|---|---|
| | **Without primary cardioprotection** | **With primary cardioprotection** |
| Average number of CVD risk factors | 3.06/person | 1.71/person |
| CVD-related death | 9/62 (14.5) | 0/35 (0) |
| Systolic dysfunction at the end of therapy (only patients with final echocardiography assessment included) | 8/15 (53.3) | 3.03 (1/33) |
| New CVD-related symptoms | 24.2 (15.62) | 2.86 (1/35) |

**446**
**EVALUATION OF A PANEL OF CIRCULATING BIOMARKERS DURING DEVELOPMENT OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN LYMPHOMA AND BREAST CANCER PATIENTS**

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**Introduction:** Heart damage from cancer therapy is a significant problem for lymphoma survivors. Some of the most effective treatments such as anthracyclines cause heart toxicity that leads to significant morbidity and mortality later in life. Methods of detecting heart damage at an early reversible stage are lacking. This project explored a panel of circulating biomarkers alongside cardiac magnetic resonance imaging (CMR) in patients receiving anthracyclines with the aim of finding cardiotoxicity biomarkers to aid clinical decision making. The biomarkers were chosen to reflect the different pathological processes occurring in anthracycline related cardiotoxicity; myocyte degradation, metabolism changes, inflammation, fibrosis and strain.

**Methods:** Following ethical approval, 30 patients with lymphoma (25) or breast cancer (5) receiving standard first-line anthracycline-based chemotherapy (doxorubicin or epirubicin respectively) were recruited December 2011 to May 2013. Serial CMR scans were performed before, during, after and 12 months post-treatment to assess cardiac function. Blood was taken prior to each cycle and 2, 24 and 72 hours after the first, middle and last cycles to measure transient and cumulative changes in a panel of 11 potential cardiotoxicity biomarkers (MMP9, MMP2, TIMP1, MPO, IL8, PAPPA, FABP3, NTproBNP, TNFa, IL1b and Trop I) using multiplex ELISA.

**Results:** A decline in left ventricular ejection fraction (LVEF) was seen in all patients. Cardiotoxicity, (a fall in LVEF of ≥10% to ≤55%) was seen in 7 patients (23%) by the end of study but 17 patients (57%) had a drop of ≥10% during the study. LVEF decline was dose related. Sixty-three per cent of patients receiving >280 mg/m$^2$ had a decrease in LVEF ≥ 10% compared to only 36% receiving ≤280 mg/m$^2$. Significant elevations of Troponin I were seen during the last cycle of therapy ($p < 0.0001$) and peak levels correlated with decline in cardiac function (Spearman $r = 0.4$, $p = 0.05$). MMP9 levels fell during treatment but showed no correlation with cardiac function and the other biomarkers did not change significantly.

**Conclusions:** LVEF deteriorated in all patients during and after anthracycline therapy and a clinically significant fall was seen in 23% within 18 months of therapy. Cardiotoxicity was dose related even in the 'safe' doses currently received by lymphoma and breast cancer patients. Troponin I was the only biomarker showing significant potential although levels did not peak until the final cycle of chemotherapy, too late to prompt alteration/cessation of therapy. Findings support work by others (Cardinale *et al.*, 2004) showing that troponin I could be used to risk stratify patients for monitoring or cardio-protective strategies following therapy. Despite utility in several cardiac conditions the other biomarkers were not informative highlighting the need to find alternatives capable of identifying anthracycline cardiotoxicity early during treatment.

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**EARLY DETECTION OF ANTHRACYCLINE INDUCED CARDIOTOXICITY IN ADULT LYMPHOMA PATIENTS USING BIDIMENSIONAL STRAIN IMAGING AND CARDIAC BIOMARKERS: A SINGLE-CENTER EXPERIENCE**

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**Introduction:** Cardiotoxicity is a major threat in haematological patients treated with anthracyclines eventually evolving towards hypokinetic cardiomyopathy and heart failure. Reduction in left ventricular ejection fraction (LVEF) is a late toxic effect, therefore more sensible techniques are needed. Bidimensional strain echocardiography (2DST) associated with biomarkers monitoring has been developed for early detection of myocardial changes: a 10% to 15% early reduction in Global Longitudinal Strain (GLS) has been proposed as predictor of cardiotoxicity both in oncological and pediatric haematological patients. Clear data on adult haematological patients are missing.

**Methods:** All adult lymphoma patients admitted to our institution to receive an anthracycline containing regimen were prospectively included in the study. They underwent a baseline evaluation including cardiac biomarkers (NT-PROBNP, troponin I) and 2DST. Biomarkers were tested before and after each course of chemotherapy and 2DST was repeated halfway (T1) and at the end of chemotherapy (T2). According to American Society of Echocardiography criteria, cardiotoxicity was defined as a decrease in the LVEF >10% or to a value <53%.

**Results:** From May to December 2014 14 patients were included: 13 Non-Hodgkin and 1 Hodgkin Lymphoma (6 males, 8 females), aged 23–81 (median 60.5 years). The median dose of anthracycline administered was 204 mg/mq (min 100 mg/mq, max 304 mg/mq). At baseline median LVEF was 64% (min 51%, max 75%) and median GLS was -19% (min -15%, max -22%). Three/14 patients developed cardiotoxicity, only one clinically significant. However, no significant reduction in LVEF was observed at T1 (n: 14 patients, median value 63%, min 44%, max 74%, $p = 0.99$) and at T2 (n: 9 patients, median value 64%, min 56%, max 69%, $p = 0.34$). Median GLS was -18% (min 11%, max 22%) at T1 and -18% (min -13%, max -22%) at T2. Comparing GLS at baseline with GLS at T1 and T2 a downward tend has been noticed. At T1 12 patients were evaluable with median GLS −18%, $p = 0.15$. At T2 8 patient were evaluable with median GLS −17.5%, $p = 0.05$. Notably, only patients who received a total dose of anthracycline greater than 150 mg/mq developed a GLS reduction >10%. In our series no alterations in troponin I were seen. Two/14 patients had elevated baseline NT PROBNP that remained stable during treatment. None of the patients with normal baseline NT PROBNP had it elevated at the end of treatment, while it could be temporarily elevated between one course and another.

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**Conclusions:** GLS seems to have high sensibility to recognize myocardial damage, since it showed a downward trend during treatment without concomitant decrease in LVEF. Biomarkers did not prove to be helpful in our series. Hereafter, it is necessary to expand the studied population and to evaluate longer in the follow-up the evolution of cardiotoxicity, especially in patients with GLS reduction >10%.

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### CARDIAC DAMAGE FROM LYMPHOMA TREATMENTS: AN AUDIT OF CONSENT AND COUNSELLING AT THE CHRISTIE NHS FOUNDATION TRUST, UK

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**Background:** Heart toxicity is an important cause of late morbidity and mortality in patients who have been treated for lymphoma. The majority of lymphoma survivors will have received anthracycline-based chemotherapy which is also well known to cause heart damage and/or radiotherapy to the heart. Oncology clinicians have responsibility for informing patients about the potential side effects of treatments including the potential of late effects like cardiac disease. Patients should be educated about risk factor modification, and close liaison with primary care givers is vital to optimize the cardiovascular health of cancer survivors following therapy.

**Methods**: Consent, information giving and cardiac events were audited in 93 lymphoma patients who received anthracycline chemotherapy and/or radiotherapy involving cardiac tissue at the Christie NHS Foundation Trust, UK. A list of patients diagnosed with lymphoma in 2009 was generated using the hospital clinical coding system. Case notes were manually reviewed to identify patients who received anthracycline chemotherapy and/or potentially cardiac damaging radiotherapy. The pre-treatment consent process and subsequent counselling about cardiac effects during the 5-year follow-up period were audited. Standards were audited against regional (Greater Manchester and Cheshire Cancer Network haemato-oncology clinical subgroup) and good clinical practice guidelines. Data about cardiac events were collected, and a further cohort of 70 patients treated in 1999 (with 15 years of follow-up) were also audited to estimate the cardiac event rate in this centre.

**Results**: Only 42% of patients receiving anthracycline chemotherapy were consented for the potential of cardiotoxicity prior to treatment whereas 61% of patients were consented for cardiac damage from radiotherapy. Only 18% of patients received counselling about late cardiovascular effects and risk factor reduction following therapy and just 14% of general practitioners (GPs) were informed about the cardiotoxic potential of the treatment. Twenty-three per cent of patients treated in 1999 developed cardiac events, the majority of which occurred in the first 2 years after therapy (42%), whereas only three cardiac events (4%) were recorded in the 2009 cohort.

**Conclusions**: Consent for cardiac damage from lymphoma treatment, particularly chemotherapy was poor in this centre therefore 'cardiac damage' was added to the list of pre-printed side effects on standard consent forms. Patients were rarely counselled about late cardiotoxicity following therapy and communication with primary care givers was poor. To address this, the centre now provides treatment summaries detailing the key late toxicities to patients and GPs within a community-based follow-up programme. A relatively high number of cardiac events were seen in the 1999 cohort but fewer in the 2009 cohort. This may reflect the shorter follow-up and improvements in lymphoma treatment during this time.

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### RITUXIMAB INDUCED INTERSTITIAL LUNG DISEASE—CMOG EXPERIENCE

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**Introduction:** Rituximab has revolutionized the treatment of B-cell lymphomas. Apart from rare anaphylactoid reactions, the treatment is usually well tolerated. We discuss rituximab-induced lung disease seen in our clinical practice.

**Methods:** We analysed the details of all patients who developed rituximab induced lung toxicity. Retrospective analysis of case records was done. The study period was between 2011 and 2014.

**Results:** Five patients developed rituximab induced interstitial lung disease during this period. All of them were elderly (>60 years) with median age being 68 years. Three patients were males and two were females. All had non-Hodgkin lymphoma —B-cell lymphoma (DLBCL—3, mantle cell lymphoma—1, follicular lymphoma Grade 3B—1). The toxicity was seen during the fourth cycle or later in all patients. Three of them expired in spite of best of care which included IV steroids. Diagnosis was purely on radiological basis.

**Conclusions:** Rituximab-induced interstitial lung disease is seen later in the course of treatment of B-cell lymphomas. Elderly patients may be more vulnerable. It is very important to think about this often fatal toxicity in patients who undergo rituximab-based treatment and complaints of dyspnoea and cough. Elderly patients may require dose adjustment.

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### APREPITANT PREVENTS NAUSEA AND VOMITING AFTER HD-CTX FOR PBSC HARVESTING: A PHASE III, DOUBLE-BLINDED, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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**Purpose:** Peripheral blood haematopoietic stem cells for autologous transplantation are often mobilized by means of highly emetogenic, intermediate doses of cyclophosphamide which are administered in an out-patient regimen. As prophylaxis of chemotherapy induced nausea and vomiting (CINV) Aprepitant, a 5-HT3 receptor antagonist, is recommended in combination with dexamethasone and a 5-hydroxytryptamine 3 (5-$HT_3$) receptor antagonist.

Since aprepitant moderately inhibits CYP3A4, concomitant administration with cyclophosphamide might decrease cyclophosphamide clearance, thus impairing efficacy of autologous stem cell mobilization.

This study was designed to determine whether the aprepitant-palonosetron-dexamethasone regimen was better than palonosetron-dexamethasone therapy in preventing CINV and to assess its impact upon stem cell harvesting and toxicity.

**Patients and Methods**: This single centre, randomized, double-blinded, placebo-controlled phase III trial was conducted in patients who received a highly emetogenic cyclophosphamide IV chemotherapy (3 $g/m^2$) for autologous PBSC harvesting. Efficacy of retching and vomiting, rescue medication, severity of nausea and overall quality of and safety data were obtained from the patient's daily diary (Days 1–5) which reported episodes life. The Functional Living Index-Emesis questionnaire was completed on Days 1 (before starting chemotherapy) and 6 (after chemotherapy). All side effects were recorded daily.

**Results:** A total of 122 patients were enrolled and randomized. The analysis was performed according to the intention-to-treat principle.

Primary endpoint: When the 2 groups were compared, the number of patients with no emetic episodes and no rescue medication in the first 120 hours post-chemotherapy, was significantly lower in the aprepitant group ($p = 0.0114$).

Secondary endpoints: Acute ($p = 0.0272$) and delayed ($p = 0.0039$) complete response rate, complete control rate ($p < 0.0001$), number of emetic events ($p = 0.0003$) and the impact of nausea and vomiting on daily life ($p < 0.0001$) were also significantly better in the aprepitant group. The adverse event rate and stem cell harvest ($p = 0.821$) were not significantly different.

**Conclusions:** The aprepitant regimen more effectively prevented CINV and weakened its impact on daily life without impairing the efficacy of autologous PBSC mobilization and harvesting in patients treated with highly emetogenic, intermediate doses of cyclophosphamide.

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**DEVELOPMENT OF A PREDICTION SCORE FOR THE IDENTIFICATION LYMPHOMA PATIENTS AT RISK FOR THROMBOEMBOLISM**

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**Introduction**: There is a paucity of data that pertain to thrombosis in patients with lymphoproliferative diseases. In few published studies the rate of thrombotic complications in lymphoproliferative disease is highly variable.

Aim of our study was to determine incidence of thromboembolic (TE) events in patients with non Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and chronic lymphocytic leukemia (CLL) who were treated in our institution. Also, we assessed predictive model for chemotherapy-associated thrombosis developed by Khorana and create new model for the identification of lymphoma patients at risk for thromboembolism

**Methods:** We reviewed all medical records of patients with NHL, HL and CLL diagnosed according to the World Health Organization classification and treated at our institution between January 2006 and December 2014.

**Results:** A total of 1054 patients, with malignant lymphoma were eligible for analysis: 576 patients were men (54.6%) and 478 were women (45.4%), and mean age was 54.0 years. A total of 510 patients (48.4%) had high-grade lymphoma, 211 had low-grade lymphoma (20.0%), 153 had Hodgkin lymphoma (14.5%), and 75 (7.1%) had other forms of lymphoma; HLL had 105 (10.0%) patients. Out of our lymphoma patients, 72 (6.8%) had at least one TE. In 49 patients (68%), thrombosis occurred during treatment or up to 3 months after completion of therapy, whereas in 23 patients (32%) thrombosis was diagnosed prior to therapy. Patients with aggressive NHL had increased risk for TE (8.6%) compared to all other types of lymphoma patients (RR = 2.1; 95% CI for RR 1.2–3.6; $p = 0.009$) (incidences of TE in low NHL, HL and other forms of lymphoma were 4.3%, 3.3% and 6.7%). Patients with HLL had an 8.6% incidence of TE. Patients with high grade lymphomas, overweight patients (BMI > 25 kg/m$^2$), patients with reduced mobility (ECOG > 2), and patients with recent operative procedure (<4 weeks) had increased risk for thrombosis ($p < 0.05$ for all). No difference regarding age, gender or disease stage was found. Based on previously mentioned risk factors a new TE risk model was developed for patients with lymphoma (excluding HLL). Patients who had at least two out of four risk factors fall into high-risk group for TE (RR = 13.0; 95% CI for RR 7.4–23.0; $p < 0.001$). Predictive model for chemotherapy-associated TE developed by Khorana was also evaluated on this group of patients (RR = 7.0; 95% CI for RR 3.3–14.7; $p < 0.001$). In multivariate analysis our newly developed model for TE events in lymphoma patients were predictor of TE events and independent from Khorana model.

**Conclusions:** Prediction tools for estimating the risk of TE events in lymphoma patients have not been established but our newly developed model should be used in addition to chemotherapy-associated model in guiding best practice in the prevention of thromboembolism in patients living with lymphoma.

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**SERUM LEVELS OF CCL22 AND CCL25 MIGHT PREDICT SKIN RASH INDUCTION, THE COMMONEST ADVERSE EVENT BY BENDAMUSTINE IN THE TREATMENT OF MALIGNANT LYMPHOMA**

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**Introduction:** Bendamustine has been recently approved for the treatment of low-grade malignant lymphoma. In spite of the better efficacy, skin rash is the commonest adverse event by bendamustine in the treatment of malignant lymphoma. To understand the mechanism of the skin rash, we prospectively examine the relationship between skin rash and chemokines.

**Methods:** After informed consent to the patients who would received bendamustine (B) or bendamustine + rituximab (BR), the sera were collected before and after the treatment of B or BR. Cytokine/Chemokine array was performed by Bio-Plex system.

**Results:** 20 patients with FL ($n = 18$), MCL ($n = 1$), and low-grade B-cell lymphoma ($n = 1$) were enrolled in this study. All patients wee treated with B or BR regimen. Nine patients showed skin rash after treatment of bendamustine. In cytokine/chemokine array, serum levels of CCL22 and CCL25 before the treatment were significantly higher in skin rash group, and serum levels of CXCL10 and CXCL11 after skin rash were higher significantly.

**Conclusion:** Serum levels of CCL22 and CCL25 might predict skin rash induction, the commonest adverse event by bendamustine and CXCL10 and CXCL11 might contribute to induction of skin rash by bendamustine.

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**OSTEOPOROSIS IN ADULTS WITH HEMATOLOGICAL MALIGNANCIES AT A LOCAL HOSPITAL, INTERIM ANALYSIS**

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**Introduction:** Osteoporosis is known as an important consequence of cancer and its treatment in the pediatric age group. Its effects may persist throughout the lifetime of survivors and negatively impact their quality of life.

Contributing factors include bone marrow involvement, large doses of steroids, prolonged bed rest and infections.

Though osteoporosis/osteopenia has been well documented in the pediatric age group, only sparse reports exist for the adult population, all involving small cohorts of patients.

The purpose of this study is to evaluate the prevalence of osteoporosis/osteopenia as measured by bone density in a cross-section of patients with hematological malignancies, and to identify which subgroup of malignancies has the highest prevalence and severity of osteoporosis. We report an interim analysis of the first 84 patients.

**Methods:** Patient population: 200 patients with hematological malignancies from the Hematology Department at SZMC.

Inclusion: all patients with hematological malignancies, excluding multiple myeloma/MGUS, before, during or after therapy, who were willing to undergo bone density testing and give informed consent.

DXA (bone density) adjusted for age, weight and height was measured in the lumbar vertebrae and hip.

Osteoporosis was defined by DXA T-score below −2.5 in at least one of the studied lumbar vertebrae and hip measurements.

The Z-score was used to compare study subjects with the normal population.

**Results:** Of 84 patients with hematological malignancies, 52% were females. The median age of diagnosis was 63.3 years. As the number of patients in each disease subgroup was small, we divided the patients into 3 major diagnostic groups: AML 18 (21.4%), CLL 15 (17.9%), NHL 43 (51.2%). Eight patients could not be grouped.

86% of the females were in menopause. The median time from diagnosis until DXA testing was 1.85 years (range 0–22.6), and median age at DXA testing 65.2 (range 22.8–89.8).

Osteoporosis of the spine as measured by T-score was detected in 30.12% and osteoporosis of the hip in 15.66% of all patients. Lymphoma patients were the most susceptible to osteoporosis, followed by AML, while CLL had normal scores.

In order to compare our subject population with the normal population we used the Z-score. In the normal population only 2.14% have a *Z*-score lower than −2. In our subject population 13.9% had a *Z*-score lower than −2 (more than 6 times greater prevalence in our subjects).

**Conclusions:** 1. We found a high prevalence (rate) of osteoporosis as measured by spinal T-score, 30.1% among patients with hematological malignancies who were tested.

2. Lymphoma patients were at the highest risk for osteoporosis and associated morbidity.

Further investigation in a larger group of patients is needed to confirm these results.

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**PROPHYLACTIC LAMIVUDINE PLUS ADEFOVIR DIPIVOXIL VERSUS LAMIVUDINE IN PREVENTING HBV REACTIVATION IN HBSAG-POSITIVE LYMPHOMA PATIENTS AFTER CHEMOTHERAPY**

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**Introduction:** Lamivudine (LAM) is wildly used in preventing hepatitis B virus (HBV) reactivation in hepatitis B surface antigen (HBsAg)-positive patients after chemotherapy, but its major limitation is drug-resistant mutants. Adefovir dipivoxil (ADV), a synthetic adenine nucleotide analog, plus LAM had higher efficacy and lower resistance rates than LAM alone, however, data of the efficacy of LAM + ADV as prophylaxis in HBsAg-positive patients receiving chemotherapy are still limited. The aim of this study was to evaluate the efficacy of prophylactic LAM + ADV compared with LAM alone in preventing HBV reactivation in HBsAg-positive lymphoma patients undergoing chemotherapy, especially in those with baseline high HBV DNA load ($\geq 2 \times 10^3$ IU/ml).

**Methods:** Twenty-one HBsAg-positive lymphoma patients undergoing chemotherapy and received LAM (100 mg/d) plus ADV (10 mg/d) (LAM + ADV group) were analyzed by comparing with 47 control patients who received prophylactic LAM (100 mg/d) (LAM group).

**Results:** The rates of HBV reactivation in the LAM + ADV group and LAM group were 19% and 27.7%, respectively ($p = 0.449$). In patients with pre-chemotherapy HBV DNA load $\geq 2 \times 10^3$ IU/ml, the rate of HBV reactivation was significantly lower in the LAM + ADV group compared with LAM group (17.6% vs 55.0%; $p = 0.020$). Among the patients with HBV DNA load $< 2 \times 10^3$ IU/ml, no significant difference was observed in the rate of HBV reactivation (7.4% vs 25.0%; $p = 0.267$) between the two groups. Pre-chemotherapy HBV DNA load $\geq 2 \times 10^3$ IU/ml was an independent risk factor for HBV reactivation in HBsAg-positive patients. LAM + ADV treatment was the only independent protective factor for HBV reactivation in patients with pre-chemotherapy HBV DNA load $\geq 2 \times 10^3$ IU/ml.

**Conclusion:** LAM + ADV and LAM are both efficacious in the prophylaxis of hepatitis B reactivation in HBsAg-positive patients. LAM + ADV may be used in preference to LAM in patients with pre-chemotherapy HBV DNA load $\geq 2 \times 10^3$ IU/ml.

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**HBV REACTIVATION AFTER WITHDRAWAL OF PROPHYLACTIC ANTIVIRAL THERAPY IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA**

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**Introduction:** Prophylactic antiviral therapy can prevent hepatitis B virus (HBV) reactivation and reduce mortality in HBV-infected lymphoma patients. However, the exact incidence and severity of HBV reactivation after the withdrawal of prophylactic antiviral therapy is unknown.

**Methods:** We retrospectively analyzed 107 newly diagnosed diffuse large B-cell lymphoma patients with HBV infection who received chemotherapy (with or without rituximab). Prophylactic antiviral therapy was administered during chemotherapy and for 6 months after in all patients. Delayed HBV reactivation was defined as a confirmed increase in serum HBV DNA level of 1 $\log_{10}$ IU/ml from nadir (lowest value) or a new appearance of serum HBV DNA after withdrawal of antiviral therapy.

**Results:** The incidence of delayed HBV reactivation was 21.7% (10/46) in HBsAg-positive group and 0 (0/61) in HBsAg-negative/anti-HBc-positive group ($p < 0.001$). HBV-related hepatitis flares occurred in 5 HBsAg-positive patients and no HBsAg-negative/anti-HBc-positive patients ($p = 0.008$). No HBV-related fulminant hepatitis or hepatitis-related death occurred. No difference in overall survival was observed between patients with and without HBV reactivation after a median follow-up of 24.8 months (range: 2.6–62.8). The multivariate analysis showed that female gender and lengthy cycles of chemotherapy (more than eight cycles) were independent risk factors of HBV reactivation in HBsAg-positive patients.

**Conclusions:** Prophylactic antiviral therapy could be withdrawn 6 months after the cessation of chemotherapy in HBsAg-negative/anti-HBc-positive patients. However, a longer course of prophylactic antiviral drug administration may be an optimal option to prevent delayed HBV reactivation for HBsAg-positive patients.

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**DEVELOPMENT OF EORTC DISEASE SPECIFIC QUALITY OF LIFE QUESTIONNAIRES IN PATIENTS WITH HODGKIN LYMPHOMA, NON-HODGKIN LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKAEMIA**

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**Introduction:** Advances in the management of patients with lymphoma or chronic lymphocytic leukaemia (CLL) have changed dramatically the survival outcomes the last two decades. There is increasing number of new agents for these patients and quality of life (QoL) is becoming primary objective. Unfortunately, disease-specific QoL questionnaires to assess the impact during or after treating patients with Hodgkin lymphoma (HL), high-grade non-Hodgkin lymphoma (HG-NHL), low-grade non-Hodgkin lymphoma (LG-NHL) and CLL are lacking. This prospective EORTC study describes the combined development of four disease-specific QoL questionnaires for patients with HL, HG-NHL, LG-NHL and CLL to supplement the European Organization for Research and Treatment of Cancer (EORTC)-QLQ C30 core cancer questionnaire.

**Methods:** Questionnaire development was conducted according to guidelines from the EORTC Quality of Life Group. Phase I comprised generation of QoL issues relevant to patients. Phase II included operationalization and assessment of item relevance. In phase III, items were pretested in a cross-cultural sample representing all four malignancies to assess issues such as comprehensibility and intrusiveness of items. Data were analysed per malignancy.

**Results:** Seventy-five QoL issues were identified through focus groups and systematic literature searches. Semi-structured interviews with 80 health-care professionals and with 245 patients (75 HL, 66 HG-NHL, 41 LG-NHL and 63 CLL) resulted in a provisional module of 39 items representing items relevant for all or at least one of the four malignancies. In phase III this was further tested in 67 HL, 117 HG-NHL, 67 LG-NHL and 86 CLL patients with different phases of disease and treatment from five European countries. Results from the interviews, clinical experiences and statistical analyses resulted in a questionnaire with 27 items for HL (EORTC QLQ-HL27), 29 items for HG-NHL (EORTC QLQ-NHL-HG29), 20 items for LG-NHL (EORTC QLQ-NHL-LG20) and 17 items for CLL (EORTC QLQ-CLL17). The items are conceptualized in several multi-item scales: symptom burden, physical condition/fatigue, worries/fears health

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and functioning, emotional impact (not in the CLL module), neuropathy (only in the HG-NHL module).

**Conclusions:** This prospective study provides four modules for use in clinical trials and observational research in conjunction with the EORTC QLQ-C30 for assessment of QoL for patients with HL, HG-NHL, LG-NHL and CLL. The Phase IV prospective international EORTC study of the questionnaire development, implementing and validating the four questionnaires (EORTC QLQ-HL27, QLQ-NHL-HG29, QLQ-NHL-LG20 and QLQ-CLL17) has been approved and will start recruitment in 2015.

**Funding:** The project was allocated an EORTC Quality of Life Group (QLG) grant for module development, Phases I–III.

**457**
**INCIDENCE OF SECONDARY PRIMARY NEOPLASMS IN A COHORT OF PATIENTS WITH FOLLICULAR LYMPHOMA. SINGLE CENTER REPORT**

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**Background**: Follicular lymphoma (FL) account approximately one third of all non-Hodgkin lymphomas, it is know that primary affect males and white population; also is established that any patient diagnosed for a primary cancer have an increased risk to be diagnosed for a second primary neoplasm (SPN), respect to follicular lymphoma there are few reports about a 25% increased risk for all non-Hodgkin lymphomas not related with histologic subtype. Although is recognized that chemotherapy, radiotherapy and radioimmunotherapy (RIT) increased this risk; there are recent reports about the use of radioimmunotherapy with $^{90}$Y ibritumomab tiuxetan ($^{90}$Y-IT) that show an increased risk for SPN. RIT is available in our center since 2005 and we have been accumulated a long experience. Considering this, we analyze our data and compare the incidence of SPN in all patients with FL treated in our institution with different schedules including or not $^{90}$Y-IT.

**Aims**: To analyze the incidence of second primary neoplasm in FL patients diagnosed and treated in our center, searching for relationship with RIT.

**Patients and Methods**: A chart review was carried using the registry of diagnosed patients, from the Department of Hematology; all consecutive patients diagnosed of follicular lymphoma of any grade according to WHO since 2001 were included; a review of clinical records was conducted: demographic and clinical data, incidence of previous cancer and therapies, SPN (basocellular skin and *in situ* cervix carcinoma were excluded), number of chemotherapies, relapses, therapy with $^{90}$Y-IT, actual status, and cause of death were recorded.

**Results**: A total of 251 FL patients were registered, male/female ratio: 107/144, mean age 59.9 years (15–86), Stage I: 7.2%, II: 11.0%, III: 24.1%, IV: 57.8%, FLIPI: low risk: 62.15%, intermediate: 13.9%, high risk: 10.3%. A 27.8% of patients receiving two or more chemotherapy schedules, 10.5% had underwent an auto-SCT. One hundred patients (39.8%) received $^{90}$Y-IT (55 as a second or third line of therapy and 45 as a consolidation therapy). The mean follow-up for all patients is 108 months (median 49 months). Respect to incidence of neoplasms we found that 38 (15.1%) patients have a registry of at least 2 primary cancers, in 16 (42.1%) of them FL diagnosis were the second primary neoplasm. In 22 patients who developed a SPN, 11 had been received two or more lines of therapies and 5 of them including RIT. According to relationship with $^{90}$Y-IT, in 3 SPN were diagnosed before the use of RIT and in one at the same time of RIT, and in 8 patients the diagnosis of SPN occur after RIT at a mean time of 32.3 months. For all patients the SPN were recorded at a mean time of 24.5 months after FL diagnosis.

**Conclusion**: This work summarizes relevant information outside clinical trials about the incidence of second primary neoplasm in one cohort of follicular lymphoma patients treated (100) or not (151) with $^{90}$Y-IT. In our experience, $^{90}$Y-IT did not increase significantly the risk of SPN.

A more exhaustive analysis will be presented in case of acceptance.

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**THE IMPACT OF COMORBID DISEASE HISTORY ON THE SURVIVAL OF NON-HODGKIN LYMPHOMA AND MYELOMA—A SWEDISH POPULATION-BASED STUDY**

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**Introduction:** Comorbid diseases are prevalent in patients diagnosed with hematological malignancies and increase overall mortality. The impact of comorbid diseases on cancer-specific mortality in lymphoma and myeloma, taking competing risks into account, has not been evaluated in population-based settings.

**Methods:** Using the Swedish Cancer Register, we identified patients >18 years of age with a first diagnosis of diffuse large B-cell lymphoma (DLBCL, $N = 2384$), follicular lymphoma (FL, $N = 1561$), T-cell lymphoma ($N = 704$) or myeloma ($N = 4584$) 2002–2009. Prior comorbid disease history was assessed through the National Patient Register using hospital discharge and visit codes of diseases included in the Charlson comorbidity index, adding psychiatric disease. Mortality rate ratios (MRR) were computed through 2012 using Poisson regression. Probability of cancer-specific death in the presence/absence of comorbidity by age was computed using flexible parametric survival models.

**Results:** The most prevalent comorbid diseases among the DLBCL, FL, T-cell lymphoma and myeloma patients were history of another cancer (10–13%) and cardiovascular disease (7–9%). Comorbidity was associated with statistically significant moderately increased risk of cancer-specific death ($MRR_{DLBCL}$: 1.33, 95% CI: 1.19–1.48; $MRR_{FL}$: 1.27, 95% CI: 1.03–1.56; $MRR_{T\text{-}cell}$: 1.20, 95% CI: 0.98–1.46; $MRR_{myeloma}$: 1.17, 95% CI: 1.08–1.28). Cardiovascular, cerebrovascular, chronic pulmonary and psychiatric disorders increased cancer-specific mortality in patients with DLBCL and myeloma, diabetes and liver disorders in patients with DLBCL and FL, and renal disorders and dementia in myeloma patients. In absolute terms, accounting for competing risks, the differences in cancer-specific survival proportions among DLBCL patients with and without comorbidity were greater at ages 60–69 years than among older patients, but greater for FL patients 80–89 years than younger patients. Among patients with myeloma, difference in absolute survival proportions by comorbidity did not vary by age.

**Conclusions:** Cardiovascular, cerebrovascular, liver, renal, chronic pulmonary and psychiatric diseases, diabetes and dementia are associated with adverse outcomes in lymphoid neoplasms, likely due to lower treatment tolerance in these patients. The impact of comorbidity varied by age, with notable differences among subtypes of lymphoid neoplasms. The results indicate the need for more efficient and less toxic treatment regimes in most groups of comorbid and elderly DLBCL, FL, T-cell and myeloma patients.

## IMAGING

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**CONCORDANCE BETWEEN FDG-PET/CT AND BONE MARROW BIOPSY IN EARLY HODGKIN LYMPHOMA PATIENTS**

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**Introduction:** The high sensitivity of PET-CT for bone marrow involvement (BMI) has recently called into question the continued use of bone marrow biopsy (BMB) in several common histologies. In early-stage Hodgkin´s Lymphoma (HL) patients rarely have BMI and those with advanced stage would benefit from image-guided biopsies rather than blinded BMB. In HL, diffuse and homogeneous increased uptake often represents reactive hyperplasia and should not be mistaken with lymphomatous involvement. Thus, if PET/CT is performed, a BMB is may be no longer required for the routine evaluation of patients with HL. The aim of this study was to correlate the results of BMB in early-stage HL patients selected by FDG-PET/CT images in order to obviate the BMB.

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**Methods**: One hundred sixty-seven patients with HL were referred for FDG-PET/CT and BMB (unilateral) at the initial staging. According to PET/CT results, only early HL patients were selected in order to correlate with BMB (considered as the reference standard). Involvement of a single extralymphatic site, supra or infradiaphragmatic nodes, and eventual presence of a bulky lesion were recorded. Focal bone marrow uptake was considered positive (BMI). Diffuse and homogeneous bone marrow FDG uptake below liver uptake was considered negative, and equal or higher than liver uptake was considered more likely reactive BM changes.

**Results:** Ninety-one out of 167 patients were classified as early HL stage (54%). Five patients were excluded due to absence of BMB. Mean age patient of the remaining 86 (39 males) was 39 years old (range 16–82). PET/CT images classified these patients in the stages: I, 12 (14%); and II, 74 (86%). PET/CT images showed supradiaphragmatic lymph nodes involvement in 77/86 patients (89%), infradiaphragmatic in 7/86 (8%) and single extralymphatic site in 2/86 (2%). A Bulky mass was present in 32 patients (37%). In 38/86 patients (44%) PET/CT showed a BM uptake below liver and the remaining 48 (66%) had diffuse uptake equal or higher than liver uptake. The results of BMB in all these patients (86) were negative. Concordance between PET/CT and BMB was 100%.

**Conclusion:** Diffuse and homogeneous BM uptake detected by PET/CT reflects more likely reactive BM changes in early HL patients. Excellent concordance between PET/CT and BMB has been found, so we believe there is evidence to obviate BMB in this group of patients.

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### WHAT DO WE MISS AND GET IF WE REPLACE BONE MARROW BIOPSY IN DLBCL WITH STAGING PET/CT?

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**Introduction**: DLBCL is the most frequent type of lymphoma taking around 40% of all non-Hodgkin lymphomas. Assessing of bone marrow involvement (BMI) during staging of the disease is important for clinical stage determination. According to new guidelines (Cheson *et al*., *J Clin Oncol* 2014; 32: 3059–3067) bone marrow biopsy (BMB) is no longer required and FDG PET substitutes it.

**Methods:** We analyzed retrospectively 217 [112 men and 105 women, median age 65 years (21–87 years)] consecutive patients with newly diagnosed DLBCL between 2011 and 2014 who had available staging BMB and FDG PET. PET was evaluated visually. Increased FDG uptake was considered as positive finding. Positive PET findings were divided into 2 groups, focal and diffuse. BMB was evaluated histologically. Type of involvement (concordat or discordant) and percentage of malignant cells was determined. Particular findings were compared; impact to clinical stage (CS) and outcome of treatment was analyzed.

**Results:** BMI by BMB only, PET only and PET/BMB were found in 32 (14.7%), 51 (23.5%) and 61 (28.1%) patients, respectively. Corresponding BMI by PET and BMB was observed in 178 (82.0%) patients, 156 were negative and 22 (10.1%) were positive (4 of them had diffuse PET pattern). Different findings were observed in 39 (17.9%) patients, out of these 29 (13.4%) had positive PET and negative BMB, and 10 (4.6%) had negative PET and positive BMB. In the 29 PET+/BMB− patients were 3 patients with diffuse PET pattern, and these we considered as false positive, PET driven clinical stage (CS) was downgraded by BMB from CS IV to localized one in 2 out of 3 patients. Among 10 PET−/BMB+ 8 patients had discordant BMI according to histology and 2 only 5% of DBCL cells. All these PET findings can be considered as false negative, and PET-driven CS was upgraded from localized to CS IV in one patient. After immunochemotherapy 30 of 32 patients with initial BMI had BMB, 2 of them (6.7%) remained BMB+. Of 51 patients with initial PET+ finding, 46 had restaging PET after CHT, and 4 (8.7%) had positive findings. In PET+/BMB+ patients (at staging), after immunochemotherapy 2 of 22 remained PET+/BMB+ none of them remained positive only with one method.

**Conclusions:** PET has very good correlation with BMB and increases finding of BM involvement from 14.7% to 23.5%, combining the methods (excluding false-positive PET findings) we get on 26.7% BMI findings. PET however misses BM involvement (especially discordant and small volume concordant) which represents 4.6% of patients. On the other hand almost half of the diffuse PET+ cases are false positive (three out of seven). We recommend to perform BMB in all cases with diffuse PET pattern of positivity, regarding the false-positive findings. In response assessment we suggest to repeat BMB after therapy if initially positive. Although PET can replace BMB in the majority cases and increases the finding of BMI, some cases remain to be evaluated by BMB as well.

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### THE ROLE OF BONE MARROW INCREASED FDG UPTAKE ON PET/CT IN PATIENTS WITH LYMPHOMA-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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**Introduction**: In detecting bone marrow involvement (BMI) the $^{18}$F-2-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (FDG-PET/CT) may be an appropriate technique to replace bone marrow biopsy (BMB) or complementally detect positive BMI patients missed by BMB in malignant lymphoma, including newly diagnosed Hodgkin lymphoma, diffuse large B-cell lymphoma and extranodal NK/T cell lymphoma. The role of FDG uptake of BM on PET/CT in patients with lymphoma-associated hemophagocytic lymphohistiocytosis (LA-HLH) remains uncertain.

**Methods:** Thirty-four LA-HLH patients were retrospectively enrolled from August 2009 to March 2014 in our study. All patients underwent both PET/CT and comprehensive BM examinations prior to treatment. SUVmaxBM is defined as the maximum of the SUVmax of the thoracic (T10–12), lumbar (L2–4) vertebral bodies, limb long bone (the humerus and femurs) and the marrow rich in the skeleton (the sternum, anterior superior spine and spina iliaca posterior superior) on PET/CT. Moreover, SUVmeanBM represents the average of the SUVmax of the above mentioned skeleton. The SUVmaxBM with hypermetabolism showing a value higher than $SUV_L$ was considered BMI detected by FDG-PET/CT. Presence of BMI was confirmed by marrow examinations in any of the following findings: (1) abnormal lymphocytes were observed by morphology or immunohistochemical staining; (2) monoclonal lymphocytes of B-cell, T-cell, NK-cell were found through flow cytometry or molecular biology analysis.

**Results:** A total of 32 (94.1%) patients were diagnosed with BMI by PET/CT and the BMI was confirmed by marrow comprehensive examinations in 25 patients (73.5%). Comparison between PET/CT and BM examinations for the assessment of BMI indicated statistical difference ($p = 0.039$). And PET/CT had an elevated rate of false-positive results, showing diffusely increased FDG uptake of BM. The specificity of PET/CT in detecting BMI was 11.1% compared to BM examinations. However, significant positive correlations of SUVmaxBM with the level of CRP ($r = 0.515$, $p = 0.004$), ferritin ($r = 0.370$, $p = 0.031$) and sCD25 ($r = 0.508$, $p = 0.008$) were observed. The SUVmeanBM revealed strong positive correlations with CRP ($r = 0.403$, $p = 0.030$), ferritin ($r = 0.386$, $p = 0.024$) as well as sCD25 ($r = 0.458$, $p = 0.019$), and strong negative correlation with fibrinogen ($r = -0.378$, $p = 0.027$). A SUVmaxBM of 5.35 and a SUVmeanBM of 4.38 were the optimal cut-offs by ROC curves for predicting patients' outcome. By univariate analysis, significant difference was found between high group and low group of SUVmaxBM ($p = 0.017$) and SUVmeanBM ($p = 0.013$) using log-rank test statistics.

**Conclusions:** Our findings suggest that FDG uptake of BM failed to detect lymphomatous BMI, but might reflect the level of cytokines storm to a certain extent and might be as prognostic factors in patients with LA-HLH.

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### THE NOVEL ROLE OF BONE MARROW INVOLVEMENT DETECTED BY BOTH STAGING AND INTERIM FDG PET-CT IN DIFFUSE LARGE B-CELL LYMPHOMAS

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**Introduction**: The aim of our study was to confirm the diagnostic and prognostic value of bone marrow involvement (BMI) assessed by PET(0)-CT [PET(0)-BMI], evaluate the impact of PET(0)-BMI on the classical International Prognostic Index (IPI) and the significance of BMI detected by PET(inter)-CT (two or three cycles of standard R-chemotherapy) [PET(inter)-BMI] in diffuse large B-cell lymphoma (DLBCL).

**Methods:** One hundred thirty-five DLBCL patients were retrospectively enrolled in our study, for whom underwent both a PET(0)-CT and staging bone marrow biopsy (BMB). The presence of BMI in patients was determined by BMB and PET(0)-CT results.

**Results:** BMI was detected by PET(0)-CT in 35 (25.9%) and by BMB in 18 (13.3%) cases with the total number of BMI in 38 (28.2%) according to our criteria. Both pretreatment PET(0)-BMI(+) ($p < 0.0001$) and BMB(0)-BMI(+) ($p = 0.032$) were significantly associated with worse progression-free survival (PFS). However, by multivariate analysis, only IPI > 2 (HR, 3.033; [95% CI, 1.351–6.807]; $p = 0.007$) and PET(0)-BMI(+) (HR, 2.521; [95% CI, 1.327–4.781]; $p = 0.005$) remained independent predictive factors of PFS. Among the 60 patients with inter-risk of IPI (2 − 3), the patients with PET(0)-BMI(+) (19 patients) had significantly inferior PFS than patients with PET(0)-BMI(−) (41 patients) ($p = 0.005$). However, the 41 patients with PET(0)-BMI(−) had similar PFS with the initial overall 60 patients ($p = 0.285$). Moreover, we can find that the other 19 patients had similar PFS to the patients whose IPI scores were 4–5 ($p = 0.661$). Among the 35 patients with positive PET(0)-BMI(+), patients with PET(inter)-BMI(−) had better PFS than patients with a positive BMI ($p = 0.009$). In contrast, patients with ΔSUVmax more than 67.5% did not significantly differ in PFS comparing with patients with ΔSUVmax less than 67.5% ones ($p = 0.052$)

**Conclusions:** PET(0)-BMI demonstrates a better diagnostic and prognostic performance. The patients with IPI of inter-risk meanwhile with PET(0)-BMI(+) should be treated as IPI score of high-risk. Furthermore, PET(inter)-BMI(−) appears vital significance of predicting better prognosis in DLBCL patients with PET(0)-BMI(+).

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**PET/CT FOR THE EARLY INTERIM EVALUATION OF RESPONSE IN ADVANCED HODGKIN LYMPHOMA AFTER ABVDX2: EFFECTIVE SALVAGE WITH BEACOPP BUT LOW NEGATIVE PREDICTIVE VALUE FOR STAGE IV**

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**Introduction:** A positive PET/CT after two cycles with ABVD (PET-2) is an unfavorable prognostic factor for patients with intermediate-stage and advanced-stage HL. The aim of this study was to report our experience with PET-2 in patients with advanced HL, describe further treatment approach in 'real life' and evaluate the accuracy of PET-2 in predicting the outcome.

**Methods:** 86 patients (85/86, <60 years) with advanced HL according to GHSG (Stage III/IV or IIB with bulky mediastinum and/or extranodal disease) who were treated with ABVD and underwent PET-2 in six centers in Athens, Greece, were retrospectively evaluated. PET-2 was assessed according to Deauville criteria and was considered as positive when graded as 4 or 5 (residual uptake greater than the uptake of liver). Treatment modification (switch to BEACOPP) was at the discretion of the treating physician. Radiotherapy was administered according to standard practice.

**Results:** The median age of the 86 patients was 30 years (16–73), 56% were males, 83% suffered from NSCHL, 43%, 41% and 16% were classified as Stages IV, III and II and 70% had B-symptoms. The median value of IPS was 2 (0–6). Based on initial PET/CT, 51% of patients had Stage IV. The characteristics of the present patient group were more unfavorable compared to other published studies. PET-2 was negative in 65 (76%) and positive in 21 (24%) patients. Overall, 3-year PFS was 76%. The strong prognostic value of PET-2 was verified, since 3-year PFS was 86% in PET-2(−) and 43% in PET-2(+) patients ($p < 0.0001$), despite switch to BEACOPP in 8/21 patients. *PET-2(+) patients*: 8/21 PET-2(+) patients received BEACOPP, while 13 continued with ABVD (6 received six cycles with BEACOPP-esc and 2 received six and four cycles of BEACOPP-base, respectively). Three-year PFS was 73% vs 25% for BEACOPP and ABVD, respectively ($p = 0.09$). *PET-2(−) patients*: Based on conventional staging 3-year PFS did not differ significantly between Stage II–III and Stage IV (91% vs 79% respectively, $p = 0.13$). However, when staging was based on the initial PET/CT, 3-year PFS was significantly shorter in patients with Stage IV compared to patients with Stage II–III (73% vs 97%, $p = 0.008$).

**Conclusions:** The results of the present study confirm the strong prognostic value of PET-2, as the possibility of relapse was much higher in PET-2(+) patients, despite the more aggressive therapeutic approach adopted for some of them. In case of PET-2(+) physicians were frequently reluctant to intensify treatment, possibly due to the availability of potentially effective salvage in case of definite ABVD failure. However, patients treated with BEACOPP upon PET-2(+) tended to have superior outcomes. PET-2(−) patients with Stage IV had inferior outcome with >20% relapse rate. The last observation may have a significant impact on the design of treatment strategies.

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**INTERIM AND 'END OF CHEMOTHERAPY' FDG-PET ARE STRONG PROGNOSTIC FACTORS IN EARLY-STAGE HODGKIN LYMPHOMA PATIENTS TREATED WITH ABVD FOLLOWED BY RADIOTHERAPY**

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**Purpose:** To evaluate the prognostic role of both interim-PET (iPET) and end of chemotherapy-PET (fPET) in patients with early stage Hodgkin Lymphoma (HL) treated with ABVD followed by Involved Fields/Involved Sites radiotherapy (RT).

**Methods and Materials:** We included 257 patients with Stage I–IIAB HL treated between March 2003 and July 2011. All were staged with FDG-PET before chemotherapy and after 2 ABVD cycles; 165 patients were also evaluated by PET at the end of ABVD before RT. All images were retrospectively reviewed by local nuclear medicine physicians, and re-scored using the Deauville 5-point scale criteria. We considered as positive a score ≥3.

**Results:** After a median follow-up of 56 months 250 patients are alive and without evidence of disease, one patient is alive with disease relapse and 6 patients died. The 5-year overall survival (OS) was 97.5% and the 5-year disease-specific survival (DSS) was 98.3%. The 5-year progression-free survival (PFS) was 95.6%. After

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iPET revision, the number of patients with a positive iPET was 43/257 (16.7%). Analyzing patients with both iPET and fPET, 142/165 patients (86%) achieved a final complete metabolic response after ABVD, whereas 23/165 (14%) had still a positive fPET. A concordance between the iPET and fPET (iPET/fPET−/− or iPET/fPET+/+) was observed in 149/165 patients (90%). A change from iPET negative to fPET positive was observed in 1.5% of patients (2/130). The sensitivity, specificity, and negative and positive predictive values of iPET for predicting fPET negativity were 0.91, 0.90, 0.60 and 0.98, respectively. For iPET-negative and iPET-positive patients, the 5-year PFS rates were 98.1% and 83.7% respectively ($p = 0.0001$); for fPET-negative and fPET-positive patients, the 5-year PFS rates were 97% and 78%, respectively ($p = 0.0001$). Combining the iPET and fPET results, the 5-year PFS were 98%, 92% and 78% in iPET/fPET−/−, iPET/fPET+/−, iPET/fPET+/+ groups, respectively ($p = 0.0005$). At univariate analysis, positive iPET ($p = 0.02$) and fPET (0.0001) resulted associated with a worse OS; the same prognostic factors (B symptoms ($p = 0.011$) and positive iPET ($p < 0.0001$) and fPET ($p < 0.0001$)) resulted associated also with a worse PFS. The 5-year OS rates were 98% and 82% in fPET-negative and fPET-positive patients ($p = 0.0001$), respectively. At Cox regression analysis, adjusted by age, B-symptoms, stage, histology and CHT cycles, the fPET positivity was correlated with a worse OS [HR (95% CI) 12.1 (2.11–68.8), $p = 0.005$].

**Conclusions:** This retrospective study confirms the prognostic role of interim PET in early-stage HL patients treated with combined modality therapy, giving new information on the role of the 'final' PET performed before RT. Negative patients show optimal outcomes both in terms of PFS and OS. Patients with only i-PET positivity show satisfactory outcomes following the continuation of ABVD and radiotherapy. Conversely, patients with a persistent PET positivity after four to six ABVD cycles are at higher risk of relapse and death, albeit the use of radiation.

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### THE IMPACT OF PERIPHERAL BLOOD ABSOLUTE LYMPHOCYTE/ABSOLUTE MONOCYTE RATIO ON THE PROGNOSTIC EFFECT OF INTERIM PET/CT SCAN RESULTS

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**Background:** It is a well-known fact that in advanced-stage Hodgkin lymphoma (HL) patients, the interim 2-[$^{18}$F] fluoro-deoxy-D-glucose positron emission tomography (PET2) carried out after the second cycle of adriamycin, bleomycin, vinblastine, and dacarbazine has a higher prognostic value, compared to the traditionally used International Prognostic Score; however, the negative predictive value of PET2 examination is excellent, higher than 90%, but its positive predictive value is not strong enough, usually between 50% and 70%. A decreased peripheral absolute lymphocyte/monocyte ratio (LMR) was reported to be an unfavorable prognostic marker in HL. We aimed to investigate whether combining LMR and interim PET/CT scan result (PET2) holds a stronger prognostic value than using PET2 alone.

**Methods:** One hundred twenty-two HL patients were investigated, who were diagnosed and treated between 2007 and 2013. Absolute lymphocyte and monocyte values measured at the time of diagnosis were considered as LMR. PET2 examination was carried out after the second cycle of chemotherapy between Days 11 and 14. Two-sample *t*-test was used to examine homogeneity of the samples; survivals were calculated according to the Kaplan–Meier method. Log-rank test was used to determine level of significance. The effect of variants on survival results was examined using the univariate and multivariate analyses of the Cox regression hazard model. The best LMR cutoff value was determined by the receiver operating characteristic curve.

**Results:** The best LMR cutoff value was 2.11 in the case of our patients (LMR > 2.11: favorable, LMR ≤ 2.11: unfavorable). Overall and progression-free survivals (OS/PFS) were significantly worse in both the lower LMR (≤2.11) (OS: $p = 0.041$, PFS: $p = 0.05$) and PET2-positive groups (OS: $p < 0.001$, PFS: $p < 0.001$). In the PET2-positive patient group ($n = 32$), the low LMR result meant a significantly worse OS (0.03) and PFS (0.001). Both LMR and PET2 proved to be independent prognostic factors by multivariate analysis, which strengthened each other.

**Conclusion:** LMR measured at the time of diagnosis could be an easily available, cheap prognostic factor in the daily routine, which may increase the positive and negative predictive values of PET2 results, thus selecting those patients who may benefit from non-standard treatment.

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### EARLY $^{18}$F-FDG PET/CT RESPONSE ASSESSMENT IN DIFFUSE LARGE B-CELL LYMPHOMA. COMPARISON BETWEEN VISUAL AND SEMI-QUANTITATIVE ANALYSES

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**Introduction:** The aim of this study was to compare visual and semiquantitative analyses in the early response evaluation with PET/CT (interim PET) in diffuse large B-cell lymphoma and its correlation with the final response.

**Methods:** Forty-six patients with newly diagnosed diffuse large B-cell lymphoma (43) and follicular 3B (3) were included in this prospective study. Median age patient was 59 years (range 19–81) and 23 of them were men. Stages were I in 1, II in 11, III in 5, and IV in 29. All patients underwent PET/CT three times: at initial staging (baseline), at midtherapy or interim (after three to four cycles), and at the end of therapy. Interim PET studies were interpreted according to visual and maximal standardized uptake value reduction compared to baseline PET. Interim PET images were considered negative (good responders) when FDG uptake in the residual lesion was less than liver uptake (DS1–3 in Deauville criteria for visual analysis) and ΔSUVmax ≥ 65% (after three cycles) or ≥72% (after four cycles) for the semiquantitative analysis. Correlation study was performed between two methods. The results of interim PET were compared with the end of therapy (complete metabolic response) and the follow-up (>6 months). Statistical analysis were calculated: negative and positive predictive values (NPV and PPV).

**Results:** All baseline PET studies had positive lesions. Thirty-two out of 46 patients (69%) were classified as negative interim PET according to visual analysis and 40/46 according to ΔSUVmax (32 concordant results and 8 discordant). All of them achieved complete response at the end of treatment and remained free of disease in the follow-up. All patients classified as positive interim PET (six according to visual and semiquantitative analyses) finally progressed. Discordant results were observed in eight patients (positive visual analysis and negative ΔSUVmax); all these patients remain in complete metabolic response. The correlation between two methods was moderate (kappa = 0.5). NPV and PPV for visual analysis were 93% and 42% and for ΔSUVmax were 95% and 100%, respectively.

**Conclusion:** Interim PET is a good predictor of the response at the end of treatment. A moderate correlation between visual and ΔSUVmax reduction analysis was found. NPV and PPV for ΔSUVmax were better than those for visual analysis.

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### USE OF PET/CT FOR TAILORED TREATMENT OF YOUNGER ADULTS WITH HIGH-RISK DLBCL—RESULTS OF PET-RIMCEB TRIAL OF THE CZECH LYMPHOMA STUDY GROUP

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**Introduction:** We prospectively studied if PET/CT after two cycles of induction can be used for tailored treatment of younger adult patients with high-risk diffuse large B-cell lymphoma (DLBCL).

**Methods:** Patients with DLBCL aged 18–60 years, with aaIPI 2–3, were treated with two cycles of R-MegaCHOP (rituximab, 375 mg/m$^2$, Day 0 + 1; cyclophosphamide, 2 g/m$^2$, Day 1 in the first cycle and 3 g/m$^2$ in the second cycle; adriamycin, 75 mg/m$^2$, Day 1; vincristine 2 mg, Day 1; prednisone 60 mg/m$^2$, Days 1–5 + G-CSF) and PET/CT scanned (PET2). Deauville criteria were used for PET evaluation, and patients with higher than liver residual uptake were considered PET positive. All patients received a third R-MegaCHOP. PET2-negative patients were randomized to three cycles of R-CHOP or three cycles of R-CHOP followed by BEAM + ASCT, while PET2-positive patients underwent salvage treatment with platinum-based regimen and BEAM + ASCT. Radiation therapy was scheduled for initially bulky disease (>10 cm) or for PET-positive residual disease at the end of treatment. Seven centres enrolled patients into this study between 05/2010 and 08/2012.

**Results:** After enrolment of 46 patients, the study was prematurely closed for slow accrual and higher percentage of PET2-positive patients than expected. Twenty-one patients (46%) were males, and median age was 46.5 years (range, 20–60 years). Of 44 patients evaluable, 19 patients (43%) were PET2 negative; 11 of them were randomized to R-CHOP and 8 to R-CHOP + ASCT arm (7 were transplanted and 1 died due to toxicity before ASCT). The other 25 patients (57%) were PET2 positive after two cycles and underwent salvage treatment. Six patients (13%) received radiation as a part of initial therapy. Progression-free survival (PFS) at 2 years was 67% for the whole cohort of patients, and overall survival (OS) was 80%. Altogether, there were 10 progressions/relapses and three toxic deaths. Of 10 progressing patients, 4 were successfully salvaged. Disturbingly, six progressions relapses occurred in 11 patients who were PET2 negative and were randomized to R-CHOP without transplantation, compared to only one relapse in eight patients randomized to R-CHOP + ASCT. Although there was no difference in PFS between PET2-positive and PET2-negative patients ($p = 0.83$), there was a trend towards better PFS in PET2-negative patients randomized to R-CHOP + ASCT compared to patients randomized to R-CHOP only (87.5% vs 45%, $p = 0.096$), and PET-negative patients randomized to R-CHOP + ASCT had better 2-year lymphoma-free survival (100% vs 45%, $p = 0.02$).

**Conclusion:** In this prematurely closed study was a trend towards better PFS and statistically better lymphoma-free survival in PET2-negative patients randomized to R-CHOP + ASCT compared to patients randomized to R-CHOP only. PET2-positive patients had comparable survival to PET2-negative patients after salvage treatment.

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## CONTROVERSIAL ROLE OF POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY FINDINGS IN NON-HODGKIN LYMPHOMA PATIENTS BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION

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**Introduction:** The role of fluorine-18-deoxyglucose positron emission tomography/computed tomography (PET/CT) as a prognostic factor prior to allogeneic stem cell transplantation (allo-SCT) for non-Hodgkin lymphoma (NHL) is not yet clearly defined. While it has been suggested that allo-SCT can overcome PET/CT positivity, there are some reports on the contrary that PET/CT-positive patients have poor outcome regardless of allo-SCT. We performed a retrospective analysis of 66 patients with NHL who underwent allo-SCT in our center between March 1997 and September 2014.

**Methods:** Patient characteristics were as follows: 45/66 were males, 27 patients received myeloablative and 39 reduced-intensity conditioning, and median age was 50 years (range, 21–63). Histological subtypes were as follows: FCL 16 (24%), DLBCL 12 (18%), T-NHL 20 (30%), MCL 14 (21%), and 4 others. Twenty-two (33%) patients had an identical sibling donor, and 44 (67%) patients were transplanted from an unrelated donor. Twenty-nine (44%) patients had previous autologous SCT (ASCT). Median of previous chemotherapy lines was 2 (range, 1–9). At allo-SCT, 40 (61%) patients were chemosensitive. PET/CT scans before allo-SCT were performed in 33 (50%) patients, with 20 patients being PET/CT positive and 13 PET/CT negative.

**Results:** Median follow-up of survivors was 54 months (range, 4–156). Overall, 33 (50%) patients died, 8 of disease progression and 25 of non-relapse mortality (NRM). Estimated overall survival (OS) and event-free survival (EFS) at 5 years were 43% and 35%, respectively. The cumulative incidence of NRM and relapse incidence at 5 years were 48% and 31%, respectively. Acute GVHD occurred in 45% patients and chronic GVHD in 47% patients. Significantly worse results ($p = 0.002$) were observed in patients with DLBCL (3-year OS 18%), while in MCL, PTCL, and FCL, the results were better with achievement of plateau on survival curves (3-year OS of 49%, 60%, and 75%, respectively). Analysis of other pre-transplant factors showed a significantly better OS ($p = 0.01$) and EFS ($p = 0.005$) in patients without prior ASCT. We found no significant differences in terms of donor type, number of previous chemotherapy lines, or conditioning intensity. Significantly better results were observed in patients with chemosensitive disease according to standard CT criteria at time of allo-SCT; 5-year OS was 52% and 28% for chemosensitive and chemoresistant disease ($p = 0.01$) and 5-year EFS was 47% and 17%, respectively ($p = 0.003$). Overall, 9 out of 20 PET/CT-positive patients achieved PET/CT negativity after allo-SCT. We found no significant difference between PET/CT-positive and PET/CT-negative patients in terms of OS and EFS.

**Conclusions:** The results suggest that allo-SCT can overcome poor prognosis of PET/CT-positive patients. The limitation of our study is the small number of patients, heterogenous NHL histology, and the fact that majority of failure events were NRM in the context of GVHD. Chemosensitivity is prognostically essential, and it is necessary to search for new procedures and treatment modalities to achieve the best possible response before allo-SCT.

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## CHARACTERIZATION OF RESIDUAL MASSES IN LYMPHOMAS USING WHOLE-BODY DIFFUSION-WEIGHTED IMAGING WITH APPARENT DIFFUSION COEFFICIENT MAPPING

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**Introduction:** Diffusion-weighted imaging (DWI) probes tumour and tissue cell density. Previously, we have shown that DWI is able to demonstrate post-treatment apparent diffusion coefficient (ADC) changes of lymphoma lesions. The aim of this study was to further assess its potential value in characterizing residual lymphoma masses early during the first-line chemotherapy, with integrated $^{18}$F-fluoro-2-deoxyglucose positron emission tomography/computed tomography (PET/CT) as the reference standard.

**Methods:** Twenty adult patients with newly diagnosed lymphomas (13 DLBCL, 5 HL and 2 FL) and bulky disease (>7 cm) underwent whole-body DWI ($b = 50$,

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400 and 800 s/mm$^2$) and PET/CT before initiation and after two cycles of chemotherapy (except one consent withdrawal after the baseline scans). ADC of the main bulky mass for each patient was assessed both visually and quantitatively. On visual ADC analysis, any focal signal intensity lower than that of muscle, that is, with diffusion restriction, was considered positive for viable tumour. Volume-of-interest encompassing the entire mass was manually drawn to obtain the mean ADC. The 2014 Lugano classification was used for PET/CT interpretation, and a score of 4 or 5 was considered positive.

**Results:** Before treatment, all of the 20 main bulky masses showed diffusion restriction with a mean ADC of $0.946 \times 10^{-3} \pm 0.310$ mm$^2$/s (standard deviation; range, $0.617 \sim 1.594 \times 10^{-3}$ mm$^2$/s). They were all FDG avid with a maximum SUV of $20.7 \pm 8.7$. At interim, among the 19 patients who had undergone follow-up DWI scans, at least focal diffusion restriction on visual ADC analysis was noted within 10 of the residual masses. On PET/CT, they were all considered positive for viable tumour based on the 2014 Lugano classification with a maximum SUV of $7.0 \pm 3.5$. There were two additional patients, for whom main masses remained positive on PET/CT after chemotherapy but demonstrated no more restricted diffusion, one gastric DLBCL and another anterior mediastinal HL. Overall, for interim response assessment, DWI was concordant with PET/CT in 17 of 19 patients (89%) based on visual ADC analysis. The mean ADC values of PET-positive ($n = 12$) and PET-negative ($n = 7$) residual masses were $1.389 \times 10^{-3} \pm 0.412$ vs $2.361 \times 10^{-3} \pm 0.381$ mm$^2$/s (unpaired $t$-test, $p < 0.0001$).

**Conclusions:** Whole-body DWI with ADC mapping seems useful in characterizing residual masses in lymphomas. Further studies are required to explore its potentials in individualized patient care.

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### UTILITY OF CAPSULE ENDOSCOPY FOR DETECTING INTESTINAL INVOLVEMENT OF MALIGNANT LYMPHOMA

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**Introduction:** Malignant lymphoma sometimes involves the small intestine. Intestinal involvement impacts the disease stage and can occasionally cause intestinal bleeding and perforation, making it difficult to detect on existing imaging modalities. Capsule endoscopy (CE) is expected to be potentially valuable to evaluate small bowel disease.

**Methods:** Patient characteristics and the CE results of patients with lymphoma were retrospectively obtained from the consecutive CE cohort at our institute. Images obtained using other modalities (CT scan, Ga scintigraphy, and FDG-PET/CT) were compared with the findings on CE.

**Results:** Of the 210 patients who underwent CE between April 2010 and December 2013, 16 patients had lymphoma at the time of examination [eight males and eight females; median age at CE examination, 65.5 years (range, 58–78 years); diffuse large B-cell lymphoma (DLBCL): six, follicular lymphoma (FL): five, mantle cell lymphoma (MCL): two, MALT lymphoma: two, and nodal marginal zone B-cell lymphoma (nMZL): one]. Eleven patients had been diagnosed with gastrointestinal lesions on upper and lower gastrointestinal endoscopies (stomach, two; duodenum, five; terminal ileum and cecum, two; colorectum, two). At least five patients had intraperitoneal lymphadenopathies, but only one patient had been detected to have small bowel involvement on CT, Ga scintigraphy, and FDG-PET. CE revealed that 11 of 16 patients (68.8%) had abnormal findings in the small intestine (DLBCL: six, FL: three, MCL: one, and nMZL: one). White villi in the nodes were the most common findings in patients with FL and MCL. Ulcerations were found in two patients with DLBCL. Two lesions were matched between the findings on CE and those on other imaging modalities: one patient with DLBCL had bulky involvement of the intestine detectable on CT, and the other patient with FL was found to have marked FDG uptake, which was initially thought to be a physiological uptake in the intestine.

**Conclusions:** Capsule endoscopy could detect small intestinal lesions even in patients who were found to be negative using other modalities. A large-scale study is warranted not only to assess the actual incidence of small bowel involvement in malignant lymphoma but also to evaluate the risk of complications.

## PRECLINICAL STUDIES

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### MEK1 INHIBITOR SELUMETINIB SENSITIZES PRECURSOR B-CELL LEUKEMIA/LYMPHOMA CELLS TO DEXAMETHASONE THROUGH MODULATION OF MTOR ACTIVITY AND STIMULATION OF AUTOPHAGY

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**Introduction**: Glucocorticoids (GC) are fundamental drugs used in the treatment of lymphoid malignancies. GC's mechanism of action involves induction of apoptosis, but recent studies highlight the role of autophagy upstream of apoptotic cell death. Herein, we sought to elucidate the molecular mechanisms driving GC resistance in precursor B-cell tumours and *in vitro* characterize the therapeutic potential of targeted intervention in these mechanisms.

**Methods:** Bioinformatic analyses of GC-sensitive and GC-resistant tumours were carried out using publicly available datasets and GENEPATTERN program. Primary leukaemia cells were isolated from peripheral blood of newly diagnosed ALL patients. Cells were incubated with dexamethasone (DEX, 0.05, 2 or 30 μg/mL) +/− MEK1 inhibitor, selumetinib (SEL, 200 nM). Apoptosis was assessed using annexin V/PI staining. Protein expression and phosphorylations were assessed by western blotting or by flow cytometry. Autophagy was monitored by western blot, MDC staining and GFP-LC3 relocalization. Beclin 1 (BCN1) knockdown was achieved with shRNA. Vectors were transduced using retroviral infections.

**Results:** To identify mechanisms involved in GC resistance, we first analysed gene expression profiles of GC-resistant and GC-sensitive tumours and found that resistant cells exhibit significantly higher expression of MAPK/ERK pathway components ($p < 0.001$, FDR = 0.17). In GC-resistant cell line SEMK2, inhibition of MEK1 with SEL completely abrogated pERK and increased sensitivity to GC by 1.8-fold to 2.6-fold. A similar pattern was observed in primary ALL blasts from 19 of 23 tested patients. Overexpression of a constitutively active MEK mutants in GC-sensitive cells (RS4-11) induced resistance to DEX. Since GC in lymphoid cells induce autophagic cell death, we assessed LC3 processing, formation of autophagolysosomes (MDC staining) and GFP-LC3 relocalization in cells incubated with either DEX, SEL or a combination of drugs. Either drug alone caused only marginal change in the level of these markers, but their combination markedly increased autophagic flux. Since mTORC1 is the critical regulator of autophagy, we assessed the activity of mTORC1 following DEX/SEL treatment and found that the combination resulted in a marked decrease of p-4EBP1. Finally, we silenced BCN1, a gene required for autophagosome formation, and assessed cellular responses to DEX-SEL. In control cells transduced with non-targeting shRNA, SEL sensitized cells to DEX, but in BCN1-deficient cells, the synergy of DEX and SEL was markedly decreased.

**Conclusion:** Taken together, we show that MEK1 inhibitor selumetinib enhances DEX toxicity in GC-resistant B-ALL cells. The underlying mechanism of this interaction involves inhibition of an mTORC1 signalling pathway and induction of autophagy that leads to apoptotic cell death.

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**THE COMBINATION OF A NOVEL BRUTON TYROSINE KINASE INHIBITOR PLS123 AND MTOR INHIBITOR EVEROLIMUS SYNERGISTICALLY INDUCES APOPTOSIS IN MANTLE CELL LYMPHOMA**

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**Introduction:** Mantle cell lymphoma (MCL) is an aggressive and incurable malignant lymphoma with a median survival of 5 years that accounts for approximately 6–8% of B-NHL patients. Given the lack of successful treatment options for MCL, the development of new combination strategies for the treatment of this debilitating disease is urgently needed. The B-cell antigen receptor signal pathway has gained significant attention as a potential therapeutic target in B-cell lymphoma. We recently developed a novel covalent irreversible Btk inhibitor PLS-123, which exhibits a dual-action mode of inhibition for both the catalytic activity of Btk and its own activation. This study aims to investigate whether PLS-123 potentiates the growth inhibitory effect of everolimus, a specific mTOR inhibitor, in human MCL cells.

**Methods:** *In vitro* cell viability was analyzed using CellTiter-Glo Luminescent Cell Viability Assay. Induction of apoptosis and cell cycle arrest were measured by flow cytometry. Western blotting analysis was used to detect the essential regulatory enzymes within related signaling pathways after combination of PLS-123 and everolimus treatment.

**Results:** Co-administration of PLS-123 and everolimus synergistically induced antiproliferative effect in MCL cell lines (Granta519, Mino, and Z138). Exposure of these MCL cells to minimally toxic concentrations of everolimus and PLS-123 resulted in more significant inhibitory activity than each agent alone. Interestingly, combined treatment of MCL cells with everolimus and PLS-123 resulted in marked induction of apoptosis monitored by flow cytometry compared to single-agent treatment, which were accompanied by marked upregulation of apoptosis-related proteins (cleaved caspase 3, caspase 8, caspase 9, and PARP) and pro-apoptotic proteins Bax and Bad. The combination of PLS-123 and everolimus also substantially increased the cell population of G1 phase and decreased that of G2 in cell cycle, which indicated that combined treatment blocked cell cycle progression at the G1-S phase. The expression of CDK4 and CDK6, which regulated G1/S-phase cell cycle transition, was reduced after combination treatment. Furthermore, the possible impacts of combination treatment towards related signaling cascades were next investigated by immunoblotting analysis. Combined exposure of MCL cells to everolimus and PLS-123 resulted in marked inactivation of the mTOR/AKT pathway compared to single-drug treatment. The activation of p-ERK and p-p65 was also more significantly diminished after treatment of PLS-123/everolimus.

**Conclusions:** The combination of novel Btk inhibitor PLS-123 and everolimus synergistically induced anti-tumour activity in MCL cell lines, suggesting a new combination direction for the treatment of MCL patients.

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**NOVEL STRATEGIES FOR TARGETING THE MTOR-EIF4F-MYC AXIS IN LYMPHOMA**

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**Introduction:** Deregulated Myc is associated with resistance to chemotherapy and poor survival in aggressive lymphomas. No drugs that directly target Myc have yet been approved for the treatment of any cancer. In fact, since Myc is involved in many essential functions in normal cells, direct Myc inhibitors may be associated with significant toxicity. Alternatively, it may be advantageous to inhibit upstream cancer-specific signals that converge on Myc. The translation of Myc is dependent on the translation initiation factor eIF4F, which in turn is regulated by mTOR. Both the proteasome and PI3K-AKT pathways are involved in the activation of mTOR, and both have been successfully targeted for cancer treatment. We hypothesized that combinations of PI3K and proteasome inhibitors could lead to synergistic inhibition of Myc and Myc-dependent cancer, via inhibition of mTOR-eIF4F.

**Methods:** Cytotoxicity of the PI3K-delta inhibitors CAL-101 (idelalisib) and TGR-1202 and proteasome inhibitors bortezomib and carfilzomib was studied in a high-throughput format. Growth inhibition of the single agents and combinations was determined using the CellTiter-Glo assay. Synergy was determined by calculating the excess over bliss (EoB). EoB values of 0 indicate additivism, with values below or above 0 indicating antagonism or synergism, respectively. Protein expression was determined by Western blot, and mRNA expression was determined by RT-qPCR.

**Results:** The PI3K inhibitors TGR-1202 and Cal-101 had minimal and comparable cytotoxic effects. Bortezomib and carfilzomib caused dose-dependent inhibition of cell growth and were comparable. Surprisingly, the combinations displayed markedly different degrees of synergy. The TGR-1202 and carfizomib combination was consistently synergistic across different concentrations tested, and the other three combinations showed varying and overall much lower degrees of synergy. The mechanistic basis of the synergy and differences between the combinations were determined through a systematic interrogation of the PI3K/AKT/mTOR pathway and Myc expression. Only the TGR-1202 and carfilzomib combination produced profound Myc protein downregulation with concordant downregulation of eIF4F subunits and inhibition of mTOR and marked induction of PARP cleavage. These were observed in cell lines representing different histologic types of lymphoma, as well as in primary samples isolated fresh from patients. Importantly, the TGR-1202 and carfilzomib combination was not toxic to healthy lymphocytes at concentrations known to be highly potent for primary lymphoma samples.

**Conclusion:** Targeting the mTOR-eIF4F-Myc axis through specific combinations of PI3K and proteasome inhibitors is a novel, effective, and safe strategy to mitigate the poor risks of Myc in patients with aggressive lymphomas.

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**A NOVEL BISPECIFIC APPROACH TO SAFELY AND EFFECTIVELY TARGET CD47 FOR THE TREATMENT OF B-CELL LYMPHOMA**

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**Introduction**: Many cancers upregulate the expression of CD47 in order to evade immune surveillance and killing. This 'don't eat me' signal is a molecular means for healthy cells to limit their phagocytic elimination by macrophages and other innate immune cells. This cell-purging mechanism is mediated by the interaction of CD47 on the target cell and signal regulatory protein alpha (SIRPα) on myeloid cells. Development of anti-CD47 monoclonal antibodies may be hindered due to the ubiquitous expression of CD47, leading to rapid drug elimination kinetics through target-mediated drug disposition, an unfavourable pharmacokinetic profile, and target-related toxicity including chronic anaemia.

**Methods:** We have developed a panel of anti-CD47 antibody arms with a range of affinities to afford rapid engagement/disengagement kinetics when binding CD47 in a monovalent setting. These arms were paired with a high-affinity anti-CD19 arm in a fully human bispecific format, the κλ-body™, and used to probe the affinity balance that succeeded in selectively killing tumour cells. CD47+ CD19+ human B-cell lymphoma lines and patients' cells were used to demonstrate binding and killing through Fc region-dependent effector functions, such as antibody-mediated cellular phagocytosis (ADCP) and antibody-mediated cellular cytotoxicity (ADCC). These findings were extended to *in vivo* studies using a tumour xenograft model. Safety was assessed *in vitro*, and pharmacokinetics and hematological parameters were evaluated following a single dose in non-human primates.

**Results:** Using human B-cell lymphoma lines, the κλ-body™ demonstrated selective binding and targeted blockade of the CD47-SIRPα interaction and cell killing through ADCP and ADCC. We extended these observations using B-lymphoma patient samples as target cells. *In vitro* safety studies demonstrate a favourable binding profile of κλ-bodies™ to B cells compared with erythrocytes, no evidence of platelet activation or aggregation and no haemagglutination at concentrations of the

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anticipated effective clinical dose. Moreover, studies in non-human primates demonstrated favourable elimination kinetics (i.e. comparable to human IgG) and no effects on hematological parameters (e.g. red blood cell and platelet counts) at the doses tested.

**Conclusions:** Taken together, our efforts to explore a novel therapeutic format have illustrated that combining the right potency of an anti-CD47 blocker with a certain affinity for a tumour-associated antigen can lead to impressive tumour killing as well as acceptable pharmacokinetic profiles, characteristics required for the development of a therapeutic candidate. Our lead κλ-body™, NI-1701, is in preclinical enabling studies.

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**INHIBITION OF PIM KINASES IMPAIRS NFKB ACTIVITY AND INDUCES APOPTOSIS IN CLASSICAL HODGKIN LYMPHOMA REED–STERNBERG CELL LINES**

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**Introduction:** The classical Hodgkin lymphoma (cHL) Reed–Sternberg (HRS) cells depend on constitutive activity of the JAK-STAT and NFκB pathways. Since these pathways induce expression of the oncogenic PIM1/2/3 serine/threonine kinases, we hypothesized that PIM kinases may be overexpressed in HRS cells and be involved in disease pathogenesis. Herein, we investigated PIM1/2/3 expression in cHL, determined mechanisms underlying their expression and characterized biochemical and biological consequences of PIM inhibition in HRS cells.

**Methods:** Expression analyses in cell lines was performed by immunoblotting and real-time PCR (RT-PCR). PIM1/2/3 expression in a group of 67 cHL patients was assessed by immunohistochemistry. Impact of NFκB inhibition on PIM expression was assessed by RT-PCR in HRS cells expressing IκB super-repressor or with siRNA-silenced NFκB subunits. JAK2 inhibition was achieved with a small molecule inhibitor (CYT387). PIM1/2/3 expression knockdown was achieved by siRNAs. MTS assay and FACS–annexin V were used to investigate cellular proliferation and apoptosis. NFκB p65 activity was measured using DNA binding assays.

**Results:** Our analyses revealed that PIM1/2/3 are ubiquitously expressed in primary and cultured HRS cells. At least one PIM isoform was expressed in each cell line and in 97% of primary cHL biopsies investigated. HRS cells treated with the JAK1/2/3 inhibitor displayed reduced PIM1 and PIM2 levels, and genetic NFκB suppression led to a decrease of PIM2 and PIM3, but not PIM1 expression, suggesting PIM1/2/3 dependence on JAK-STAT and NFκB in cHL. To assess the role of PIM kinases in cell viability, we silenced expression of each PIM isoform (individually or simultaneously) in HDLM-2 cells. Knockdowns of individual PIM isoforms were associated with a marked increased in remaining isoform expression and were not associated with toxicity. In marked contrast, downregulation of all three isoforms increased cellular apoptosis by 17%. For this reason, for subsequent studies, we used a novel pan-PIM inhibitor (SEL24-B489). The inhibitor was toxic to all cells with IC50 ranging from 3 to 5 μM. To determine mechanisms underlying toxicity, we assessed the activities of specific PIM substrates: 4EBP1, S6, and p65 (RelA). SEL24-B489 rapidly decreased PIM-dependent phosphorylation of these molecules in all tested cell lines. Furthermore, it reduced DNA binding activity of the NFκB–p65 complexes and decreased NFκB-dependent gene expression.

**Conclusions:** Herein, we demonstrate that the oncogenic PIM-1/2/3 kinases are expressed in primary HRS cells and cHL-derived cell lines, and their activity can be specifically blocked using a pan-PIM inhibitor SEL24-B489. PIM inhibition significantly reduced activity of specific PIM substrates and impaired NFκB-dependent gene expression. Taken together, these results demonstrate that PIM kinases are promising targets in cHL.

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**BCL2-TARGETING AGENTS REVEAL VARIOUS BCL2-DEPENDENT AND MCL-1-DEPENDENT CATEGORIES OF DIFFUSE LARGE B-CELL LYMPHOMA**

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**Introduction:** While BCL2 gene deregulation was repeatedly associated with poor prognosis in diffuse large B-cell lymphoma (DLBCL), the role of MCL1 remains largely unknown. We used highly selective BCL2 inhibitor ABT199 and homoharringtonine (HHT), whose principal antitumour activity is mediated by downregulation of MCL1 protein, to study the roles of BCL2 and MCL1 for the survival of DLBCL cells.

**Methods:** Immunophenotype of primary DLBCL samples was determined by immunohistochemistry (IHC) using the Hans algorithm. Sensitivity of 18 established DLBCL cell lines and 8 clones with targeted knockdown or targeted (over)expression of BCL2 or MCL1 to ABT199 and HHT at clinically relevant concentrations (1 mM ABT199 and 30 nM HHT) was determined by WST8-based cell proliferation assay.

**Results:** By western blot or IHC, we demonstrated that while MCL1 protein was detectable in all DLBCL cell lines ($n = 18$) and primary samples ($n = 114$, GCB = 51, ABC = 63), BCL2 protein was not detectable in 6/18 (33%) cell lines and 32/114 (28%) primary samples. Of 12 (67%) BCL2+ cell lines, 8 were sensitive to ABT199. All six BCL2− cell lines were resistant to ABT199. Of 18 (94%) cell lines, 17 were sensitive to HHT. Significant anti-lymphoma synergism between ABT199 and HHT was observed in most BCL2+ cell lines and was confirmed on a mouse xenograft model of BCL2+ primary DLBCL cells. Targeted knockdown of BCL2 or MCL1 was lethal for those cell lines that were highly sensitive to ABT199 or HHT, respectively. Clones of selected cell lines with knockdown or transgenic (over)expression of BCL2 demonstrated that BCL2 expression negatively correlates with sensitivity to HHT. Transgenic expression of BCL2 in BCL2− cell lines did not impact resistance of the clones to ABT199. BCL2 knockdown increased sensitivity to ABT199 in one of two clones. Knockdown and overexpression of MCL1 protein increased and decreased sensitivity to HHT in one of two clones, respectively, but did not impact their sensitivity to ABT199. In addition to cytotoxic tests, we analysed protein interactions of MCL1 and BCL2 in DLBCL cell lines with different sensitivities to HHT and ABT199. By immunoprecipitation, we detected high amounts of MCL1-bound BIM protein in HHT-sensitive DLBCL cell lines. No apoptosis activators were bound on MCL1 protein in HHT-resistant cell lines.

**Conclusions:** Based on our data acquired from 18 DLBCL cell lines, 2 biological categories of DLBCL might be assumed based on protein expression of BCL2: (1) BCL2-negative DLBCL, which are MCL1 dependent (HHT sensitive) and BCL2 independent (ABT199-resistant) and (2) BCL2-positive DLBCL. The BCL2-positive category can further be divided into two subcategories: 2A = BCL2 dependent (ABT199 sensitive) and MCL1 independent (HHT resistant) and 2B = BCL2 dependent (ABT199 sensitive) and MCL1 dependent (HHT sensitive). BCL2-resistant and MCL1-resistant DLBCL appear to be very rare.

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**DEVELOPMENT OF RESISTANT LYMPHOMA CELLS TO HDAC6 INHIBITOR, ACY-1215, UNCOVERS POTENTIAL SYNERGISTIC DRUG PARTNERS**

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**Introduction:** HDAC inhibitors possess marked activity in lymphoma and induce pleiotropic effects. HDAC6 is cytoplasmic and binds poly-ubiquitinated misfolded proteins to facilitate their transport to the aggresome for proteasome-independent degradation. To better understand the function of HDAC6 in lymphoma, we developed two lymphoma cell lines resistant to the selective HDAC6 inhibitor ACY-1215 (Acetylon Pharmaceuticals, Inc.).

**Methods:** The DLBCL cell line, OCI-Ly10, and the T-cell line, H9, were exposed to systematically incremental increasing concentrations of ACY-1215 to develop distinct cell lines with $IC_{50}$ values 20-fold greater than that for parental lines (P). Cytotoxicity was assessed with CellTiter-Glo Assay, protein expression by immunoblot assay, and GEP with RNA Seq techniques. Patient samples were collected under an IRB-approved protocol.

**Results:** The resistant (R) R-LY10 achieved an $IC_{50}$ of 20 μM compared to P-LY10 of 0.9 μM while the R-H9 achieved an $IC_{50}$ of 35 μM compared to P-H9 $IC^{50}$ of 1.2 μM at 48 h. Resistance was maintained after repeated passages for >1 month. Resistance was not conferred by upregulation of efflux pumps as determined by co-exposure with verapamil. Interestingly, the R-LY10 was also resistant to vorinostat, which strongly inhibits HDAC6 (R-LY10 $IC_{50}$ 7 μM vs. P-LY10 $IC_{50}$ 2 μM), and sensitive to romidepsin, which is known to predominantly inhibit HDAC1, HDAC2, and HDAC3 with minimal activity against HDAC6 (R-LY10 $IC_{50}$ 5.2 nM vs. P-LY10 $IC_{50}$ 4.5 nM). In addition, treatment of R-LY10 with bortezomib demonstrated intermediate activity, underscoring the concept that although the drug targets are of vastly different biology, their mechanisms converge on the processing of misfolded proteins (R-LY10 $IC_{50}$ 4.5 nM vs. P-LY10 $IC_{50}$ 3.3 nM).

The GEP of R-LY10 was compared to that of P-LY10 and revealed 931 genes significantly upregulated and 391 genes significantly downregulated ($p < 0.05$). Genes of interest in R-LY10 include upregulation of JNK3, IKZF2, HDAC9, and FYN, as well as downregulation of SH3BP5 and LCK. These results were confirmed in a second R-LY10 line. Given the upregulation of FYN, a tyrosine kinase in the B-cell receptor pathway, and downregulation of SH3BP5, a negative regulator of BTK, R-LY10 cells were treated with ibrutinib and compared to P-LY10. Treatment with ibrutinib 0.1 μM was able to overcome resistance with 70% viability in R-LY10 compared to 64% in P-LY10 at 72 h. P-LY10 cells treated with ibrutinib in combination with ACY-1215 demonstrated synergy with RRR = 0.46 (<1 = synergy) and increased FYN, SH3BP5, and IKF2 and decreased p-BTK and p-AKT protein expression. Primary human lymphoma samples treated with the combination also demonstrated strong synergy (RRR = 0.51).

**Conclusions:** The development of these ACY-1215-resistant cell lines has proven to be a powerful tool to better understand the mechanistic role of HDAC6 in lymphoma and may be useful in guiding rational drug partners that target complimentary pathways.

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### AUTOPHAGY IS PIVOTAL FOR HODGKIN AND REED–STERNBERG CELLS' SURVIVAL, REVEALING A NEW TREATMENT STRATEGY FOR CHL

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**Introduction:** Twenty per cent of patients with classical Hodgkin lymphoma (cHL) do not respond to standard chemotherapy, providing incentive to pursue new therapeutic approaches. Autophagy, a regulated catabolic pathway to degrade cell's own components, is in cancer linked with both tumour suppression and promotion. The finding that autophagy enhances tumour cell survival would thus lead to immediately testable strategies for novel therapies in cHL because a number of drugs affecting autophagy are available. Thus, we studied the status quo of autophagy and its importance in the pathogenesis of cHL.

**Methods and Results:** We found constitutively activated autophagy in cHL cell lines and primary tissue. The expression of key autophagy-relevant genes (e.g. Beclin-1 and ULK1-3) and LC3 processing was increased in cHL cells, even in lymphoma primary cases. Consistently, cHL cells exhibited elevated numbers of autophagic vacuoles and intact autophagic flux. Autophagy inhibition with chloroquine or inactivation of ATG5 induced apoptosis and reduced their proliferation. We further found that autophagy protects cHL cells from cytotoxic levels of ROS and plays a pivotal role in sustaining mitochondrial function.

**Conclusion:** We conclude that cHL cells require autophagy for growth, survival and sustained metabolism, making them sensitive to autophagy inhibition. This suggests autophagy as a useful target for new strategies in cHL treatment.

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### ERK-MEDIATED AND AMPK-MEDIATED AUTOPHAGY PROTECTS BURKITT LYMPHOMA CELLS FROM OXIDATIVE STRESS

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**Introduction:** Burkitt's lymphoma (BL) is a B-cell non-Hodgkin lymphoma that is highly metabolically active. Cellular control of ROS by autophagy is important for protection from oxidative damage in highly metabolically active cells, where much ROS occurs. In this study, we investigated the role of autophagy in BL cells under oxidative stress.

**Methods:** To investigate the status quo of autophagy under oxidative stress, we analyzed autophagy-relevant proteins by Western blot and quantified autophagosomes by FACS in BL cell lines that were treated with pyrogallol, antimycin A, or hydrogen peroxide to induce ROS.

**Results:** We found that after 12 h of incubation with antimycin A/pyrogallol, LC3II increased. Hydrogen peroxide induced elevation of LC3II after only 6 h of incubation. The number of autophagosomes elevated after 24 h of antimycin/pyrogallol treatment and after 12 h of hydrogen peroxide treatment. To investigate whether BL cells are dependent on autophagy under ROS, we treated ROS-induced cells with the autophagy inhibitor chloroquine (CQ). Treatment led to increased apoptosis after 24-h incubation under antimycin A/pyrogallol and after 12-h treatment with hydrogen peroxide. In addition, cell proliferation was significantly reduced. Interestingly, activities of the ROS-transforming enzymes SOD1/SOD2/catalase increased significantly when autophagy is activated under antimycin A/pyrogallol. After hydrogen peroxide treatment, the activity of catalase elevated. CQ treatment prevented elevated enzyme activities. To further delineate the mechanism of ROS-mediated autophagy, we analyzed the expression levels of phospho-ERK, phospho-JNK, and phospho-AMPK in ROS-induced BL cell lines. Phospho-ERK increased significantly at the latest after 8 h of antimycin A/pyrogallol treatment in both BL cell lines tested, while phospho-AMPK and phospho-JNK remained unchanged over the whole 12 h. Hydrogen peroxide treatment led to elevated levels of phospho-AMPK and a slight increase of phospho-JNK, reaching the peak after only 6 h. Over a time period of 36 h, autophagy is not initiated after treatment of cells with the ERK inhibitor FR180204 under antimycin A/pyrogallol. Under hydrogen peroxide, autophagy initiation failed in AMPK-inhibited cells.

**Conclusion:** We conclude that ERK/AMPK-mediated autophagy helps BL cells to survive and proliferate under ROS by increasing the activity of ROS-transforming enzymes.

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### ROLE OF NK CELL IN THE ANTI-TUMOUR RESPONSE INDUCED BY IFNA DENDRITIC CELLS LOADED WITH APOPTOTIC FOLLICULAR LYMPHOMA CELLS

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**Introduction:** Although the survival of patients with FL has improved greatly since the introduction of anti-CD20 therapy (rituximab), FL is still considered virtually incurable.

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Novel immunotherapeutic strategies based on apoptotic tumour cell-loaded dendritic cells (DC) seem to be promising for inducing an effective antitumour response. In previous studies, we have widely characterized a new type of monocyte-derived DC generated after 3 days of *in vitro* treatment with IFN-α and GM-CSF, known as IFN-DC. IFN-DC are highly efficient in internalizing tumour antigens, mediating cross-presentation and cross-priming of $CD8^+$ T cells. Furthermore, since IFN-DC are extraordinarily competent in preserving internalized protein from degradation, tumour antigens could be retained in lymphoid organs for long periods after uptake, favouring the recruitment of rare antigen-specific $CD8^+$ T-cell precursors.

**Methods:** IFN-DC derived from peripheral blood monocytes of FL patients were tested for differentiation and tumour cell uptake. $CD8^+$ T and NK cells were obtained by culturing FL patient PBL for 2 weeks with IFN-DC loaded with autologous apoptotic FL cells. Phenotype, frequency and antitumour response towards autologous lymphoma cells were tested by flow cytometry, cytotoxic assay, ELISPOT and confocal microscopy (CLSM). Lytic granule release and NK/IFN-DC synapses were also analysed. Cytokine production and cell proliferation were evaluated by ELISA and CFSE assays.

**Results:** A preliminary set of experiments was carried out on PBL from healthy donors, followed by a preclinical study utilizing blood samples and tumour specimens from FL patients. We showed that IFN-DC loaded with apoptotic lymphoma cells and cultured with autologous PBL induced a remarkable increase in $CD8^+$ and $CD56^+$ cell frequency and proliferation, with a preferential expansion of the $CD56^{bright}$ population. Huge amounts of IFN-g were detected after 3 days of IFN-DC/PBL cocultures, preserving high levels until Day 14. IFN-DC efficiently activated NK cells showing enhanced expression of NKp30, NKp44 and NKp46 and enhanced killing of target cells. Of note, both CD8 and NK cells exhibited increased granzyme B and perforin expression and improved cytotoxic activity against autologous lymphoma cells. CLSM and neutralization assays highlighted a critical role of IL-15 and MICA/B on the IFN-DC membrane in NK-cell activation and IFN-g production, events that precede the revitalization of the CD8 T-cell response *in vitro* against lymphoma cells.

**Conclusions:** Our data strongly suggest that IFN-DC, instead of conventional DC, represent a highly promising tool to improve the clinical efficacy of patient-specific vaccination strategies for the treatment of FL, by activating NK cells and thus improving host cellular antitumour immune response.

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### IDENTIFICATION OF MUTATIONS PREDICTIVE FOR SENSITIVITY TO DASATINIB IN LYMPHOMA CELLS USING NEXT-GENERATION SEQUENCING AND CRISPR SCREENING

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Dasatinib is a compound that inhibits a range of protein kinases including the Bruton's tyrosine kinase (BTK) and Bcr-Abl fusion kinase. It has demonstrated high efficiency for the treatment of chronic myeloid leukemia (Kantarjian *et al*., 2010). In addition, it was found that dasatinib is a strong inhibitor of various other kinases including the family of sarcoma kinases (Karaman *et al*., 2008). Patients showing mutations in these kinases including DDR2 mutations benefit from dasatinib treatment (Hammerman *et al*., 2011; Sen *et al*., 2012). One patient who had an inactivating mutation for BRAF for example remained tumour free after 4 years (Sen *et al*., 2012). We have collected four sensitive B-cell lymphoma cell lines and three resistant cell lines to dasatinib. In the sensitive cell lines, cell growth is strongly blocked and apoptosis rapidly induced by dasatinib.

We hypothesize that the sensitivity to dasatinib might be attributed to mutations in kinases regulated by dasatinib. We aim to identify mutated kinases by whole-exome sequencing and the functional knockout screen CRISPR.

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### PROGNOSTIC VALUE OF PD-L LIGANDS EXPRESSION IN LYMPHOMAS

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**Introduction:** Programmed cell death ligand 1 (PD-L1, also known as B7H1) is a cell-surface protein that suppresses the cytotoxic CD8(+) T cell-mediated immune response. Expression of PD-L1 is not well studied in lymphomas. Despite recent advances in targeted therapy of lymphomas, especially diffuse large B-cell lymphoma (DLBCL), some patients do not benefit from these therapies. Agents inhibiting PD-1 and PD-L1 are showing compelling antitumour activity in current clinical trials in some solid and hematological cancers.

**Methods:** Sixty-two patients with cHL (21 men and 41 women, median age 32.8 years) and 28 patients with DLBCL (11 men and 17 women, median age 49.5 years) were included in this study. The patients with cHL received chemotherapy regimen ABVD or BEACOPP-14/esc ± radiotherapy. The patients with DLBCL were treated by R-CHOP-like regimens. PD-L1 and PD-L2 mRNA expression levels were analysed in pre-treatment fresh biopsies of lymph nodes using real-time RT-PCR. PD-L1/2 connection with histological type, stage of disease and prognostic role between complete response (CR) and progression-free survival (PFS) was analysed. Tumours were categorized into two groups (high or low PD-L1 expression) based on a cut-off point—optimal criterion, which was determined by ROC analysis.

**Results:** Among the group of patients with Hodgkin lymphoma, there are 77.5% (48/62) PD-L1-positive cases and 22.5% (14/62) PD-L1-negative cases. PD-L1 expression levels were slightly higher in nodular sclerosis cHL and advanced stages of disease ($p = 0.12$). PD-L2 expression level was not associated with either histological cHL variant or disease stage. Any association between PD-L1/2 expression level and treatment response was not noticed. High PD-L1 expression was associated with reduced PFS in cHL patients. The 3-year PFS rate for cHL patients with high PD-L1 expression was 66.6% compared to 85.1% for low or absent of PD-L1 expression ($p < 0.05$).

Of the DLBCL cases, 82.2% (23/28) were PD-L1 positive and 17.8% (5/28) were PD-L1 negative. Significant association between PD-L1 expression level and clinical characteristic of DLBCL (histological subtype and stage of disease) was not found. However, high PD-L2 expression level was associated with a risk of relapse in patients with DLBCL regardless of disease stage ($p < 0.05$).

**Conclusions:** The obtained results suggest that the high level of PD-L1 is associated with unfavourable prognosis for patients with cHL. PD-l might provide a possible application of this genetic marker as an independent prognostic factor of lymphomas.

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### CXCR5, CXCR4 AND CXCL13 POLYMORPHISM IN CENTRAL NERVOUS SYSTEM LYMPHOMA - SNP PROFILE IS RELATED TO TROPISM

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**Introduction:** Central nervous system (CNS) relapse (secondary CNS involvement of systemic lymphoma (sCNSL)) occurs in around 5% of diffuse large B-cell lymphoma (DLBCL) cases, and primary CNS lymphoma (PCNSL) occurs around 1–2 %. Chemokines and their receptors control normal migration and homing of lymphocytes. In this study, we investigate single nucleotide polymorphisms (SNPs) of lymphocyte guiding chemokine receptors and chemokines in systemic and CNS lymphoma. We focus on chemokine (C-X-C motif) receptor 5 (CXCR5) also known as Burkitt lymphoma receptor 1 (BCR1) and its ligand, C-X-C motif chemokine 13 (CXCL13) and chemokine (C-X-C) receptor 4 (CXCR4).

**Methods:** We analyzed eight PCNSL tumor biopsies and normal bone marrow DNA from 12 DLBCL, 10 sCNSL and three PCNSL cases. Custom ampliseq panels (Life Technologies) covering coding DNA sequence (CDS) and untranslated region (UTR) regions were first designed for CXCR5 and CXCR4, chemokine (C-C motif) receptor 7 (CCR7), CXCL13, and chemokine (C-C motif) ligands 19 and 21

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**Abs 483 - Table 1.** SNP incidence in PCNSL, sCNSL and DLBCL. Global minor allele frequency (MAF)

| CXCR5 | MAF | PCNSL (*N*=11) % | sCNSL (*N*=10) % | DLBCL (*N*=12) % |
|---|---|---|---|---|
| rs613791 | 0.3189 | 72.7 | 60 | 50 |
| rs598207 | 0.3217 | 72.7 | 60 | 50 |
| rs665648 | 0.0084 | 9.1 | 0 | 0 |
| rs3922 | 0.4433 | 81.8 | 70 | 58.3 |
| rs676925 | 0.1542 | 54.5 | 60 | 33.3 |
| rs45543933 | 0.1522 | 54.4 | 50 | 33.3 |
| rs10892307 | 0.0952 | 27.3 | 40 | 8.3 |
| rs113967672 | 0.0010 | 0 | 0 | 8.3 |
| rs199805189 | 0.0001 | 9.1 | 0 | 8.3 |
| rs73575405 | 0.0308 | 9.1 | 0 | 16.7 |
| rs373932544 | NA | 0 | 20 | 0 |
| rs2230321 | 0.0308 | 9.1 | 0 | 8.3 |
| **CXCR4** | | | | |
| rs2680880 | 0.1697 | 36.4 | 50 | 75 |
| **CXCL13** | | | | |
| rs144898428 | 0.0016 | 9.1 | 0 | 16.7 |
| rs1052563 | 0.0439 | 0 | 0 | 8.3 |

SNP, single nucleotide polymorphism; PCNSL, primary CNS lymphoma; sCNSL, secondary CNS involvement of systemic lymphoma; DLBCL, diffuse large B-cell lymphoma; MAF, minor allele frequency.

(CCL19 and CCL21) genes. Five PCNSL samples were sequenced and studied for sequence variants. Based on results, hotspot panel containing a total of 14 variants in CXCR4, CXCR5, and CXCL13 genes was designed and used to sequence the rest of the samples. All sequence libraries were prepared with Ampliseq Library Kit 2.0 and sequenced with Ion Torrent PGM system (Life Technologies). Sequencing results were imported to Ion Reporter analysis software (Life Technologies) and analyzed for potential sequence variants.

**Results:** For reliable results, coverage must be over 40. In our analysis, coverage was 500–2000. *P*-value is 0.

**Discussion:** Polymorphism in CXCR5 and CXCR4 chemokine receptors expose to malignant B-cells migrates into CNS. We suggest that the development of CNS lymphoma is dependent on disease characteristics and host CXCR5 and CXCR4 profiles.

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