

Non-Hodgkin lymphoma - Biology and clinical

P832

HIGHLY SPECIFIC QPCR ASSAYS USING A CONSENSUS LNA PROBE FOR THE DETECTION OF T(11;14) AND T(14;18) IN NON-HODGKIN LYMPHOMA WITH A SENSITIVITY OF 10^{-5} FOR QUANTIFICATION OF MINIMAL RESIDUAL DISEASE

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Background: In mantle cell lymphoma (MCL) the reciprocal translocation t(11;14)(q13;q32), juxtaposing the *BCL1* gene to the immunoglobulin heavy chain (*IgH*) locus, is present in about 80% of the patients. This results in an overexpression of cyclin D1 giving rise to increased cell proliferation. In follicular lymphoma (FL), the t(14;18)(q32;q21) balanced translocation causes an elevated expression of *BCL2* rendering the cells less prone to apoptosis. In addition, a subpopulation of diffuse large B-cell lymphomas also carry t(14;18). Importantly, the above aberrations can be employed for the detection of minimal residual disease (MRD), but optimal MRD measurements in both peripheral blood (PB) and bone marrow (BM) requires a validated and sensitive assay which can, moreover, be easily applied in a clinical setting. Employing a very sensitive assay enables the use of PB samples for analysis in addition to BM or lymph node biopsies facilitating easy access to cancer cells.

Aims: By employing chemically modified RNA nucleotides, such as locked nucleic acids (LNA), we wanted to design and validate highly sensitive and specific qPCR assays to detect t(11;14) and t(14;18). We set out to use consensus primers avoiding the generation of patient-specific priming in order to monitor MRD in subgroups of NHL patients over time.

Methods: We obtained PB and BM samples at the time of diagnosis and/or relapse as well as during treatment. A consensus reverse primer and probe targeting all six J-segments of the *IgH* locus were applied in the qPCR assays. The probe, consisting exclusively of LNA bases, enabled the design of a short amplicon, thus improving the efficiency of the assay. The forward primer targeting the *BCL1* gene was located in the major translocation cluster. Likewise, the *BCL2* forward primer was designed to cover the various breakpoints of the major breakpoint region. The *TCF20* gene was used as a reference gene, and the cell lines JVM2 and DOHH2 were employed as positive controls. All clinical samples were normalized to a diagnostic or a relapse sample defining the MRD levels at diagnosis or relapse as 1. Samples with Cq values >40 were defined as negative.

Results: We employed the JVM2 and DOHH2 cell lines to establish the t(11;14) and t(14;18) qPCR assays and subsequently validated the assays in 22 MCL and 9 FL patients. The assays successfully quantified MRD in 11/22 MCL patients and 9/9 FL patients. Quantification of t(11;14) and t(14;18) was possible down to less than 10^{-5} and 10^{-4} , respectively, as illustrated in Figure 1 which shows representative longitudinal MRD curves of two patients. The level of MRD reflects the clinical courses of the patients based on pathology and flow cytometry. As indicated in Figure 1, high levels of MRD correspond to clinically detectable disease, and low levels of MRD correspond to complete remission (CR).

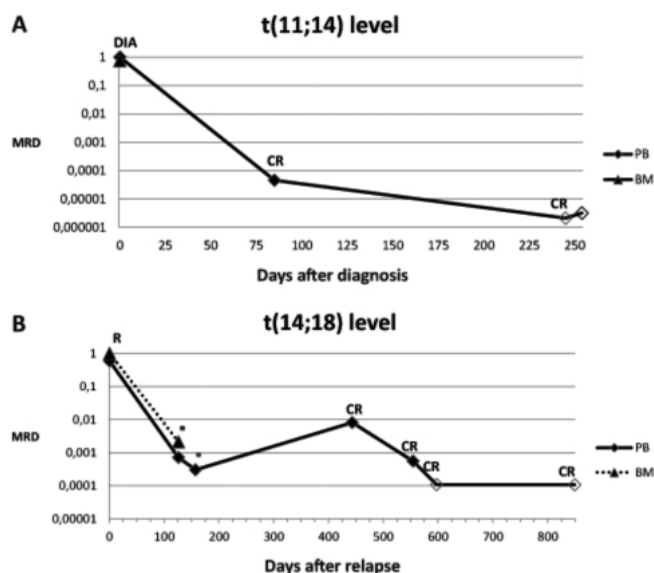


Figure 1. Representative MRD curves of A) an MCL patient and B) an FL patient. Open symbols signify values with a Cq >40. DIA: diagnosis, CR: complete remission, R: relapse, *: clinical status unknown.

Summary and Conclusions: The fraction of quantifiable patients using these assays reflects the presence of multiple translocation breakpoints within the *BCL1* and *BCL2* loci. As the assay is based on consensus primers the time-consuming process of designing and optimizing patient-specific qPCR assays is avoided, and the sensitivity is still down to 10^{-5} . Hence, the present qPCR assays for t(11;14) and t(14;18) show a high degree of sensitivity and specificity and hold great potential as an easily applicable methodology for MRD quantification.

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COMPARISON OF NEXT-GENERATION SEQUENCING AND ASO-PCR METHODS FOR MRD DETECTION IN MANTLE CELL LYMPHOMA

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Background: The prognostic value of MRD detection using allele-specific oligonucleotide (ASO)-PCR has been demonstrated in mantle cell lymphoma (MCL) patients treated with autologous stem cell transplant (ASCT) or with chemotherapy alone (Pott *et al.*, Blood 2010). ASO-PCR requires preparation of clonotype-specific primers for each individual which is laborious and time-consuming. We developed the LymphoSIGHT™ platform, a high-throughput sequencing method, which universally amplifies immune receptor gene segments and can identify all lymphoma-specific sequences at diagnosis, allowing monitoring of disease progression during therapy (Faham *et al.*, Blood 2012).

Aims: In this study, we compared the sequencing and ASO-PCR methods for measuring MRD in follow-up samples from 22 MCL patients.

Methods: Using the sequencing assay, we analyzed diagnostic blood and bone marrow samples from 22 MCL patients for clonal rearrangements of immunoglobulin (IGH-VDJ, IGH-DJ, IGK) genes. Clonal rearrangements had been previously detected in all 22 patients using ASO-PCR methods. We assessed MRD at the IGH and/or IGK locus in 159 follow-up samples, and analysis of the concordance between MRD results obtained by the sequencing method and ASO-PCR is ongoing.

Results: Sequencing detected the presence of a high-frequency clonal rearrangement of at least one receptor ("calibrating receptor") in 100% of the 22 MCL patients; 20 patients had at least 2 calibrating receptors at diagnosis, and 2 patients had 3 calibrating receptors. Following the identification of a calibrating receptor, we analyzed follow-up samples for the presence of MRD. Sequencing detected MRD levels of >10% in 1 sample, 1-10% in 3 samples, 0.1-1% in 8 samples, 0.01-0.1% in 7 samples, 0.001-0.01% in 21 samples, and <0.001% in 23 samples. MRD was undetectable by sequencing in 96 samples.

Summary and Conclusions: This high-throughput sequencing method enables MRD detection without the need for development of patient-specific reagents. Concordance data between sequencing and ASO-PCR will be presented.

P834

NR4A1 MEDIATED APOPTOSIS SUPPRESSES LYMPHOMAGENESIS IN A XENOGRFT MOUSE MODEL

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Background: NR4A1 (*Nur77*) and NR4A3 (*Nor-1*) are two members of the orphan nuclear receptors (NRs) whose function as critical tumour suppressor genes was demonstrated by the rapid development of acute myeloid leukemia (AML) in the NR4A1 and NR4A3 double knock out mouse and by their reduced expression in leukemic blasts from human AML patients.

Aims: The aim of our study was to comprehensively investigate NR4A1 and NR4A3 expression and function in lymphoid malignancies.

Methods: NR4A1 and NR4A3 expression were determined on mRNA- and protein levels in most lymphoid malignancies of B cell type by semi-quantitative RQ PCR, Western Blot and immunohistochemical analyses. For functional characterization NR4A1 was over expressed in a Sc-1 lymphoma cell line by using an inducible lentiviral construct followed by apoptotic assays (cleaved caspase3, Sub-G1 peak determination, and the Annexin V staining) and by xenograft mouse experiments.

Results: We found a more than 50% reduced expression of both, NR4A1 and NR4A3 in B-CLL (71%) in follicular lymphoma (FL) (70%), and in diffuse large B-cell lymphoma (DLBCL) (74%). In aggressive lymphomas (DLBCL, FL II) low NR4A1 expression was significantly associated with a non-germinal centre B-cell subtype and with poor overall survival. To investigate the function of NR4A1 in lymphomas, we over-expressed NR4A1 in the lymphoma cell line Sc1 by using an inducible lentiviral expression system. Induction of NR4A1 expression led to a significantly higher proportion of induced Sc1 cells undergoing apoptosis as demonstrated by caspase 3-, DNA cleavage and Annexin V staining.

To test the tumor suppressor function of *NR4A1* *in vivo*, the stably transduced Sc1 lymphoma cell lines were further investigated in the NOD scid gamma (NSG) mouse model. Induction of *NR4A1* in Sc1 abrogated tumor growth in the NSG mice, in contrast to vector control- and uninduced Sc1 cells, which formed massive tumors.

Summary and Conclusions: Our data suggest that *NR4A1* has a pro-apoptotic function in aggressive lymphoma and defines *NR4A1* as novel tumor suppressor involved in lymphoma development.

P835

EZH2 EXPRESSION DETERMINED BY IMMUNOHISTOCHEMISTRY DOES NOT CORRELATE WITH THE PRESENCE OF EZH2 MUTATIONS IN A SERIES OF DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL)

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Background: Advances in DNA sequencing technology have recently enabled the characterization of genomes and transcriptomes at sufficient resolution for identification of somatic point mutations that are involved in cancer. This knowledge has led, not only to enhance understanding of the mechanisms of pathogenesis, but also to improve the treatment of these patients by designing drugs that specifically act on these targets. Somatic mutations of *EZH2* have been involved in cancer and lymphomagenesis by affecting the mechanisms implicated in the regulation of gene expression. Gain-of-function mutations involving Y641 amino acid have been described in B-cell lymphomas derived from follicle germinal center cell phenotype (GCB) including follicular lymphoma (FL) (7%) and diffuse large B-cell lymphomas (DLBCL) of GCB type (22%). More recently, mutations in the A677 amino acid have been reported in a B-cell lymphoma cell line.

Aims: Our aim was to study the mutational status of *EZH2* gene in a series of DLBCL, and to correlate the mutational status with *EZH2* expression by immunohistochemistry in the neoplastic cells. In addition, we correlated the mutational status of *EZH2* gene with clinical characteristics at presentation and follow-up.

Methods: We included 111 cases of DLBCL diagnosed between 2000 and 2011 according to WHO 2008 diagnostic criteria, corresponding to 61 DLBCL of GCB type and 50 DLBCL of the activated B-cell type (ABC). *EZH2* mutational study was assessed in DNA extracted from paraffin-embedded tumour tissue. Regions where mutations have been previously described (amino acids Y641 and A677 of *EZH2* gene) were analysed by Sanger sequencing. Expression of *EZH2* protein was performed by immunohistochemistry using clone SP129 (Ventana Medical Systems, Tucson, AZ) in 27 cases, 14 *EZH2* mutated and 13 *EZH2* unmutated, using the detection system of Optiview BenchMark XT (Ventana Medical Systems).

Results: Of the 111 cases included, 14 samples had mutations in the *EZH2* gene (12.6%) (GCB type in 11 cases and ABC type in 3 cases). In all cases, mutations were detected in the amino acid Y641 (Y641D:n=7, Y641S:n=4, Y641H:n=3, Y641D:n=1). No mutations were observed in the A677 position. Immunohistochemical analysis of *EZH2* expression showed positivity in neoplastic cells in all cases examined (n=27) with no qualitative or quantitative differences regardless of *EZH2* mutational status. Mutational status of *EZH2* gene was not associated with the following clinicopathologic characteristics: age >60 years, gender, Ann Arbor stage, International Prognostic Index (IPI), ABC-GCB type. Overall survival at 3 years was inferior in high or intermediate-high IPI patients when compared with low or intermediate-low IPI cases (46% vs 79%, *P*=0.005), but no differences in survival were observed between patients with mutated and unmutated *EZH2* gene (80% vs 59%, respectively, *P*=0.376).

Summary and Conclusions: In our series, *EZH2* mutations were present in the 12.6% of cases, with higher prevalence in the GCB subgroup (18%) but were also observed in the ABC profile (6%). All mutations were detected in the amino acid Y641 of the *EZH2* gene. No differences were seen between the mutational status of *EZH2* and the pattern of *EZH2* protein expression determined by immunohistochemistry in the tumor tissue. Finally, mutational status of *EZH2* gene was not associated with clinicopathologic characteristics and did not have influence on outcome.

P836

DLBCL BIOLUMINESCENT XENOGRAFT MODEL FOR NON-INVASIVE FOLLOW-UP OF ANTITUMOR EFFECT

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Background: Diffuse large B cell lymphoma (DLBCL) is the most common non-

Hodgkin lymphoma subtype. Addition of rituximab to the classical chemotherapy has improved response rates. However, potential cure is achieved in only 30–50% of patients. The heterogeneous nature of DLBCL and the lack of appropriate animal models are obstacles to further understand the pathology and to identify new drugs for this disease. Although xenograft models of DLBCL are widely used to test new drugs against this neoplasia, most of them are subcutaneous xenografts which do not show a disseminated disease. We describe bioluminescent and disseminated xenograft models of both Germinal Center B cell (GCB) and Activated B Cell (ABC) subtypes of DLBCL. As described previously by our group, the subcutaneous conditioning increased GCB-DLBCL cells capacity to engraft and disseminate. Hence, we performed a subcutaneous passage of the cells before their intravenous injection in mice. The dissemination of the disease was non-invasively followed by *in vivo* imaging.

Aims: To develop bioluminescent xenograft mouse models of GCB and ABC and to validate the DLBCL models using cyclophosphamide

Methods: Toledo (GCB) and RIVA (ABC) DLBCL cell lines expressing GFP-Luciferase (GL) were subcutaneously injected in 6-10 female NOD/SCID mice. Tumors (600 mm³) were extracted and disaggregated. Then, 12-20 mice were randomly divided in two groups and intravenously injected with cultured *in vitro* cells (Toledo GL or RIVA GL) or with the subcutaneously-conditioned cells (Toledo-GL-Sc or Riva-GL-Sc). For cyclophosphamide treatment, mice were injected with subcutaneously-conditioned Toledo-GL-Sc cells and randomized in two groups. Drug (60mg/mL) or vehicle was intravenously injected every 2 days for a total of 7 administrations. *In vivo* images of bioluminescence were taken using IVIS Imaging System once a week.

Results: 1) Subcutaneous passage of Toledo-GL and RIVA-GL cell lines increases aggressiveness: Mice injected intravenously with RIVA-GL-Sc cells showed higher engraftment and dissemination rate than the RIVA-GL intravenously injected group; a significant increase of dissemination to lymph nodes, bone marrow and central nervous system was detected (Figure 1). Aggressiveness of the Toledo-GL-Sc cells also increased with the subcutaneous conditioning. Survival of mice was significantly decreased when lymphoma cells were submitted to the subcutaneous passage (Kaplan-Meier survival curves not shown). 2) Generation of xenograft bioluminescent mouse models of GCB and ABC DLBCL: The intravenously injected cells, Toledo-Sc and RIVA-Sc, generated a disseminated disease in NOD/SCID mice. As observed in Figure 2, most mice showed lymph node and bone marrow infiltration. Macroscopic infiltration was observed mostly in cervical, caudal, inguinal and renal lymph nodes. When tissues were analyzed microscopically, multiple lymph nodes, bone marrow and central nervous system infiltration was observed in most mice (Figure 2D-F) corresponding with the bioluminescent images (Figure 2C). As observed by immunohistochemistry, the infiltrated lymph nodes were CD10+ in Toledo-GL injected mice and CD10-, BCL6+, MUM1+ in RIVA-GL injected mice, confirming its germinal center and activated phenotype, respectively. 3) Validation of DLBCL bioluminescent xenograft model using cyclophosphamide: In order to validate the DLBCL bioluminescent mouse model, cyclophosphamide (Genoxal®) or vehicle were administered to mice. The bioluminescence images (Figure 3) showed decreased dissemination of cells in the treated group. In addition, mice survival significantly increased with cyclophosphamide treatment (Kaplan-Meier survival curves not shown).

	Riva GL	Riva-GL-Sc	Toledo GL	Toledo-GL-Sc
Mice dead by lymphoma, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)
Survival time, days	85.2 ± 12.2	52.7 ± 8.1	37.8 ± 6.2	27.7 ± 1.5
Mice with LN infiltration (macro or micro), n (%)	3/5 (60)	6/6 (100)	8/9 (88.8)	8/10 (80)
Mice with BM involvement, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)
Column or Legs, n (%)	0/6 (0)	5/6 (83)	9/9 (100)	10/10 (100)
Cranium, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)
Mice with CNS infiltration, n (%)	5/6 (83.3)	6/6 (100)	9/9 (100)	10/10 (100)

Figure 1.

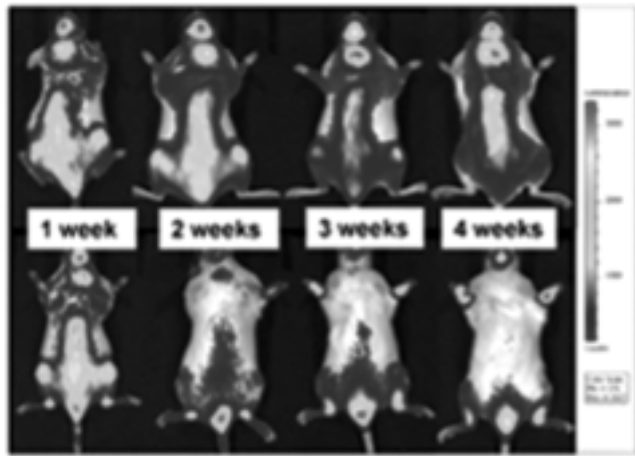


Figure 2.

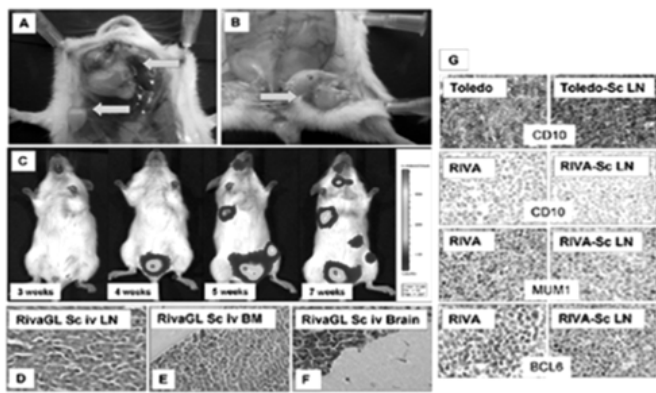


Figure 3.

Summary and Conclusions: We have successfully generated bioluminescent xenograft mouse models for the study of GCB and ABC-DLBCL. Our xenograft mouse models were successfully validated with a drug which is routinely given to patients. The obtained models may be helpful to study the mechanisms of dissemination in DLBCL pathology and to non-invasively monitor the effect of new therapies in the context of a disseminated disease.

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BLOOD AND LYMPHATIC MICROENVIRONMENT, MACROPHAGE COMPONENT AS PROGNOSTIC MARKERS IN FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) has considerable clinical heterogeneity. There is a need for easily quantifiable prognostic biomarkers. There is no consensus on the prognostic significance of microvessel density and number of macrophages.

Aims: To characterize the blood and lymphatic microvessel density, the number of macrophages of tumor tissue basing on immunohistochemical study (IHC) of sections from paraffin blocks of lymph nodes biopsies in two groups of patients with different clinical course.

Methods: The study included 59 patients with follicular lymphoma: 39 women (67%) and 20 men (33%). The mean and median age was 53 years (range: 27–83 years). 49 patients were observed in the National Research Center for Hematology (Moscow) from April 2001 to May 2011 and 10 patients - in Cancer Research Center (Moscow) at the same period of time. The first group—good outcome (1) included 28 patients followed during 2 years or more without therapy; with remission of the underlying disease; with relapse in five or more years from the start of their treatment (late relapse). The second group—poor outcome (2) included 31 patients who died due to tumor progression in the first 1–2 years from the time when this diagnosis was put; with resistance to the passage of the tumor; with relapsed FL in the first year from the start of treatment (early relapse). Patients in both groups received the same initial treatment. The vascularization of tumor tissue was assessed by IHC on sections from paraf-

fin blocks of tumor biopsies of lymph nodes. For the visualization of blood and lymphatic vessels antibodies CD34 and D2-40 were used. CD34 is an endothelial marker of bloodvessels. D2-40 is a new lymphatic marker (podoplanin), which recently was found to express at a high level in lymphatic endothelial cells. CD68 is a marker, which is used to visualize activated macrophages. Morphometric analysis was performed using light microscopy and a digital camera Leica (vol. $\times 40$). The pictures are processed by a computer program "VideoTest-Morphology 5.2" in order to estimate the specific area of vessels in relation to the tumor tissue (%) during the researcher's visual inspection. IHC - specimen evaluation was carried out using a Table of random numbers. Counting the number of macrophages was realized by using light microscopy (magnification $\times 40$) in nodular, nodular and diffuse areas of tumor growth. Macrophages were counted on 1 mm² of tumor tissue. The number of positive cells was determined intrafollicular and interfollicular space.

Results: 5-year OS rate in group 1 was significantly higher than in group 2 (83 $\pm$ 7% vs 28 $\pm$ 13%; $P=0.03$) (Figure 1A). Evaluation of the specific area of blood and lymph vessels was conducted in two comparable groups. The specific area of blood vessels in group 2 was significantly higher than in group 1: 0.04 (95% CI, 0.03–0.05) vs 0.02 (95% CI, 0.01–0.03) (Figure 1B). The specific area of the lymph vessels in group 2 was significantly higher than in group 1: 0.06 (95% CI, 0.04–0.07) vs 0.03 (95% CI, 0.01–0.04) (Figure 1C). The number of CD68 - positive macrophages with nodular and diffuse growth of tumor in group 2 was significantly higher than in group 1: 800 (95% CI: 380–1222) vs 79 (95% CI: 10–566) (Figure 1D).

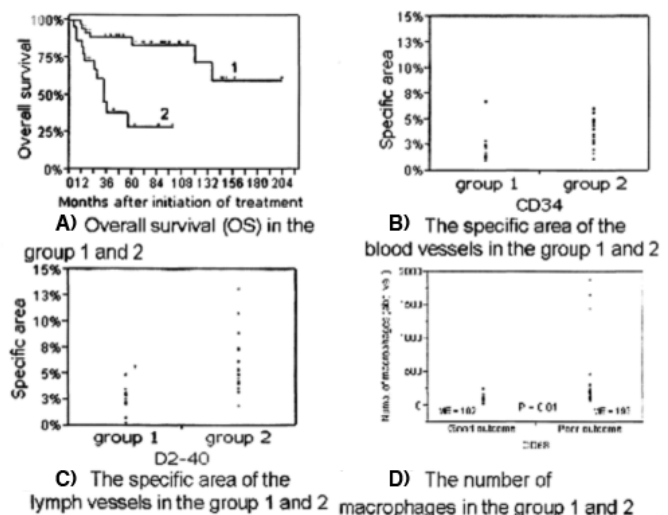


Figure 1.

Summary and Conclusions: The microvascular density can be estimated using the markers CD34 and D2-40. Our results demonstrate an association between prognosis and angiogenic sprouting and reveal higher angiogenic activity in the poor prognostic group. The higher number of macrophages is also associated with a worse prognosis. Probably, these results will let determinate the nature of the follicular lymphoma.

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ANALYSIS OF SOX11, SOX4 AND SOX12 IN MANTLE CELL LYMPHOMA

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Background: The related transcription factors SOX11, SOX4 and SOX12 share significant homology at the gene and protein level and are classified as the SOXC family. In mantle cell lymphoma (MCL), SOX11 is expressed in approximately 90% of cases. Lack of SOX11 expression has been associated to a different biological behavior in MCL. Little is known on how SOX4 and SOX12 are expressed in SOX11 positive and SOX11 negative MCL. SOX11 has been suggested to be epigenetically regulated in lymphoma, but there are partly conflicting results considering the role of promoter methylation.

Aims: To investigate the role of promoter methylation in SOX11 expression. To investigate how SOX4 and SOX12 are expressed in SOX11 positive and SOX11 negative MCL.

Methods: Expression of SOXC transcription factors was analyzed by Q-PCR

in primary MCL (n=29), in MCL cell-lines (n=4) and in non-malignant lymph node, spleen and peripheral blood B lymphocytes (PB). These samples were also investigated for promoter methylation using MspBC-digestion and pyrosequencing. The expression of SOXC transcription factors in MCL cell lines was also investigated after treatment with 5-azacytidine.

Results: SOX11 was expressed in all but three primary MCL (median 500-fold expression compared to PB) and in 3/4 MCL cell lines while non-malignant lymphocytes lacked SOX11 expression. SOX4 was variably expressed; 13/29 MCL expressed lower levels and 10/29 expressed higher SOX4 levels than PB. SOX12 was expressed at higher levels in 27/29 MCL compared to PB. Three SOX11 negative primary MCL all expressed SOX12. In SOX11 positive MCL there was a highly significant correlation between expression of SOX4 and SOX11 (Spearman's correlation coefficient 0.56; P<0.005) and between SOX12 and SOX11 (Spearman's correlation coefficient 0.63; P<0.005). The promoter region of SOX11 was hypomethylated in SOX11 positive MCL as well as in non-malignant B-lymphocytes and heterogeneously methylated in the three SOX11 negative MCL cases. Treatment with 5-azacytidine reduced SOX11 expression in 2/3 SOX11 positive MCL cell lines and induced expression of SOX4 but not SOX11 in the SOX11 negative MCL cell line JVM2.

Summary and Conclusions: SOX11 expression in MCL is not regulated by promoter methylation but methylation at other sites might be of importance for sustaining high SOX11 expression levels. SOX12 are expressed at higher levels in SOX11 positive and SOX11 negative MCL compared to non-malignant lymphocytes. Finally, the expression of SOXC transcription factors is strongly correlated in SOX11 positive MCL. This might be highly biologically relevant since the members of the SOXC family have been reported to activate similar target genes.

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HIV ASSOCIATED DOUBLE-HIT B-CELL LYMPHOMAS PREFERENTIALLY INVOLVE THE MYC AND BCL6 GENE LOCI

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Background: Double-hit (DH) lymphomas have recently attracted attention due to their aggressiveness, their possible under diagnosis previously and the need to better characterize these disease in order to develop novel treatment strategies. A number of case studies and case series on DH lymphoma show that these diseases are very aggressive, are often classified as lymphomas with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) and typically have translocations of the *MYC* and *BCL2* genes which partner with the immunoglobulin loci. Double hits involving *MYC* and *BCL6* translocations are much more rarely described in the international literature. HIV infection significantly increases the risk of developing high grade B-cell lymphoma. These often present with atypical features posing challenges in diagnosis. In particular, lymphomas presenting with L3-like morphology have other features that best fit the diagnosis of the intermediate class of lymphoma introduced in the WHO 2008 classification (B-Cell lymphoproliferative disorder, NOS, with features intermediate between BL and DLBCL).

Aims: To characterize the clinical, morphological, immunological and cytogenetic features of HIV associated double-hit B-cell lymphoma.

Methods: Reports of patients who presented with HIV-associated high grade B-Cell Lymphoma (particularly Burkitt-like and atypical Burkitt lymphoma/leukaemia), between 2008 and 2012 were reviewed for the presence of DH lymphoma on cytogenetic/FISH analysis. Cytogenetics studies were performed using standard methods and GTG banding, chromosomes were classified according to ISCN 2013. Fluorescence *in situ* hybridization (FISH) was performed using LSI c-MYC/IGH dual colour dual fusion translocation probe and/or LSI c-MYC dual-colour breakapart rearrangement probes, LSI BCL6 dual colour breakapart rearrangement probes and LSI BCL2/IGH dual colour dual fusion translocation probe. Flow cytometry and immunohistochemistry were performed as per standard protocols.

Results: Seven patients were diagnosed between 2008-2012. This included five adults (3 males and 2 females) with an age ranging from 25 to 41 years, (mean age of 35 years) and two male children (6 and 7 years old respectively). All patients were HIV positive and had systemic disease at presentation. Five patients (3 adults, 2 children) had morphology/histology with a mixture of cells of intermediate to large size best fitting the diagnosis of B-cell lymphoma with intermediate features between DLBCL and BL. Two adults patients had a more typical L3 morphology. A complex karyotype with more than 3 structural and/or numerical chromosomal aberrations was seen in every patient. *MYC* was rearranged in a classical t(8;14)(q24;q32) in four cases and in a variant Burkitt translocation t(8;22)(q24;q11) in three cases. *BCL6* was involved in a t(3;14)(q27;q32) in 4 cases and in a t(3;22)(q27;q11) in 2 cases. In one case *BCL6* rearrangement was not seen on karyotype but was detected by FISH in 50% of cells. The most common additional aberration was trisomy 1 or partial trisomy/tetrasomy for chromosome 1q. *MYC* and *BCL6* rearrangements were

all confirmed by FISH. *BCL2* rearrangement was not detected. One case had a triple hit with a t(8;14), t(3;22) and a t(2;14)(q33;q32) involving an unknown partner gene on chromosome 2. Follow-up cytogenetics obtained in case 1 and 2, (after 2 and 1 month respectively), revealed a rapidly evolving karyotype reflecting the aggressiveness of the disease. Immunophenotypically all cases had a CD19 B-cell lineage but weak CD10 and CD20 signals. Interestingly, immunohistochemistry performed in 3 cases for CD38 and CD138 showed weak positivity for CD38 in all 3 cases and a dim CD138 in 2 cases, with focal positive regions in the third case. All patients had a highly aggressive course of disease and died within few months of initial diagnosis.

Summary and Conclusions: In our series, HIV associated DH lymphoma occurred in young people, preferentially involved *MYC/BCL6* translocations and had an aggressive course of disease with extremely poor survival. There is a need to characterize these tumours to better understand their pathogenesis and develop novel therapeutic strategies.

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LOSS OF NIPA LEADS TO DEFECTS IN THE REPAIR OF DNA DOUBLE STRAND BREAKS IN MICE

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Background: Regulated oscillation of protein expression is an essential mechanism of cell cycle control. The SCF class of E3 ubiquitin ligases is involved in this process by targeting cell cycle regulatory proteins for degradation by the proteasome. We previously reported the cloning of NIPA (Nuclear Interaction Partner of ALK) in complex with constitutively active oncogenic fusions of ALK, which contributes to the development of lymphomas and sarcomas. Subsequently we characterized NIPA as a F-Box protein that defines an oscillating ubiquitin E3 ligase targeting nuclear cyclin B1 in interphase thus contributing to the timing of mitotic entry.

Aims: To study *in vivo*-function of the G₂/M checkpoint NIPA in greater detail, we inactivated the gene *Nipa* using a conditional knockout strategy.

Results: *Nipa*-deficient animals are viable, but show a lower birth rate and a reduced body weight. Furthermore, *Nipa*-deficient males were sterile due to a block of spermatogenesis during meiotic prophase. Virtually no spermatocytes progress beyond a late-zygotene to early-pachytene stage and reach an aberrant stage, with synaptonemal complex disassembly and incomplete synapsis. *Nipa*^{-/-} females are sub-fertile with an early and severe meiotic defect during embryogenesis with extensive apoptosis in early prophase (E13.5-E14.5).

Here we report, that *Nipa*^{-/-} meocytes exhibit persistent cytological markers for DNA double strand break repair proteins (like DMC1, RAD51) in meiotic prophase with more than twice as many DMC1 foci as control animals. Kinetic analysis of the first wave of spermatogenesis showed increased DMC1/RAD51 foci in *Nipa*^{-/-} cells as soon as early-pachynema cells appear (13-14 days post partum). Moreover, we show that *Nipa* deficiency does not lead to a defect in meiotic sex chromosome inactivation despite epithelial stage IV apoptosis. *Nipa*-deficient spermatocytes exhibit numerous abnormalities in staining of chromosome axis associated proteins (like SYCP3 and STAG3) indicating that chromosome axis defects were associated with compromised chromosome axis integrity leading to overt chromosome fragmentation.

Summary and Conclusions: Taken together, the phenotype of *Nipa*-knock-out mice is a definitive proof of the meiotic significance of NIPA and our results show a new, unsuspected role of NIPA in chromosome stability and the repair of DNA double strand breaks.

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IDENTIFICATION OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) SUBTYPES DEFINED BY THE EXPRESSION OF STROMAL PROTEINS AND THEIR ASSOCIATION WITH MICRORNAS INVOLVED IN ANGIOGENESIS

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Background: The identification of subtypes of DLBCL defined by expression of genes related to tumor stroma (Lenz *et al.*, 2008) may determine new paths in the treatment of these diseases using anti-angiogenic drugs. The relationship of these genes with the pro-angiomiRs is still unknown.

Aims: 1) to classify DLBCL cases according to the signature *stromal-1* and *stro-*

mal-2 using tissue microarray (TMA) and immunohistochemistry; 2) to evaluate the expression of pro-angiomiRs in paraffin embedded tissues affected by DLBCL and correlate them with the signatures of the tumor stroma.

Methods: 111 DLBCL (NOS and variants, all HIV negative) admitted to the Hospital São Paulo between 2000 and 2010 were included in this study. Expression of CD68 (*stromal-1* marker) was classified into four (0-3) levels. To analyze the expression of CD34 (MVD, *stromal-2*), we conducted a manual count of microvessels in the entire field of TMA. We performed miRNA extraction from paraffin samples using RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE Tissues (Applied Biosystems). Real-time quantitative PCR was performed using TaqMan® Small RNA kit Assays and normalized with U18. Pro-angiomiRs miR-92a, miR-126, miR-130a, miR-210, miR-378 and miR-296 were selected for assessment of angiogenesis and were considered differentially expressed when tumor samples levels were 1.2 times higher or lower than normal samples.

Results: *Stromal-1* cases had high expression of CD68 (scores 2-3) and low expression of CD34 (quartiles I-II) (34.4% of cases), and as *stromal-2* cases had low expression of CD68 (scores 0-1) and high expression of CD34 (quartiles III-IV) (12.5% of cases). The 38 cases that had high expression of both CD68 and CD34 (39.6%) were additionally considered as *stromal-2*, due to high MVD scores. Cases of low expression of CD68 and CD34 (13.5% of cases) were not scored. We observed a statistically significant relationship between the median expression of CD34 and Ann Arbor stage (I-II versus III-IV), with a predominance of high MVD expression in patients with advanced stage disease (III-IV). The median of vessels in stages I-II was 82 (range 9 to 240) and in stages III-IV was 111 (range 12-297) ($P=0.0183$, Mann-Whitney). For the marker CD68 (*stromal-1*) and for the other variables, there were no statistically significant associations between groups. 101 of 111 initial cases were considered suitable for analysis of microRNAs. We observed overexpression of miRNAs miR-296, miR-378, miR-92a, miR-210, miR-130a and miR-126 in 55.5%, 50.5%, 42.6%, 30.7%, 19% and 9.9% of cases, respectively. We also observed increased expression of miR-296 in DLBCL classified as stage III-IV ($P=0.0355$, Mann-Whitney). For other variables, there were no statistically significant differences between groups.

Summary and Conclusions: *Stromal-2* signature was found in 52.1% of cases. The higher MVD in cases of DLBCL with advanced stage (Ann Arbor III-IV) shows a possible association between angiogenesis and more aggressive/disseminated disease, being a possible target for anti-angiogenic therapy in cases that do not achieve a complete response with R-CHOP. The miR-296, directly responsible for decreased levels of hepatocyte growth factor and indirectly by the upregulation of VEGFR2 and PDGFR β , showed overexpression in 55.5% of DLBCL and association with advanced stage disease. Our study provides new information about the importance of the role of microRNAs in the development of DLBCL, which can be exploited as a therapeutic target and prognostic factor for patients with this malignant disease of high prevalence. (Supported by FAPESP 2010/17668-6).

P842

DISTINCT GENE EXPRESSION PROFILES IN A CANINE MODEL OF HUMAN DLBCL

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Background: Diffuse large B-cells lymphoma (DLBCL) is the most common type of lymphoma in adults. In humans, several studies have categorized DLBCL lymphomas into different morphological and molecular subtypes. Gene expression profiling provided a tool to reveal the presence of at least 3 subtypes of DLBCL; active B-cell (ABC-DLBCL), germinal center B-cell (GCB-DLBCL), and primary mediastinal B-cell Lymphoma (PMBL) with specific signatures correlating to the clinical course of the disease.

Aims: Evaluate the potential benefits of a canine model as an experimental platform to improve treatment and evaluate new therapies for both humans and dogs with DLBCL. In particular, the dog presents an ideal model with spontaneous occurrence of the disease. Indeed, Non-Hodgkin lymphoma is the most common hematopoietic disease in dogs that shares morphological and immunophenotypic features with human DLBCL and a highly aggressive course of the disease. Here we characterized at morphological, phenotypic, and molecular levels dogs with DLBCL to assess the factual likeness of these malignancies between human and dog patients.

Methods: More than one-hundred DLBCLs were collected during a three years period (2009-2012) at the diagnostic service of the Department of Veterinary Sciences and Public Health in Milan (IT). The diagnosis was based on morphological criteria and expression of CD20, CD21 and CD79. For twenty-one dogs with the morphological diagnosis of DLBCL that yielded high-quality extracted RNA, gene expression profiling was performed, using Affymetrix Canine 2.0 platform

(Affymetrix, Santa Clara, CA, USA). Five samples of normal lymph nodes were also analyzed. Data analyses were generated by R Bioconductor software and public databases of gene signatures (<http://Lymphochip.nih.gov/signaturedb/> and <http://broadinstitute.org/gsea/msigdb/index.jsp>) used to cross-compare human and dog signatures. Written informed consent was obtained from the dog owners.

Results: In dogs with DLBCL, the whole gene expression profiling analysis identified two clusters, a minor and a major one, with distinct signatures, providing the first example of molecular subgroups in Canine DLBCL not observed with the current standards of classification. No differences in age, gender and breed were observed for the 2 subgroups. Pathway analysis revealed activation of different pathways; the minor clusters showed decrease of apoptosis, DNA-damage response and down regulation of cell cycle progression genes suggesting a link with the human GCB-DLBCL signature. Moreover, the major cluster showed activation of *NFKB2* and *FUT8* genes together with a high expression of genes related to proliferation as revealed by cross comparison analysis between human and dog signatures, more correlated with ABC-DLBCL. Dogs DLBCL showed a general down-regulation of *BCL-6* gene compare to normal lymph nodes with a further difference between the two clusters.

Summary and Conclusions: In conclusion, in this study we analyzed gene expression profiles of dogs with DLBCL to determine if the dog with spontaneous occurrence of the disease can represent a useful study model for human DLBCL. We showed that as in human lymphoma molecular subtypes of DLBCL could be distinguished also in dogs. Further studies are needed to translate the relevance of the canine model of DLBCL to clinical research in human lymphoma.

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REAL-WORLD TREATMENT PATTERNS AND OUTCOMES IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: SEER-MEDICARE ANALYSIS

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Background: A disproportionate number of newly diagnosed diffuse large b-cell lymphoma (DLBCL) occurs in elderly patients

Aims: We set out to evaluate real-world treatment patterns and outcomes in elderly patients with DLBCL in the United States.

Methods: We utilized a retrospective cohort analysis of 9333 DLBCL patients in the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare database. Patients were diagnosed between 1/1/2000 - 12/31/2007, >66 years, and continuously enrolled in Medicare Part A and B in the year prior to diagnosis. There were 4565(49%) R (rituximab) +Chemo, 2181(23%) Chemo-only, and 467(5%) R-mono patients who initiated treatment within 3 months after diagnosis. The remaining 2120(23%) did not receive any treatment. Statistical comparisons were made between R+Chemo vs. Chemo-only, and R-mono vs. No Treatment. Logistic regression modeling assessed patient characteristics predictive of not receiving treatment. Kaplan-Meier curves and Cox proportional hazards regression assessed overall survival by treatment type. The median follow-up time was 2.4 years and date of last follow-up was 12/31/2009.

Results: Patients receiving R+Chemo were slightly younger (≤ 75 yrs: 50% vs. 46%; $P<0.05$), more likely white, and married compared to those receiving Chemo-only. Patients receiving R-mono were older (>80 yrs: 60% vs. 50%; $P<0.01$), and more likely female compared to those Not Treated). In general, untreated patients were older at diagnosis (mean age 80 vs. 77; $P<0.001$), more likely non-white (14% vs. 12%; $P<0.01$), diagnosed at late stage (51% vs. 44%; $P<0.001$), and had higher comorbidity burden than treated patients ($P<0.001$). As age increased, the treatment rate significantly decreased especially among patients >80 yrs. In the adjusted logistic regression model, increasing age, non-white race, male gender, higher comorbidity score, unmarried and lower income were predictive of not receiving any treatment. The unadjusted median overall survival was 96 months in the R+Chemo group vs. 13 months in Chemo-only group (log rank $P<0.001$); and 18 months in the R-mono group vs. 2 months in the Not treated group (log rank $P<0.001$). Among the treated, there were more R+Chemo and R-mono treatment cycles than Chemo-only cycles (5 vs. 4 cycles; $P<0.001$) delivered in the first-line. In multivariate survival analysis, patients receiving Chemo-only had a 2-fold increased risk of death (HR=2.20; 95%CI=2.04-2.37) compared to R+Chemo patients. Increasing age and comorbidity score were associated with significant increases in mortality. A higher mortality risk was noted with receipt of <6 cycles (HR=1.40; 95%CI=1.22-1.62) compared to 6 cycles. R-mono patients had a 69% lower risk of death (HR=0.31; 95%CI=0.28-0.35) compared to Not treated patients. Almost identical mortality risk reductions with R+Chemo and R-mono were also noted in a subgroup of patients >80 yrs.

Summary and Conclusions: Twenty-three percent of elderly DLBCL patients were not receiving treatment for their disease. We observed that R+Chemo and R-mono significantly decreased mortality risk in elderly DLBCL patients relative to chemo only and no treatment, respectively. The benefits were maintained even among patients >80 yrs, who were less likely to receive treatment. Sub-optimal durations of curative intent therapy (<6 cycles) were found to be associated with poorer outcomes.

CLINICAL SYMPTOM OR SIGN-DIRECTED SURVEILLANCE CAN BE MORE USEFUL TO DETECT RELAPSE COMPARED TO ROUTINE IMAGING IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AFTER COMPLETE REMISSION

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Background: Although the prognosis of patients with diffuse large B-cell lymphoma (DLBCL) has improved after introduction of rituximab, about one-third of the patients still experience eventual disease relapse. As there exists the second chance of cure by salvage therapy followed by autologous stem cell transplantation (ASCT), the optimal follow-up strategy to detect relapse early enough to proceed salvage therapy with maintenance of good performance status should be established for patients who have achieved a complete remission (CR) after frontline therapy. Despite lacking evidences, routine imaging has been widely adopted in surveillance of patients with lymphoma after CR.

Aims: The current study aimed at analyzing the patterns and their outcomes of out-patient department (OPD) follow-up for surveillance and evaluating the role of routine imaging and the result of unplanned early OPD visit in patients with DLBCL after CR.

Methods: Patients were included if they 1) diagnosed as DLBCL according to 2008 WHO criteria, 2) achieved CR by 2007 Revised Criteria after R-CHOP immunochemotherapy with or without consolidative therapy such as radiotherapy or ASCT, and 3) had at least 1 OPD visit for the surveillance of relapse. Routine imaging was defined as scheduled computed tomography (CT) or Positron Emission Tomography (PET)/CT at least 3 months prior to actual scanning by physician without any suspicious symptom or sign of the patients.

Results: From May 2004 to Feb. 2012, 106 of 151 patients with DLBCL (70.2%) obtained CR and fifteen of them (14.2%) experienced relapse, with a median follow-up duration of 38.1 months. A total of 856 OPD visits (median 6, range 1-25) were recorded from the 106 patients: 501 visits (median 4, range 0-15) were OPD surveillance with routine imaging (332 visits with CT alone, 94 with PET/CT alone, and 75 with both CT and PET/CT) and 322 visits (median 2, range 0-14) were planned OPD surveillance with only history taking and physical examination (Hx & P/Ex). Thirty-three visits (33/856=3.9%) were unplanned early visit by patients due to any abnormal symptom or sign. Routine imaging showed a perfect sensitivity and negative predictive value but low positive predictive value (PPV) due to frequent false-positive visits. Six of seven patients with false-positive routine CT scan and 17 of 23 patients with false-positive surveillance PET/CT received unnecessary further evaluation (please see the image). Compared to planned OPD surveillance with routine imaging (3 relapses out of 501 visits) and planned OPD visit with Hx & P/Ex (1 relapse out of 322 visits), early visits by patients due to any symptom or sign were highly related to disease relapse: 11 out of 33 visits (33.3%) resulted from relapse of DLBCL. Although survival from the time of relapse was not compared because of small sample size, no tendency of survival benefit in 3 patients with asymptomatic detection by routine imaging was observed (Figure 1).

Routine imaging with CT

	No Relapse	Relapse
CT normal	397	0
CT abnormal	7	3

Sensitivity: 100% (95% CI 30.5 ~ 100.0)
 Specificity: 98.3% (95% CI 96.5 ~ 99.3)
 PPV : 30.0% (95% CI 7.0 ~ 65.2)
 NPV : 100% (95% CI 99.1 ~ 100.0)

7 patients with false positive
 2 short-term CT follow-up
 2 MRI correlation
 1 biopsy with local anesthesia
 1 biopsy with general anesthesia
 1 close observation

Routine imaging with PET/CT

	No Relapse	Relapse
PET/CT normal	142	0
PET/CT abnormal	23	3

Sensitivity: 100% (95% CI 30.5 ~ 100.0)
 Specificity: 86.1% (95% CI 79.8 ~ 91.0)
 PPV : 11.5% (95% CI 2.6 ~ 30.2)
 NPV : 100% (95% CI 97.4 ~ 100.0)

23 patients with false positive
 7 CT or ultrasonogram correlation
 3 ENT exam correlation
 2 gynecologic exam correlation
 3 biopsy with local anesthesia
 1 biopsy with general anesthesia
 1 diagnosed as infection (liver abscess)
 6 close observation

Figure 1.

Summary and Conclusions: Surveillance routine imaging has limited value because of low PPV and its resultant unnecessary procedures such as additional imaging or biopsy. Considering the risk of radiation exposure, financial cost, and anxiety of the patients (Thompson CA *et al.*, *Ann Oncol* 21:2262-2266), routine imaging seems not to be an appropriate strategy for patients with DLBCL after CR. It is mandatory to perform thorough Hx & P/Ex, and adequate further active investigation when a patient visit OPD earlier than planned schedule and it have to be emphasized that patients should not hesitate to visit hospital earlier if they have any discomfort.

VIROLOGICAL SUCCESS IS ASSOCIATED WITH FAVORABLE PROGNOSIS IN PATIENTS WITH HEPATITIS C ASSOCIATED B-CELL NON-HODGKIN LYMPHOMAS, NATIONAL ANRS HC-13 LYMPHO-C STUDY RESULTS.

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Background: Hepatitis C virus (HCV) associated B-cell non-Hodgkin lymphomas (B-NHL) are a model of lymphoma induced by chronic antigenic stimulation. Lymphomagenesis mechanisms and treatment management remains matter of debate.

Aims: The national Lympho-C study collected clinical and biological data from HCV associated B-NHL aiming to better understand lymphomagenesis, treatment and outcome.

Methods: Adult patients with B-NHL and active HCV infection were included in this observational multicenter study. Patients co-infected with HIV could not be included. Data were collected from patients with either ongoing (prospective) or past (retrospective) history of HCV associated B-NHL. Cytological and histological samples were collected for centralized review and molecular analyses. Informed consent was required for each patient.

Results: Between 2006 and 2012, 138 consecutive patients were enrolled in 26 French hospitals. Subsequently 13 patients were excluded from analysis. 59 out of 125 (47%) patients were included at B-NHL diagnosis. At lymphoma diagnosis, median age was 61 years [IQR 50-71] and gender ratio was 0.9. Genotype HCV distribution was genotype 1 (51%), 2 (19%), 3 (9%), 4 (10%), 5 (1%) or unknown (10%). Median delay between HCV infection and B-NHL diagnosis was 25 years [IQR 25-75] [21-31] (unknown in 41/125 patients). Cyto-histological pattern was: diffuse large B cell lymphoma (DLBCL): 49/125 (39%), marginal zone lymphoma (MZL): 48/125 (38%), follicular lymphoma: 16/125 (13%) and others types 13/125 (10%). Among DLBCL, 17/49 (35%) were transformed from underlying indolent lymphoma. Patients in DLBCL group displayed frequent digestive tract (17/49; 35%) and hepatic (11/49; 22%) involvements. Patients in MZL group showed frequent spleen (23/48; 48%) and hepatic (13/48; 27%) involvements. The MZL patients had higher levels of rheumatoid factors (P=0.001) and more frequent mixed cryoglobulinemia vasculitis (11/48; 23%) (P=0.042). Median follow-up since lymphoma diagnosis was 31 months [IQR 25-75] [19-71]. At the end of follow-up, 79/125 (63%) had received antiviral therapy since lymphoma diagnosis, and sustained virological response (SVR) was obtained in 48/79 (61%). Significant association was shown between SVR and hematological response in LZM group (P<0.001) but not in DLBCL group (P=0.83). However in the overall population, patients with SVR had better event free survival (EFS) (P=0.06) and overall survival (OS) (P=0.006) than patients without SVR (Figure 1). During the follow-up, 16 (13%) deaths were recorded, related to lymphoma progression (n=7), infection (n=5), cirrhosis (n=3) or cardiovascular disease (n=1).

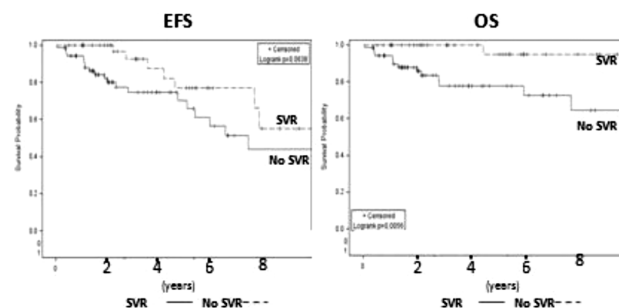


Figure 1. Impact of virologic response on HCV associated B-NHL prognosis.

Summary and Conclusions: This study underlines the heterogeneity of HCV associated B-NHL subtypes, with predominance of DLBCL and LZM. Virological success and hematological response are associated among LZM patients. Moreover, virological success appears to improve prognosis of HCV associated B-NHL patients.

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HIGH-DOSE METHOTREXATE, HIGH-DOSE CYTARABINE AND TEMOZOLOMIDE FOR THE TREATMENT OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: treatment of primary central nervous system lymphoma (PCNSL) associates with low response and survival rates using conventional radio and chemotherapy. Due to its favorable toxicity profile, Temozolomide has emerged as a new option for the treatment of PCNSL. In this study we report a consecutive series of PCNSL patients treated with an innovative regimen combining high-dose of both Cytarabine and Methotrexate with Temozolomide without Radiotherapy or intra-theal chemotherapy

Aims: to evaluate a new intensive chemotherapy with a new drug Temozolomide, trying to assess response rate and progression free survival and if the results are promising we are aiming at evaluating the overall survival rate at 5 years taking into consideration the toxicity profile. The study was performed at al Bairouni university hospital and al Shahbandar medical center in Damascus (SYRIA).

Methods: we have included 40 patients under 60 years old newly diagnosed with PCNSL histologically confirmed by a reference pathologist, biopsies were also cultured and Karyotype was performed for almost all patients. An induction chemotherapy was started (Methotrexate 3 gr/m² infused over 12 hours on day 1 + Cytarabine 3 gr/m² twice daily, short infusion on day 1 + Temozolomide 100 mg/m² from day 2 through day 6). A total of 6 cycles were given on a monthly basis.

Results: among the 40 patients included in the study, a complete response was observed in 34 patients (85%) versus a partial response seen in the remaining 6 patients (15%). Disease was progressed in 8 out of 40 patients (20%) while 31 patients were still living after 5 years making the overall survival reaching (77%). Grade II nephrotoxicity was observed in 2 patients while grade II and IV hematologic toxicity were seen in 5 patients.

Summary and Conclusions: our study has reached the 5-years overall survival comparing with other regimens not exceeding 35 months taking into consideration the lack of both radiotherapy and intra-theal chemotherapy, also the good toxicity profile makes it an acceptable regimen. However, more prospective studies are warranted to confirm or reject such results.

P848

MALE SEX EVOLVES AS A SIGNIFICANT RISK FACTOR IN DLBCL TREATED WITH RITUXIMAB ONLY IN ELDERLY, BUT NOT IN YOUNG PATIENTS: CORRELATION WITH RITUXIMAB (R) PHARMACOKINETICS

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Background: Sex and weight influence R clearance in elderly DLBCL patients (Mueller *et al.*, Blood 2012).

Aims: Therefore, we now investigated the influence of sex and weight on R pharmacokinetics and outcome in young DLBCL patients.

Methods: We analyzed the impact of sex on R pharmacokinetics and outcome of 1222 elderly pts of the RICOVER-60, 823 young (18 to 60 years) aalPI=0,1 pts of the MInT, and 275 aalPI=2,3 pts of the Mega-CHOEP trials. R pharmacokinetics was determined in 33 young and 49 elderly patients. Population pharmacokinetic modeling was performed with nonlinear mixed-effect modeling software (NONMEM VI).

Results: R clearance was independent of tumor mass (IPI), but weakly correlated (0.2, $R^2_{\text{linear}}=0.045$) with increasing age in male, and moderately inversely correlated (-0.5, $R^2_{\text{linear}}=0.207$) with age in female DLBCL patients, resulting in similar R clearances in young female and male patients (9.88 vs. 10.38 mL/h; $P=0.238$), but a significantly slower R clearance in elderly males compared to females (10.50 vs 8.25 mL/h; $P=0.006$). In the RICOVER-60 trial, elderly females had a higher 3-year PFS (68% vs. 61%) and OS (74% vs. 68%) than male pts. due to a greater outcome improvement by the addition of R in females. In a multivariable analysis adjusting for IPI, the male hazard for progression was not significantly increased after CHOP (HR=1.1; $P=0.348$), but was significantly higher after R-CHOP (OR=1.6; $P=0.004$). In contrast, young males treated in the MInT and Mega-CHOEP trials benefitted as much as females from the addition of rituximab, with a similar male hazard after CHOP and R-CHOP (HR=1.2) with no significant difference to female patients (HR_{PFS}=1.2, $P=0.552$; HR_{OS}=1.0; $P=0.898$).

Summary and Conclusions: While no differences in R clearance and bene-

fit from rituximab were found in young female compared to male patients, the reduced benefit of adding R to CHOP in elderly male DLBCL pts. who have a shorter rituximab serum half life and hence lower serum levels suggests that this subpopulation is suboptimally dosed when R is given based on body surface area at 375 mg/m². Appropriately designed prospective DSHNHL studies investigate whether higher R doses for pts. with a shorter R serum half life can improve the outcome of the respective patients.

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THE BCL-2 INHIBITOR ABT-199 (GDC-0199) IS ACTIVE AND WELL-TOLERATED IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA AND OTHER NON-HODGKIN LYMPHOMAS

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Background: New treatment options are needed for patients with relapsed or refractory lymphomas, particularly those such as mantle cell lymphoma (MCL) where curative options are limited. The anti-apoptotic protein BCL-2 is highly expressed in many non-Hodgkin lymphoma (NHL) subtypes, including MCL. Because BCL-2 plays a critical role in NHL pathogenesis and treatment resistance, it is a promising target for therapeutic intervention. A first-generation BCL-2 inhibitor, navitoclax, showed modest activity in indolent lymphoma, but co-inhibition of BCL-2 resulted in dose-limiting thrombocytopenia, precluding the full exploration of BCL-2 inhibition with this agent in NHL. ABT-199 is an orally bioavailable, second-generation BH3-mimetic that potently inhibits BCL-2 ($K_i < 0.10$ nM). Unlike navitoclax, which has similar activities against BCL-2 and BCL-2 $\times_L$, ABT-199 has 500-fold less activity against BCL-2 $\times_L$ ($K_i = 48$ nM). It demonstrates potent activity against a variety of human cell line and xenograft models of a range of B-cell NHL, including MCL.

Aims: The primary objectives of this phase-I dose-escalation study are to evaluate the safety and pharmacokinetics (PK) of ABT-199, determine a maximum tolerated dose and recommended phase 2 dose, and assess efficacy and biomarkers. We report results to date for patients in the R/R NHL arm.

Methods: Adult patients (pts) requiring therapy, with ECOG performance status ≤ 1 , and adequate marrow function (ANC $\geq 1.0 \times 10^9/L$, Plts $\geq 50 \times 10^9/L$) received ABT-199 on Week 1 Day -7 (W1D-7), followed by continuous once-daily dosing from W1D1, until progressive disease or unacceptable toxicity. Due to concerns for potential tumour lysis syndrome (TLS), a 2 to 3 wk lead-in period with stepwise escalation to the target cohort dose was implemented. Evaluations include adverse events (AE; NCI-CTCAE-V4), PK parameters and disease responses (IWG 2007 criteria). Dose cohorts up to 900 mg have been evaluated to date.

Table 1.

Characteristic	All NHL n=31	MCL Subset n=8
Age (yr) [†]	68 [35-85]	72 [35-75]
Male [‡]	20 (64.5)	6 (75)
Number of prior therapies [†]	3 [1-7]	2.5 [1-7]
Bulky disease (≥ 5 cm) [‡]	13 (42)	6 (75)
Bulky disease (≥ 10 cm) [‡]	4 (13)	1 (13)
Time on study (days) [†]	143 [14-448]	160 [56-291]
Response		
Time to first 50% reduction in nodes (days) [†]	42 [36-113]	42 [36-113]
Best response [§]	n=29	n=8
CR	1 (3)	0 (0)
PR	15 (52)	8 (100)
SD	8 (28)	0 (0)
PD	5 (17)	0 (0)
Response Rate (CR + PR)	55%	100%

[†]Median [range]; [‡]n(%); [§]Evaluable pts completed at least a W6 assessment
CR achieved in 1/7 pts with diffuse large B-cell lymphoma (DLBCL)
PRs achieved in 3/3 Waldenström macroglobulinaemia, 2/9 follicular lymphoma and 2/7 DLBCL pts

Results: As of January 2013, 31 pts have been enrolled, 8 (26%) with MCL (Table 1). The most common AEs of any grade ($\geq 15\%$ of pts) were nausea (36%), diarrhea (26%), dyspepsia, vomiting, fatigue, pyrexia and cough (16% each). Grade 3/4 AEs occurring in >1 pt were anaemia, neutropenia (4 pts each), febrile neutropenia (2 pts) and thrombocytopenia (3 pts). Gr 3/4 throm-

bocytopenia was not dose-dependent. Two of 14 pts in cohort 5 experienced DLTs (Gr 3 febrile neutropenia and Gr 4 neutropenia) at the target dose of 600 mg. Gr 3 TLS was seen after the initial dose in 1 pt with bulky MCL (largest lymph node >10 cm). 17 pts have discontinued drug: 13 due to progression, 2 due to AEs and 2 proceeded to stem cell transplantation in ongoing response. After a single dose with high-fat meal, ABT-199 had $T_{max} \sim 8$ hrs and $T_{1/2} \sim 15$ hrs. Food increased ABT-199 bioavailability by 3-4 fold. Preliminary efficacy data are summarised (Table 1).

Summary and Conclusions: ABT-199 shows a high efficacy signal in MCL, with all 8 pts to date achieving PR. The drug is generally well-tolerated, and shows activity in several NHL subtypes, with an overall response rate of 55% in this R/R population. Additional dosing and scheduling modifications are being explored in both monotherapy and combination studies in NHL to optimize the balance between the safety and efficacy of this active new agent. Updated results will be presented. (study status see clinicaltrials.gov).

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LENALIDOMIDE PLUS RITUXIMAB-CHOP21 IN ELDERLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA IS PROMISING: LONG TERM RESULTS OF THE PHASE II REAL07 STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: Standard treatment for elderly diffuse large B-cell lymphoma (DLBCL) patients is R-CHOP21, however up to 40% of patients fail. In the phase I trial REAL07 conducted by FIL, 15 mg lenalidomide from day 1 to day 14 was identified as the maximum tolerated dose (MTD) in combination with R-CHOP21, showing that the association of Lenalidomide plus R-CHOP21 (LR-CHOP21) is safe and feasible in elderly untreated DLBCL patients.

Aims: Based on the phase I results, FIL conducted a phase II trial REAL07 aimed at evaluating efficacy of LR-CHOP21 combination. Primary endpoint was an improvement of overall response rate (ORR) of 15% in LR-CHOP21 compared to 70% of standard R-CHOP21.

Methods: Phase II was designed according to Simon's two stage design. Response was evaluated according to 2007 Cheson criteria. PET scan was mandatory at the end of the treatment; patients in partial remission (PR) who underwent radiotherapy were considered as failure in progression free survival (PFS) analysis. Inclusion criteria were: age 60-80 FIT at the comprehensive geriatric assessment; untreated CD20+ DLBCL; Ann Arbor stage II/III/IV; international prognostic index (IPI) at low-intermediate/intermediate-high/high (LI/IH/H) risk. Treatment plan was: R-CHOP21 plus 15 mg lenalidomide from day 1 to 14 for 6 courses. All cases were reviewed by expert pathologists and tissue samples were centrally collected. With the aim to correlate outcome with cell of origin profile and biological markers, immunohistochemistry for Hans' and Choi criteria and gene expression profile were planned and are in advanced status of analysis at the time of the submission of the present abstract.

Results: From April 2010 to May 2011, 49 patients were enrolled. Clinical characteristics were: median age 69 years (range 61-80); stage III/IV 43 (88%), performance status >1 31 (63%), IPI IH/H 30 (61%). At the end of 6 LR-CHOP21, ORR was 45/49 (92%). Complete remissions (CR) were 42 (86%) and PR 3 (6%); 3 patients (6%) did not respond and one (2%) died for violent death. At a median follow-up of 22 months, 2-year overall survival (OS) was 92% (95%CI: 79-97) and 2-year PFS was 73% (95%CI: 57-84). According to IPI risk, 2-year PFS for IPI LI was 84% (95%CI: 59-95) and for IPI IH/H 65% (95%CI: 41-81). PFS analysis according to cell of origin profile is ongoing. Hematological toxicity was mild: grade III/IV thrombocytopenia occurred in 13% of the 277 courses performed, anemia in 5% and neutropenia in 31%, with only 4% of febrile neutropenia. No grade IV extra-hematological toxicities were observed. Grade III non-hematological toxicities were reported in 7 patients: cardiologic, gastroenteric and renal in one patient respectively, grade III neurological toxicities, sensory and motor neuropathy in two, thromboembolic event in one and infection in one. No toxic deaths occurred during treatment. One patient died three months off therapy while in CR, due to aeromonas hydrophila sepsis and multi-organ failure.

Summary and Conclusions: With a still limited follow-up time, our data compare favorably with historical R-CHOP21 data. LR-CHOP21 is able to induce a high 2-yr PFS (73%) and it is effective also in poor risk patients (65%). The addition of lenalidomide to R-CHOP21 is safe without unexpected toxicities. These encouraging data warrant a future phase III randomized trial comparing LR-CHOP21 vs. R-CHOP21 in elderly untreated DLBCL.

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PHASE 1B STUDY COMBINING IBRUTINIB WITH RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, VINCRISTINE, AND PREDNISONE (R-CHOP) IN PATIENTS WITH CD20-POSITIVE B-CELL NON-HODGKIN LYMPHOMA (NHL)

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Background: Ibrutinib, a first-in-class oral Bruton's tyrosine kinase inhibitor, has demonstrated single-agent activity in a variety of relapsed or refractory B-cell malignancies with limited toxicity, making it an appropriate drug to combine with standard R-CHOP chemotherapy in patients with previously untreated NHL.

Aims: The primary objective of this study was to determine the recommended phase 2 dose (RP2D) of ibrutinib in combination with standard R-CHOP (IR-CHOP). Secondary objectives were to assess safety, overall response rate, pharmacokinetics, and pharmacodynamic biomarkers.

Methods: Patients received a daily oral dose of ibrutinib (280, 420, or 560 mg) in combination with standard doses of R-CHOP (rituximab, cyclophosphamide, doxorubicin, and vincristine on day1, and prednisone on days 1 through 5 of each 21-day cycle for up to 6 cycles).

Results: Seventeen patients (7, 4, and 6 in increasing ibrutinib doses) were enrolled: 59% male, median age 65 (range 46-81) years, diffuse large B-cell lymphoma 47%, mantle cell lymphoma 29% and follicular lymphoma 24%. In the 280 mg cohort, 2 patients had dose-limiting toxicity (DLT): 1 with transient syncope and 1 with periorbital cellulitis; at 560 mg, 1 patient had gastritis (grade 2). The RP2D was established at 560 mg ibrutinib. The most common (≥20% of patients) adverse events (AEs) were neutropenia (77%), thrombocytopenia (65%), vomiting (59%), anemia (53%), nausea (47%), fatigue (35%), headache (29%), constipation (24%), diarrhea (24%), and dizziness (24%). To date, 6 patients completed 6 cycles of treatment, and 2 patients discontinued treatment (1 due to noncompliance with study drug and 1 due to non-DLT AE). At the time of this analysis, of the 10 patients with at least one post-baseline tumor assessment, the overall response rate was 100% (7 complete and 3 partial responses).

Summary and Conclusions: The combination of IR-CHOP has an acceptable safety profile. No new toxicities were noted with adding ibrutinib to R-CHOP. An expanded 560 mg ibrutinib cohort (RP2D) is being opened to further explore the safety and efficacy of IR-CHOP in patients with newly diagnosed diffuse large B-cell lymphomas.

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PROSPECTIVE COHORT STUDY FOR SECONDARY CENTRAL NERVOUS SYSTEM INVOLVEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH RITUXIMAB-CHOP

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Background: Secondary central nervous system (CNS) involvement is a serious complication encountered during the management of diffuse large B-cell lymphoma (DLBCL) patients. Although many studies were performed to evaluate the secondary CNS involvement in DLBCL, the majority of data were from retrospective analyses. Thus, there is a few data about the prospectively monitored patients who were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone).

Aims: We analyzed the incidence of secondary CNS involvement in pathologically confirmed DLBCL patients enrolled in the Prospective Cohort Study (Risk-adapted Central Nervous System Evaluation in DLBCL, PROCESS study, NCT01202448).

Methods: Patients should be treated with at least one cycle of R-CHOP, and provide written informed consents. We assessed the risk of CNS involvement based on previously reported risk factors: serum LDH elevation, the number of extranodal involvements, serum albumin, bone marrow invasion, HIV positivity, the involvement of testis, breast, paranasal sinus, bone, retroperitoneal lymph nodes, orbit, and epidural space. If patients had any of these risk factors, they underwent CSF study to screen the CNS involvement. If necessary, brain MRI was also done. CNS prophylaxis was done with intrathecal chemotherapy with methotrexate for patients who had positive findings of screening evaluation or were determined to have a risk of CNS involvement based on physicians' decision.

Results: 600 patients were enrolled between 2010 and 2012 from 27 institutions belonged to the Consortium for Improving Survival of Lymphoma. Seven patients including withdrawal of informed consents were excluded. Thus, 593 patients were prospectively monitored with the median follow-up duration of 12.8 months. The median age was 59.5 years old (range 20–89 years), and stage III/IV accounted for 49.2% (109/186). 208 patients (34.7%) involved two or more than two extranodal sites including bone marrow (n=76, 12.7%) and 280 patients showed the elevation of serum LDH (46.7%). Thus, 204 patients (34.4%) belonged to high or high-intermediate risk of the International Prognostic Index (IPI). 390 patients had at least one of risk factors for CNS involvement (65.8%), and 198 patients underwent CNS evaluation at diagnosis. The screening CNS evaluation including CSF exam and brain MRI revealed that 19 patients initially had the evidence of CNS involvement even though they did not have any neurologic symptoms: positive cytology (n=15) and brain parenchyma lesion (n=4). During follow-up, 14 cases of additional CNS involvement including brain parenchyma (n=8), leptomeningeal (n=5), and ocular invasion (n=1) were observed. The median time to CNS event in these 14 patients was 7.5 months (range 1.2–15.9 months). Thus, 33 cases of secondary CNS involvement were documented in our study population at the time of analysis (5.5%) including 19 cases at diagnosis (3.2%) and 14 cases during follow-up (2.4%). Out of 390 patients with any risk factors for CNS involvement, 28 patients developed CNS involvement (28/390, 7.2%) whereas only 5 cases of CNS involvement were found in 203 patients without risk factors (2.5%). The univariate analysis for evaluation of risk factors demonstrated serum LDH, the number of extranodal involvements, bone marrow invasion, and the involvement of retroperitoneal lymph node, breast, paranasal sinus and orbit were significantly associated with CNS involvement. The high/high-intermediate risk of IPI was also predictive of CNS involvement ($P<0.05$).

Summary and Conclusions: The incidence of secondary CNS involvement in DLBCL patients treated with R-CHOP was around 5%, and a half of cases had CNS involvement at diagnosis. Considering a particular risk of CNS involvement of disease-related factors, risk-adapted active screening against CNS involvement may help to improve treatment outcome of patients with DLBCL.