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POSTER PRESENTATIONS

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THE IMPACT OF THE MICROENVIRONMENT ON TRANSFORMATION AND PROGNOSIS OF NON-HODGKIN LYMPHOMA IN THE RITUXIMAB ERA

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Introduction: Follicular lymphoma (FL) is second to diffuse large B-cell lymphoma (DLBCL) the most frequent non-Hodgkin lymphoma (NHL) and is characterized by an indolent disease course. Although many patients experience long-term survival, transformation to a more aggressive phenotype, most often DLBCL, occurs in 2–3% of patients per year. Previous gene expression studies and immunohistochemical (IHC) studies have shown that the tumour microenvironment influences the course of the disease and outcome in patients with FL, while the role of the tumour microenvironment in transformation is less studied. We examined by immunohistochemistry the impact of several immunologic markers expressed on cells in the tumour microenvironment on transformation and survival in patients with indolent NHL.

Methods: Fifty-six patients with histologic transformation of indolent NHL were included: 52 patients had FL grades 1–3A and four patients had marginal zone lymphoma at primary diagnosis. Twenty-eight patients (50%) received rituximab for low-grade lymphoma before transformation, and all patients received rituximab at transformation except from eight patients that were either CD20 negative at transformation or had progressed to transformation shortly after rituximab. All the patients were considered for high-dose chemotherapy with autologous stem cell transplantation (HD-ASCT), of whom 38 patients proceeded to HD-ASCT.
Tissue microarrays and whole tissue slides were used for IHC staining of CD3, CD4, CD8, CD68, CD57, FOXP3, TIA-1, PD1, PD-L1, PAX5 and CD21. The percentage of positive cells, as well as the pattern of distribution, was semiquantitatively assessed. CD21, PD-L1 and CD68 were scored according to their networks or 'network-like' presence in nodular areas of the lymphoma.

Results: The median overall survival (OS) from diagnosis, from transformation and the time to transformation (TTT) for the 56 patients were 112, 42 and 43 months, respectively. Absent intranodular (IN) CD68+ cells at diagnosis was a positive predictive factor for OS from diagnosis ($p = 0.039$) and TTT ($p = 0.005$). Absent demonstrable IN networks of CD21+ cells at diagnosis showed a trend for superior OS from diagnosis ($p = 0.052$) and longer TTT ($p = 0.067$). Absent CD21+ cells at transformation was a positive predictive factor for OS and progression-free survival (PFS) from transformation ($p = 0.005$). Absent IN PD-L1+ cells, a perifollicular and IN presence of FOXP3+ cells and a total score of TIA-1+ cells $<10\%$ at diagnosis showed a longer TTT ($p = 0.032$, $p = 0.006$ and $p = 0.039$, respectively).

Conclusions: We show for the first time in the current treatment era that the absence of CD21+ follicular dendritic cells influences OS, the TTT and post-transformation PFS in FL. Furthermore, the findings confirm a role of macrophages (CD68) for OS and TTT. T-regulatory cells (FOXP3), PD-L1+ macrophages and TIA-1+ cytotoxic T cells were associated with TTT.

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CO-EXPRESSION OF PD-1, TIM-3 AND LAG-3 DEFINES EXHAUSTION OF INTRATUMOURAL CD8+ T CELLS IN B-CELL NON-HODGKIN LYMPHOMA

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Introduction: T-cell exhaustion plays an important role in attenuating the function of immune cells in B-cell non-Hodgkin's lymphoma (NHL). In the present study, we attempt to determine what surface marker set defines exhaustion of intratumoural $CD8^+$ T cells and to test whether exhaustion of intratumoural $CD8^+$ T cells can be reversed in B-cell NHL.

Methods: To define exhaustion of intratumoural $CD8^+$ T cells, we determined co-expression, regulation and function of PD-1, TIM-3 and LAG-3 on $CD8^+$ T cells by flow cytometry. To test whether the function of exhausted intratumoural $CD8^+$ T cells can be enhanced by blocking signalling through TIM-3 or LAG-3, we treated $CD8^+$ T cells with blocking anti-TIM-3 or anti-LAG-3 Ab alone or in combination and measured cytokine production of $CD8^+$ T cells.

Results: We observed that PD-1, TIM-3 and LAG-3 were variably expressed on intratumoural $CD8^+$ T cells in B-cell NHL with a median of 48.5% (range: 12.8–81.7%, $n = 32$), 39.3% (14.7–78.4%, $n = 41$) and 11.7% (range: 6.5–18.1%, $n = 5$), respectively. While the vast majority of $TIM\text{-}3^+$ T cells displayed PD-1 expression, $LAG\text{-}3^+$ T cells almost exclusively came from $PD\text{-}1^+ TIM\text{-}3^+$ cells, forming a defined population of intratumoural $PD\text{-}1^+ TIM\text{-}3^+ LAG\text{-}3^+ CD8^+$ T cells. Functionally, the intratumoural $PD\text{-}1^+ TIM\text{-}3^+ LAG\text{-}3^+$ T cells exhibited reduced capacity to produce cytokines (IL-2 and IFN-γ) and granules (perforin and granzyme B). These three co-receptors are strongly up-regulated on $CD8^+$ T cells by IL-12, a cytokine that has been shown to induce T-cell exhaustion. Interestingly, we observed that while expression of PD-1 and TIM-3 on $CD8^+$ T cells was up-regulated by IL-12 at an early time point, LAG-3 was only induced after TIM-3 up-regulation and almost exclusively on $TIM\text{-}3^+$ T cells. We found that TIM-3 blockade was able to reverse the exhausted phenotype of $CD8^+$ T cells resulting in increased IFN-γ and IL-2 production. This effect was further enhanced when $CD8^+$ T cells were treated with both anti-TIM-3 and anti-LAG-3 Abs.

Conclusions: Taken together, these results suggest that PD-1, TIM-3 and LAG-3 were involved in the induction of exhaustion of $CD8^+$ T cells in B-cell NHL. We find that PD-1, TIM-3 and LAG-3 are expressed on the same $CD8^+$ cells and that blocking TIM-3 and LAG-3 can reverse T-cell exhaustion signalling. These results suggest that PD-1, TIM-3 and LAG-3 play a synergistic role in the development of $CD8^+$ T-cell exhaustion in NHL.

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CHARACTERIZATION OF INHIBITORY RECEPTORS ON TUMOR-INFILTRATING T CELLS IN FOLLICULAR LYMPHOMA TO REVEAL NOVEL TARGETS FOR IMMUNOTHERAPY

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Introduction: Follicular lymphoma (FL) is an incurable indolent lymphoma that can transform to a more deadly diffuse large B-cell lymphoma or develop fatal drug

resistance. Immune cells in the lymphoma tumour microenvironment have been implicated in disease progression (Dave et al., *NEJM* 2004) and may play a role in transformation. We recently discovered that expression of the inhibitory receptor PD-1 was associated with suppressed cytokine signalling in FL tumour-infiltrating T cells (Myklebust et al., *Blood* 2013). Furthermore, it has been suggested that PD-1^{int} cells are the truly exhausted T cells and that PD-1^{high} cells are normal T follicular helper cells (T_{FH}) (Yong et al., *Blood Cancer J* 2015). Antibody immunotherapy targeting PD-1 has shown significant promise in aggressive malignancies (Topalian et al., *NEJM* 2012), suggesting that exhausted T cells can regain functionality, including anti-tumour effects.

Methods: Three mass cytometry (CyTOF) panels were designed to detect 30 markers per cell and used to characterize FL tumour biopsies ($n = 9$) and human tonsils. In the first panel, inhibitory and co-stimulatory receptors were quantified across 5 major T-cell subsets and maturation stages (Nicholas and Greenplate et al., manuscript in preparation). In the second panel, key surface markers were combined with antibodies to detect 12 phosphoproteins to correlate signalling responses with inhibitory receptor profiles. The last panel was designed to characterize healthy and malignant B cells. The dimensionality-reduction tool viSNE was used to analyse the high-dimensional mass cytometry data. Fluorescent flow panels were used in parallel to measure 9 inhibitory receptors in selected T-cell subsets.

Results: viSNE analysis of 23 surface markers revealed a high degree of phenotypic similarity between T cells infiltrating FL and T cells in tonsils. In contrast, viSNE characterized the significant phenotypic differences between malignant B cells and healthy B cells within the same tumour. PD1^{+} T cells from FL samples displayed reduced cytokine signalling compared to PD1^{-} cells, confirming previous results. T_{FH} cells were identified as CXCR5^{hi} ICOS^{+} CD4 memory T cells. Among the ICOS^{+} cells in tonsils, a distinct CXCR5^{-} population was identified with intermediate PD1 expression, suggesting an exhausted phenotype. These cells expressed less TIGIT, BTLA and LAIR1 than T_{FH} but contained a subpopulation of TIM3^{+} cells that was not seen within the T_{FH} population.

Conclusions: Striking similarities in phenotype and signalling response of the T cells infiltrating FL tumours and T cells from healthy tonsil observed by mass cytometry suggest active immune responses in these tissues. These results provide further support for characterizing relationships between receptor signalling and T cell function and for researching into combination immunotherapies for FL focused on modulating adaptive immune responses.

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T-CELL SUBPOPULATIONS QUANTIFIED BY FLOW CYTOMETRY IN LYMPH NODE CELL SUSPENSIONS IDENTIFY A GROUP OF PATIENTS WITH FOLLICULAR LYMPHOMA WITH FAVORABLE OUTCOME

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Introduction: Tumour microenvironment plays an important role in the behaviour of follicular lymphoma (FL). By gene expression and immunohistochemistry, an increase in macrophages has been associated with poor outcome, while an increase in T cells is associated with good prognosis. The aim of the study was to explore the prognostic impact of subpopulations of T cells using flow cytometry and to identify different groups of risk in FL patients.

Methods: Seventy-five patients (36 men/39 women, median age 60 years) diagnosed of FL (grades 1–2, 87%; grade 3, 13%) between 1984 and 2009 (median follow-up of 6.5 years) with sample at diagnosis were included in the present study. In 41 cases, T-cell staining were semiquantitatively analysed by immunohistochemical (IHC), including their distribution (intra, inter or perifollicular). T-cell populations from lymph node were quantified by multiparametric flow cytometry in cell suspensions in all cases. The percentage of B-cells, CD3^{+}, CD4^{+}, CD8^{+}, CD57^{+}, CD4T$_{FH}$ cells (double staining CD4^{+} CD57^{+}), as well as the ratio T/B-cells, CD3^{+}/CD4^{+}, CD3^{+}/CD8^{+}, CD4^{+}/CD8^{+} and CD4T$_{FH}$/CD4^{+} were analysed and correlated with initial features and outcome.

Results: CD57 expression by IHC was mainly intrafollicular, while CD8 was inter/perifollicular, with these patterns correlating with grades 1–2. The mean (± SD) percentage of B-cells, CD3^{+}, CD4^{+}, CD8^{+}, CD57^{+} and CD4^{+} T_{FH} cells were 59% (±15.7), 35.8% (±15.9), 27.1% (±13), 8.7% (±5.4), 6.3% (±4.6) and 3.6% (±2.9), respectively. Main associations with clinical characteristics and outcome are listed in the table. CD4T$_{FH}$/CD4 and CD4^{+}/CD8^{+} ratios were divided in 4 percentiles. Patients with CD4T$_{FH}$/CD4^{+} ratio in the 4th percentile had a better 10-year OS (92% vs 47%; $p = 0.01$) and PFS (66% vs 36%; $p = 0.04$) than the remainder. Moreover, patients with CD4^{+}/CD8^{+} ratio over median had a better 10-year OS (70% vs 48%; $p = 0.03$) but with no differences in PFS (37% vs 44%; $p =$ NS). There was no correlation of lymphocyte subpopulations with type of therapy, overall response or complete response rate. A multivariate analysis was performed including CD4T$_{FH}$/CD4^{+} ratio, CD4^{+}/CD8^{+} ratio and FLIPI, with CD4T$_{FH}$/CD4 ratio being the most important variable to predict OS in the Cox model with 52 patients (relative risk: 32; $p = 0.01$).

Conclusion: Flow cytometry allows the identification of T-cell subpopulation in FL, showing that a high percentage of CD4T$_{FH}$ cells is associated with more favourable prognosis.

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	CD3^{+} P ≥ 50	CD4^{+} P ≥ 50	CD8^{+} P ≥ 50	CD3^{+}/B-cell P ≥ 50	CD4^{+}/CD8^{+} P ≥ 50	CD4^{+} T$_{FH}$/CD4^{+} P ≥ 75
Age (%)						
<60 years	39	34	29*	21*	55	21
≥60 years	59	56	57	43	43	39
Histological grade (%)						
1–2	45*	43	35*	27	58**	29
3	89	67	89	67	0	11
Bulky disease (%)						
No	57*	53*	46	60*	46	23
Yes	22	17	33	11	56	29
Extranodal (%)						
≤1	58**	52*	51*	58*	47	23
>1	21	21	21	26	53	29
Leukemic phase (%)						
No	56*	50*	48*	32*	60	22
Yes	10	10	10	0	46	25

P, percentile.
*$p = <0.05$.
**$p = <0.01$.

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TXN OVEREXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA CELLS ATTENUATES OXIDATIVE STRESS-INDUCED PROAPOPTOTIC ACTIVITY OF FOXO1 TRANSCRIPTION FACTOR

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is a highly heterogeneous disease. Unsupervised gene expression profiling has led to the identification of a subset of DLBCLs characterized by enhanced oxidative phosphorylation. Since enhanced oxidative metabolism leads to overproduction of reactive oxygen species (ROS), which can be toxic to cells, we investigated the role of TXN system in the pathogenesis of DLBCL.

Methods: Clinical consequences of TXN expression in DLBCLs were assessed with publically available gene expression datasets. BCL6 impact on TXN expression was

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assessed with bioinformatic approaches, luciferase reporter assays and shRNA-mediated BCL6 silencing. TXN and FOXO1 knock-down was achieved with shRNA. TXN impact on FOXO1 acetylation, FOXO1 gene expression, cell cycle and apoptosis were studied in cells transfected with WT or mutant FOXO1, TXN and p300 vectors. The consequences of TXN inhibition on ROS-induced FOXO1 subcellular localization were determined with confocal microscopy. The consequences of FOXO1 acetylation were assessed in cells transduced with either WT FOXO1 or its acetylation-deficient mutants.

Results: TXN expression was significantly higher in DLBCLs classified as OxPhos subtype compared to BCR subtype. The OS of patients with high TXN mRNA expression was significantly shorter than of those with low TXN mRNA expression, regardless of treatment regimen. Consistent with our previous findings indicating that BCL6 does not exhibit repressor activity in OxPhos tumours, we demonstrated that relative differences in TXN expression between different DLBCL subsets are at least in part caused by the lack of BCL6 transcription repressor activity. We found that OxPhos cells lacking TXN were uniformly more sensitive to ROS production than control cells. Since TXN reduces disulfide bonds between FOXO4 and p300, which results in decreased FOXO4 acetylation and attenuated proapoptotic signalling, we next assessed whether p300 and TXN are involved in acetylation of FOXO1, a major FOXO member expressed in DLBCLs. TXN decreased p300-mediated FOXO1 acetylation and reduced its proapoptotic activity and expression of FOXO1-dependent genes (TRAIL, p27, Bim and GADD45A). Furthermore, TXN inhibited FOXO1 nuclear translocation in response to oxidative stress in OxPhos DLBCLs. Finally, knock-down of FOXO1 in OxPhos cells with silenced TXN expression markedly reduced DLBCL cell line apoptosis in response to ROS, demonstrating that FOXO1 is a major mediator of DLBCL cells' responses to oxidative stress.

Conclusion: Taken together, these results indicate that TXN is overexpressed in a subset of DLBCLs, and TXN overexpression is associated with shorter OS of DLBCL patients. TXN silencing augments ROS toxicity in OxPhos cell lines at least in part by facilitating FOXO1 nuclear relocalization and enhances acetylation of this transcription factor, thus potentiating its proapoptotic activity.

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MiR-155 AMPLIFIES BCR SIGNALLING BY TARGETING ITS MULTIPLE NEGATIVE REGULATORS IN DLBCL

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Introduction: The B-cell receptor (BCR)-activated PI3K pathway plays a critical prosurvival function in normal B-lymphocytes and certain B-cell malignancies. Multiple mechanisms are involved in trigger and modulation of the BCR signal amplitude. MiR-155 has emerged as a positive regulator of PI3K signalling in multiple malignancies, including DLBCL through targeting negative modulator of this pathway, SHIP-1. In the present study, we have searched for new targets of miR-155, which might be implicated in the deregulation of SYK/PI3K/AKT signalling in DLBCL.

Methods: MiR-155 target prediction was performed with PicTar, Miranda and TargetScan algorithms. Predicted miR-155 targets were validated with 3′UTR luciferase reporter assays in HEK293 cells. MiR-155 expression was modulated through transfection with miR-155 mimic or miR-155 inhibitor. The consequences of the miR-155 perturbations were assessed in DLBCL cell lines by proliferation assays (MTS) and immunoblotting with antibodies against predicted miR-155 targets and p-AKT. The DEPTOR silencing in DLBCL cell was achieved with retroviral shRNA vector, and its consequences were assessed using proliferation assays and immunoblotting. DEPTOR mRNA expression and survival of DLBCL patients were determined using publicly available microarray data (Lenz et al, 2008, GEO accession GSE10846).

Results: Using miRNA target finding algorithms, we identified miR-155-matching sequences in 3′UTRs of genes involved in SYK/PI3K/AKT pathway regulation: c-CBL (SYK ubiquitin E3 ligase) and DEPTOR (an mTOR phosphatase). MiR-155 suppressed the luciferase activities of vectors containing 3′UTR fragments from c-CBL and DEPTOR with wild type but not mutant miR-155 seed sequence. Transfection with miR-155 mimic dampened the expression of SHIP-1, c-CBL and DEPTOR proteins, augmented phospho-AKT level and increased cellular proliferation rate in a DLBCL cell line Ly19. MiR-155 inhibitor exhibited opposite effects. Since functional consequences of an mTOR phosphatase DEPTOR expression in DLBCL have not been defined, we silenced the expression of this protein with shRNA. Attenuated protein level of DEPTOR led to the increased activity of AKT and increased proliferation of DLBCL lines, suggesting that DEPTOR plays a tumour suppressor function in these tumours. Consistent with these findings, higher expression of DEPTOR mRNA in primary DLBCL biopsies was associated with longer overall survival (log rank test, $p = 0.018$).

Conclusions: Our data underscore the role of miR-155 in the regulation of SYK/PI3K/AKT prosurvival signalling in DLBCL. MiR-155 regulates SYK/PI3K/AKT signalling not only by decreasing expression of SHIP-1 but also by modulating the abundance of c-CBL and DEPTOR. DEPTOR modulates AKT activity, and its silencing promotes proliferation of DLBCL cells, suggesting that DEPTOR functions as a tumour suppressor in DLBCL.

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NR4A3 SUPPRESSES LYMPHOMAGENESIS BY INDUCTION OF APOPTOSIS AND SERVES AS A DRUG TARGET FOR LYMPHOMA THERAPY

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Introduction: Recently, we described a significant down-regulation of NR4A1 (Nur77) and NR4A3 (Nor-1)—two members of the orphan nuclear receptors acting together as critical tumour suppressor genes in acute myeloid leukaemia—in aggressive lymphoma[1]. NR4A1 overexpression proved its proapoptotic function in aggressive lymphoma cells, and its lymphoma suppressive properties *in vivo* was demonstrated in a xenograft mouse model. Since the role of down-regulated NR4A3 in aggressive lymphomas and the effects of NR4A3 agonists on lymphoma cells are unknown, we aimed to investigate the function of NR4A3 in lymphoid malignancies and the effects of two NR4A3 agonists on lymphoma cells.

Methods: For functional characterization, NR4A3 was overexpressed in a SuDHL4 lymphoma cell line by using an inducible lentiviral construct followed by various apoptotic assays and followed by a xenograft mouse experiment. Furthermore, aggressive lymphoma cells (SuDHL4, Karpas422, RI-1 and U2932) were treated with NR4A3 agonists (thapsigargin and prostaglandine A2) followed by cell growth (MTS) and apoptotic (Annexin V staining and caspase 3–7 activity assay) assays.

Results: Induction of NR4A3 expression led to a significantly higher proportion of induced SuDHL4 cells undergoing apoptosis as demonstrated by DNA cleavage, Annexin V staining and increased caspase 3–7 activity suggest a functional redundancy to NR4A1 in aggressive lymphoma. To test the tumour suppressor function of NR4A3 *in vivo*, the stably transduced SuDHL4 lymphoma cell line was further investigated in the NOD scid gamma (NSG) mouse model. Induction of NR4A3 in SuDHL4 abrogated tumour growth in the NSG mice, in contrast to vector control- and uninduced SuDHL4 cells, which formed massive tumours. Treatment of lymphoma cells with NR4A3 inhibited lymphoma cell growth in a dose-dependent manner in all four lymphoma cell lines (SuDHL4, Karpas422, RI-1 and U2932). Furthermore, an increased caspase 3/7 activity and a higher percentage of Annexin V positive lymphoma cells were detectable after treatment with NR4A3 agonists demonstrating its apoptotic effects.

Conclusions: Our data suggest that NR4A3 has a proapoptotic function in aggressive lymphoma and define that NR4A3 together with NR4A1 function as novel

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tumour suppressor involved in aggressive lymphoma development. Hence, NR4A3 and its agonists are promising targets and agents for the development of new drugs for lymphoma therapy.

1. Deutsch AJ, Rinner B, Wenzl K, et al. NR4A1-mediated apoptosis suppresses lymphomagenesis and is associated with a favourable cancer specific survival in patients with aggressive B-cell lymphomas. *Blood*. 2014.

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EXOME AND WHOLE GENOME SEQUENCING REVEALS NOVEL GENETIC ALTERATIONS IN DIFFUSE LARGE B-CELL LYMPHOMAS DERIVED FROM CHINESE PATIENTS

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Introduction: Next generation sequencing studies on diffuse large B-cell lymphomas (DLBCL) have revealed novel targets of genetic aberrations but also high inter-cohort heterogeneity. Previous studies have suggested that the prevalence of disease subgroups and cytogenetic profiles differ between Western and Asian patients.

Methods: In order to characterize the genome of Chinese DLBCL, we performed whole exome and/or whole genome sequencing of DNA derived from 43 tumours and respective peripheral blood samples. The mutation prevalence of *B2M*, *CD70*, *DTX1*, *LYN*, *TMSB4X*, *TP73* and *UBE2A* were investigated in addition to 105 tumour samples.

Results: We discovered 12 novel targets of recurrent mutations in DLBCL that included functionally relevant genes such as *LYN*, *TP73* and *TMSB4X*. Additional genes were found mutated at high frequency (≥10%) in the Chinese cohort including *DTX1*, which is a negative regulator of Notch and was the most prevalent mutation target in the Notch pathway. We furthermore mapped somatic translocation events genome-wide and identified 6 translocations involved in the immunoglobulin gene loci, each with a unique partner, including known (BCL6) and novel partners (CD58, CD274/PDL1, BTG2, MPEG1 and SH2B3).

Conclusions: Novel and previous unappreciated targets of somatic alterations in DLBCL identified in this study support the existence of additional/alternative tumourigenic pathways in these tumours. The observed differences with previous reports might be explained by the genetic heterogeneity of DLBCL, the germline genetic make-up of Chinese individuals and/or exposure to distinct etiological agents.

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IDENTIFICATION OF CANDIDATE PREDISPOSITION GENES IN A FAMILY WITH TWO FEMALE SIBLINGS AFFECTED BY MEDIASTINAL DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common malignant lymphoma in adults. By gene-expression profiling, DLBCL can be divided into three cell-of-origin subtypes with distinct molecular and clinical features. Most lymphomas arise sporadically, yet familial clustering is known but currently poorly understood at the molecular level. We study a Swiss-Japanese family with two female siblings affected by mediastinal DLBCLs (primary mediastinal B-cell lymphoma (PMBL) and DLBCL with features of PMBL, respectively) at the age <30 years. Pathological analyses revealed different (e.g. BCL2 expression) but also shared molecular features, e.g. gain of *JAK2* by FISH. The similar features of the DLBCLs suggested a shared biological background.

Methods: We performed whole-exome sequencing (WES) on matched tumour (laser-dissected formalin-fixed paraffin-embedded tissue) and healthy (peripheral blood) DNA from both siblings and DNA from their unaffected brother and parents. The exome was captured using the Illumina TruSeq 62Mb exome enrichment kit; sequencing was performed on an Illumina HiSeq2000 (2 × 100 bp reads). After quality control (FastQC), the reads were aligned to the reference genome hg19 (bowtie-2). GATK Haplotype Caller was used to detect germline variants, while somatic alterations were called by Strelka and SomaticSniper. In both DLBCLs, genome-wide copy number alterations were analysed by array comparative genomic hybridization (aCGH) using the Agilent 180k array. Expression of pJAK2, pSTAT3/6 was assessed by immunohistochemistry on paraffin tissue sections.

Results: To identify possible alterations implicated in lymphomagenesis, we focused on rare or previously undescribed germline protein altering variants that are shared by the siblings. Using this approach, a total of 547 of such germline alterations in 444 different genes were identified. For further reduction, we considered: (i) genes that have been linked to cancer; (ii) mutations segregating with the lymphomas in the investigated family; (iii) mutations that are *in silico* predicted to be deleterious; and (iv) genes mutated according to Knudson's two-hit theory by integrating somatic changes obtained by WES and/or a CGH. The germline variant annotation and Sanger validation is currently ongoing, and available results will be presented. The characterization of the somatic landscape of the DLBCLs revealed a shared 9p24 gain and mutations in *STAT6*. The 9p24 gain was associated with increased abundance of pJAK2 and JAK/STAT pathway activity.

Conclusions: The identification of alterations possibly predisposing to familial DLBCL might contribute to the understanding of the genetic basis of this complex and heterogeneous lymphoma. The analysis of the somatic changes provides evidence for aberrant activation of JAK/STAT signalling in both DLBCLs.

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PROGNOSTIC IMPACT OF CELL OF ORIGIN PROFILE IN YOUNG PATIENTS WITH HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS OF THE BIO-DLCL04 TRIAL OF FONDAZIONE ITALIANA LINFOMI

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Introduction: The role of cell of origin (COO) subtypes defined in immunohistochemistry (IHC) is controversial in rituximab era. Germinal centre (GC) subtype assessed by gene profile had better prognosis when treated with standard R-CHOP21. The Fondazione Italiana Linfomi conducted a multicentre phase III randomized trial aimed at investigating the benefit of intensification with R-high-dose chemotherapy + BEAM and ASCT (RHDC) compared to R-dose-dense therapy as first-line treatment in young patients with DLBCL at poor risk (age adjusted, aa-IPI 2–3). Clinical results were previously reported. (Vitolo, ASH 2012). The aim of BIO-DLCL04 trial was to correlate the biological markers with PFS.

Methods: From 2005 to 2010, 412 patients with aa-IPI 2–3 and new diagnoses of DLBCL were enrolled. Central histology revision was done. Three patients were screening failure; 13 were excluded because of different histologies. Biological markers analysis was performed on DLBCL NAS. COO analysis was performed by IHC; Bcl2, Bcl6 and Myc; abnormalities were tested by IHC and by FISH. Cases were deemed positive for specific antibodies if at least 30% of lymphoma cells were stained with each antibody (with the exception of at least 40% for Myc) and classified according to Hans' algorithm in GC and non-GC.

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Results: Two hundred twenty-three DLBCL NAS were analysed. Non-GC profile was reported in 131 and GC in 131. Clinical characteristics for non-GC vs GC were median age 51 years for both groups, men 49% vs 45%, aa-IPI 3 15% vs 25%, bone marrow involvement (BM) 16% vs 24%. R-HDC was performed in 45% of non-GC patients and in 49% of GC. Complete response was recorded in 105 (80%) of non-GC and in 62 (67%) GC. At a median follow-up of 49 months, the 3-year PFS for non-GC vs GC was 75% (95% CI: 67–82) vs 57% (95% CI: 46–67), with crude hazard ratio, HR 0.55 (0.35–0.87), *p* 0.01 and adjusted (for age, gender, aa-IPI and BM) HR 0.56 (0.35–0.88), *p* 0.013. No differences were observed by treatment in COO subtypes. Abnormalities of Myc by IHC had a relevant prognostic impact, with an adjusted HR 5.95 (2.3–15.4), *p* 0.001. By IHC, the 3-year PFS for double negative (20 patients) vs single Bcl2 or Myc abnormalities (76 cases) vs double positive (35 cases) was 85% vs 68% vs 51%, respectively, with an adjusted HR for double positive compared to double negative of 3.91 (1.13–13.53), *p* 0.031. The same figures were validated by FISH analysis, with a 3-year PFS for triple negative (56 patients) vs single hyt (30) vs double or triple hyt (8) of 73% vs 83% vs 25%, with adjusted HR 5.8 (2.16–15.54), *p* 0.001.

Conclusions: With the limit of the analysis performed by IHC, BIO-DLCL04 showed a better outcome for non-GC compared to GC, irrespective to treatment arm, abnormalities of Myc, recorded by IHC or FISH, single and mainly associated with Bcl2 abnormalities, had an important adverse prognostic role in young intermediate–high/high risk DLBCL regardless of intensification of treatment.

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CELL OF ORIGIN IS NOT ASSOCIATED WITH SURVIVAL AFTER DLBCL RELAPSE

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Introduction: Patients with untreated non-GCB DLBCL carry an inferior prognosis compared with GCB-type DLBCL when treated with anthracycline-based immunochemotherapy as their first treatment. The prognostic value of DLBCL genotype at relapse is less well defined. This study aimed to identify if cell of origin as determined by Hans algorithm is prognostic in relapsed DLBCL.

Methods: Newly diagnosed patients with DLBCL were prospectively enrolled in the University of Iowa/Mayo Clinic SPORE Molecular Epidemiology Resource (MER) from 2002 to 2012. Initial therapy and post-relapse management of the patients were per the treating physician. Patients were followed for outcome events including relapse, retreatment and death. Cell of origin information per Hans algorithm at initial diagnosis was abstracted from the medical record where available or assessed using available research tissue.

Results: There were 985 patients with DLBCL treated with anthracycline-based immunochemotherapy available for analysis; cell of origin per Hans algorithm was available on 583 (59%). At a median follow-up of 59 months (range 1–148) from diagnosis, 407 patients (41%) had a relapse, retreatment or death during follow-up. After excluding patients with low-grade relapse, patients receiving consolidation therapy for equivocal disease status after initial immunochemotherapy and death due to treatment complications or other causes, 128 patients with relapsed DLBCL and available cell of origin data were assessed for survival post-relapse. Median age at diagnosis for the 128 patients was 62 years (range 20–89) and 78 (61%) were men. The median time from diagnosis to relapse or retreatment was 7 months (range 1–148). IPI at diagnosis was 0–1 in 27 patients, 2 in 33 patients, 3 in 38 patients and 4–5 in 30 patients. Seventy-five (59%) patients had GCB tumours and 53 (41%) had non-GCB by Hans algorithm. At a median follow-up of 30 months (range 0–117) after relapse, 93 (73%) patients had died. Median survival after relapse was 8.2 months (95% CI: 7.0–14.5). Cause of death was almost exclusively due to lymphoma (94%) with 2% due to therapy and 4% from other (non-lymphoma) causes. There was no difference in survival of patients with GCB (median survival 7.6 months, 95% CI: 6.6–15.1; HR = 1.35, 95% CI: 0.89–2.06; $p = 0.16$) compared to patients with non-GCB tumours (median survival 11.1 months, 95% CI: 6.9-31.2).

Conclusions: Survival of patients with DLBCL relapse remains poor in the immunochemotherapy era. Cell of origin as assessed by Hans algorithm was not associated with survival after DLBCL relapse. Further studies using gene expression profiling of relapsed DLBCL are warranted to evaluate if cell of origin classification has prognostic implications in the relapsed setting.

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IMPACT ON SURVIVAL OF MYC GENETIC ALTERATIONS BUT NOT MYD88^{L265P} MUTATION IN PRIMARY TESTICULAR DLBCL

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Introduction: One of the main prognostic markers in diffuse large B-cell lymphoma (DLBCL) is *MYC* rearrangement, which is correlated with a high protein expression in most of the cases. The co-expression of MYC and BCL2 is also associated with an unfavourable response to R-CHOP. *MYD88* mutation in L265P, which is predominantly described in activated B-cell-like (ABC) DLBCL, is correlated with a worse prognosis. Primary testicular DLBCL is characterized by ABC profile, recurrent *MYD88*^{L265P} mutation and frequent BCL2 expression, but low incidence of *MYC* genetic alterations and protein expression. The impact of these features on survival is still unknown.

Methods: We studied the incidence and the prognostic significance of *MYC* and *BCL2* gene and protein and of *MYD88*^{L265P} mutation, in 33 primary testicular DLBCL patients (24 uniformly treated with R-CHOP immunochemotherapy and intrathecal methotrexate in the IELSG-10 clinical trial). *MYC* and *BCL2* genetic alterations were analyzed by FISH. BCL2 and MYC expressions were carried out by IHC. Detection of *MYD88*^{L265P} was performed by Sanger sequencing.

Results: *MYC* genetic alterations were observed in 10/33 (30%): 3 rearrangements and 7 gains. Six of 31 (19%) were considered positive for *MYC* expression (cut-off >40%). All cases with *MYC* rearrangement, 2/6 (33%) with gains and 1/20 (5%) without genetic alterations of *MYC* showed overexpression of the protein ($p = 0.001$). No rearrangement of *BCL2* was detected in any case, but gains were observed in 14/26 (54%). BCL2 expression was found in 19/30 (63%). Cases with *MYC* rearrangement did not showed *BCL2* gains; however, 5/6 (83%) of *MYC* gained also presented gains of *BCL2*. Among the 6 positive cases for *MYC* expression, 4 (67%) co-expressed BCL2. *MYD88*^{L265P} was detected in 18/33 (55%), which was more frequently observed in patients older than 70 years ($p = 0.009$). The only variable predicting an unfavorable outcome was the presence of *MYC* genetic alterations, which are correlated with a worse overall and progression-free survival ($p = 0.037$ and $p = 0.017$, respectively). Neither the presence of *MYD88*^{L265P} nor the expression of *MYC* protein (with or without the co-expression of BCL2) had an impact on the survival.

Conclusions: This study confirms that *MYD88*^{L265P} is a common event in DLBCL of the testis, but in contrast to DLBCL of other locations, this mutation has no

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impact on survival. Only *MYC* genetic alterations, but not their protein expression, were associated with an adverse outcome. *MYC* genetic alterations may be a useful marker to identify patients with poor response to R-CHOP.

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MYD88 L265P MUTATIONS, BUT NOT OTHER VARIANTS, IDENTIFY A SUBGROUP OF PATIENTS WITH ABC DIFFUSE LARGE B-CELL LYMPHOMA, EXTRANODAL INVOLVEMENT AND POOR OUTCOME

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is a biological and clinical heterogeneous disease. Most patients with unfavourable prognostic have the activated B-cell (ABC) DLBCL subtype molecularly characterized by the constitutive activation of NF-κB transcription complex. NF-κB pathway can be simultaneously activated by B-cell receptor and toll-like receptors (TLRs). Somatic mutations of *MYD88* in this TLR pathway increase the NF-κB transcription capacity.

Patients and Methods: We studied 213 patients (115 men/98 women; median age, 65 years), consecutively diagnosed with DLBCL according to the WHO classification in a single institution between 2002 and 2012. Primary mediastinal, CNS and immunodeficiency-associated lymphomas were excluded. Screening for the most frequent *MYD88* mutations (L265P, M232T, S219C, V217F and S222R) was performed by using allele-specific PCR assay for each mutation. Main clinicobiological variables, including cell of origin assessed by gene expression in 129 cases, and outcome were analyzed according to *MYD88* mutational status.

Results: *MYD88* mutations were found in 47 (22%) cases, including L265P in 39 cases and other mutations in 8 (M232T = 4; S219C = 4). The most important biological and clinical features of the 213 cases according to *MYD88* status are listed in the table. *MYD88* L265P mutations but not the others were seen more frequently in ABC DLBCL subtype. Patients with *MYD88* L265P mutations more frequently were old, had extranodal disease, particularly testis and breast, carried *BCL2* rearrangements and myc/BCL2 protein expression and showed inferior progression free and overall survival (OS). On the contrary, those patients with *MYD88* mutations other than L625P displayed a better prognostic. IPI and *MYD88* L265P were independent variables predicting OS in the multivariate analysis. No differences were found in OS according to real-time quantification analysis of *MYD88* L265P.

Conclusions: *MYD88* L265P mutations, but not other variants, identify a subpopulation of DLBCL mainly of ABC origin, extranodal involvement and poor outcome.

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	Wild type (*N* = 166)	L265P (*N* = 39)	Other (*N* = 8)
Age >60 years (%)	53	79	62*
Cell of origin (%)			
GCB	55	17	50
ABC	33	75*	50
Unclassified	12	8	0
Extranodal involvement (%)	51	69	37+
Testis (+)	0.6	10	0*
Breast (+)	0.6	5	0‡
5-year PFS (%)	54	44	75‡
5-year OS (%)	62	52	75*

ABC, activated B-cell like; GCB, germinal centre B-cell like; PFS, progression-free survival; IS overall survival.
*$p < 0.05$.
+$p = 0.053$.
‡$p = 0.09$.

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MYC OR BCL2 COPY NUMBER ABERRATIONS IS A STRONG PREDICTOR OF OUTCOME IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphomas (NHL). MYC aberrations concurrent with BCL2 and/or BCL6 aberrations constitute a specific cohort of patients with extremely poor outcome.

Methods: In this study, we retrospectively investigated the incidence and prognosis of MYC, BCL2 and BCL6 aberrations with DLBCL patients in Chinese population. We applied fluorescence *in situ* hybridization and immunohistochemical analysis in 246 DLBCL patients diagnosed between February 2006 and January 2014. Prognostic analysis was done on the whole cohort and 141 patients who treated with R-CHOP-like therapy.

Results: The results showed that patients with MYC or BCL2 copy number aberrations (CNAs) had significantly worse overall survival (OS) and progression-free survival (PFS) than negative cases (MYC CNAs: median OS: 15.8 months vs not reach, $p < 0.0001$; median PFS: 6.1 vs 29.0 months, $p < 0.0001$; BCL2 CNAs: median OS: 24.1 months vs not reach, $p < 0.0001$; median PFS: 13.2 vs 74.7 months, $p < 0.0001$). No survival differences were observed between gain (3–4 copies) and amplification (≥5 copies) with both MYC and BCL2. We indicated that BCL2 CNAs had much inferior outcome than BCL2 rearrangement or Bcl2 expression (median OS: 24.1 months vs not reach vs not reach, $p = 0.0021$; median PFS: 13.2 vs 37.7 vs 20.7 months, $p = 0.0008$) while MYC CNAs predicted similar OS (median OS: 15.8 vs 18.3 vs 23.0 months, $p = 0.1228$) and PFS (median PFS: 6.1 vs 9.7 vs 11.6 months, $p = 0.2750$) with MYC rearrangement or Myc expression. Similar results were recognized in the patients treated with R-CHOP-like therapies. Patients with both MYC and BCL2 CNAs had a similar outcome to classic DHL or protein double expression (Myc and Bcl2/Bcl6 coexpression).

Conclusions: MYC or BCL2 CNAs constituted another group of patients with extremely poor outcome. We suggest that further studies pay close attention to MYC and BCL2 CNAs to confirm our results.

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ANALYSIS OF 'DOUBLE-HIT' LYMPHOMA CASES BY GENETIC SUBTYPE

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Introduction: 'Double-hit' lymphoma (DHL), defined as a B cell non-Hodgkin lymphoma harboring rearrangements in *c-MYC* and partner genes *BCL2* and/or

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BCL6, carries a poor prognosis. The significance of these partner gene rearrangements is unclear as most published reports of DHL include cases which have not been tested for both *BCL2* and *BCL6* rearrangements and/or analyze all DHL cases in aggregate. Here we report characteristics and outcomes of DHL patients (pts) by genetic subtype.

Methods: From our previously described database of DHL pts (*Blood* 2014 124:2354-61), we identified cases that underwent metaphase karyotyping or fluorescence *in situ* hybridization for *c-MYC* as well as both *BCL2* and *BCL6* rearrangements. Cohorts were defined by the presence (+) or absence (−) of rearrangements: *c-MYC+/BCL2+/BCL6−* (BCL2-DHL), *c-MYC+/BCL2−/BCL6+* (BCL6-DHL) and *c-MYC+/BCL2+/BCL6+* (THL). Therapy was given at the discretion of the treating physician. Categorical variables were analyzed by Fisher's exact test and survival times by logistic regression. Univariate and multivariate analyses were performed using Cox proportional-hazards regression.

Results: Data from 117 cases were included. Extranodal disease was more frequent in BLC6-DHL (88%) than BCL2-DHL (58%) or THL (48%) pts ($p = 0.04$ for both), and germinal center cell of origin was more frequent in BCL2-DHL (92%) than BCL6-DHL (56%) pts ($p = 0.001$). Treatment received and outcomes are described in Table 1. Univariate analysis revealed that elevated LDH, stage ≥3 disease, IPI ≥4 and bone marrow involvement in BCL2-DHL pts and IPI ≥4 and bone marrow involvement in THL pts predicted for inferior overall survival; however, no factor remained statistically significant on multivariate analysis.

Conclusions: Analysis of the largest reported series of BCL2-DHL, BCL6-DHL and THL pts by genetic subtype revealed few significant differences in outcome, potentially due to small cohort sizes. BCL6-DHL pts may be more likely to achieve CR1 than BCL2-DHL and THL pts but may also experience shorter OS while in CR1 even if receiving SCT. Outcomes for THL pts may not differ from those experienced by BCL2-DHL and BCL6-DHL pts. High rates of primary refractory disease and relapse seen across all subtypes provide a rationale for offering novel therapeutic approaches to these pts in the front-line setting. Furthermore, comprehensive partner gene rearrangement testing may increase identification of candidates for gene-specific targeted therapies.

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DISTINCT PATTERNS OF GENETIC EVOLUTION OF RELAPSING DIFFUSE LARGE B-CELL LYMPHOMA REVEALED BY GENOME COPY NUMBER ABERRATION ANALYSIS

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Introduction: Relapses of diffuse large B-cell lymphomas (DLBCL) represent a source of significant morbidity and mortality. Although a few reports on clonally unrelated relapses have been published, second and subsequent presentations of DLBCL are generally regarded as direct outgrowths of the original neoplasm. Moreover, very little is known about the underlying genetic and biological mechanisms of recurrence. Paired sample studies of primaries and relapses are of great value in addressing these issues.

Methods: We performed DNA copy number profiling by array-comparative genomic hybridization of 20 matched primary-relapse pairs and 11 non-relapsing DLBCL cases. Clonal relationship was verified by immunoglobulin *V(D)J* rearrangement analysis. Additionally, mutations were analysed in frequently affected lymphoma genes by deep sequencing in selected pairs.

Results: Three clonally unrelated DLBCL recurrences were identified in which tumours showed distinctive immunoglobulin gene rearrangements and different copy number aberration profiles. In the remaining 17 clonally related pairs, we detected at least two different patterns of genomic evolution: (i) branching progression from a common progenitor clone in 6 cases, and (ii) linear evolution of relapses from the primary tumour clone in 11 cases. Comparison of copy number profiles from non-relapsing and relapsing DLBCL identified recurrently affected chromosomal regions with candidate genes in both groups, indicating a potential sensitivity to treatment for the former (recurrent deletions of 8q12.3 in non-relapsing lymphomas, containing *CYP7B1*) and a potential to relapse for the latter (recurrent gains of 10p15.3-p13 in relapsing lymphomas, containing *PRKCQ* and *GATA3*).

Conclusions: In conclusion, our copy number aberration profiling of paired primary and relapse DLBCL samples reliably demonstrates for the first time the existence of clonally unrelated DLBCL recurrences at the genomic level. Further, our results

Abstract 162 Table 1 Treatment received and outcomes

	DHL subtype			*p*-value		
	BCL2-DHL (*n* = 76)	BCL6-DHL (*n* = 16)	THL (*n* = 25)	BCL2-DHL vs BCL6-DHL	BCL2-DHL vs THL	BCL6-DHL vs THL
Receipt of DE[a]	55%	44%	65%	0.42	0.35	0.19
Receipt of CNS prophylaxis	39%	56%	40%	0.27	1.00	0.35
Complete response (CR1)	54%	75%	46%	0.17	0.49	0.10
If receiving DE[a]	55%	86%	63%	0.22	0.77	0.37
Stem cell transplant (SCT) in CR1	36%	22%	55%	0.47	0.31	0.18
Primary refractory disease	32%	13%	33%	0.14	1.0	0.25
If receiving DE[a]	25%	14%	14%	1.0	0.71	1.0
Relapse (if responding)	42%	50%	23%	0.75	0.33	0.23
If receiving DE[a]	36%	33%	25%	0.67	0.29	1.0
Median OS (months)[b]	34.8	14.5	17.2	0.89	0.69	0.90
If low-risk DPI[c]	NYR[d]	11.5	NYR[d]	0.16	—	0.32
If high-risk DPI[c]	13.6	—	17.2	—	0.58	—
If receiving DE[a]	37.5	12.1	NYR[d]	0.67	0.91	0.84
If achieving CR1	NYR[d]	14.5	NYR[d]	*0.02*	0.61	0.10
If receiving SCT in CR1	NYR[d]	5.3	NYR[d]	*0.008*	0.36	0.11

[a]DE is dose-escalated front-line chemotherapy (EPOCH, hyperCVAD and CODOX-M/IVAC).
[b]Median length of follow-up is 12.0 months (range 0.1–85.6).
[c]DPI is DHL Prognostic Index.
[d]Not yet reached.

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suggest two different genomic patterns of clonally related DLBCL relapses, i.e. branching evolution from a common progenitor and linear progression with or without progression. Finally, we identified differentially aberrant regions between primary relapsing and non-relapsing DLBCL containing genes that are potentially involved in sensitivity to treatment and potential to relapse.

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RELAPSED DLBCLS PRESENT FREQUENT COPY NUMBER VARIATIONS OF GENES INVOLVED IN LYMPHOMAGENESIS WITH DIFFERENT PATTERNS BETWEEN EARLY- AND LATE-RELAPSED DLBCLS

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma. Relapse may occur early, within the first year after the first-line treatment, or late, after 1 year or more. Prognosis is poor in both cases, and it is worse in early relapsed DLBCLs. Recent advancements in genomic technology have provided better understanding of the biology of DLBCL. The aim of this study was to compare the copy number variations (CNV) of patients with early and late relapses.

Methods: From 396 patients with relapsed DLBCL included in the CORAL trial (1), frozen biopsies were available for a total of 39 patients. Nineteen of them presented an early relapse and 20 a late relapse. CNV were determined from SNP6 Affymetrix DNA microarray hybridization. Total copy numbers were computed from the 1 800 000 probesets covering the whole genome according to the CRLMM algorithm (2). In order to remove artifactual values, we calculated the median value of the signal within a sliding window. CNV were defined as loss or gain of signal over at least 2 megabases.

Results: The average total CNVs number for the whole group was 15. Chromosomes 6, 12, 13 and 18 were the most frequently altered compared to other chromosomes ($p = 0.03$). We noted a great heterogeneity of CNV numbers between individuals (range 0–66 CNVs) but no difference between early relapsed and late-relapsed DLBCLs (average total CNVs 14 and 16, respectively; p-value = 0.8).

Frequent CNVs involved ITPKB, XPO1, BCL-6, IRF4, IBTK, PRDM1, TNFAIP3, FOXO1, TP53 and BCL2 genes but with no systematic difference between late-relapsed and early relapsed DLBCLs. In contrast, deletions of CDKN2A were a common event in early relapsed DLBCLs. More than 50 other genes showed significant CNV difference between early relapsed and late-relapsed DLBCLs.

Conclusion: In this series, we found an equivalent number of CNVs among early- and late-relapsed DLBCLs. Genes involved in lymphomagenesis frequently exhibited CNVs. Many genes exhibited significant CNV difference between early- and late-relapsed DLBCLs, including CDKN2A, which showed a high frequency of deletions in early relapsed DLBCLs.

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GENETIC HETEROGENEITY AND CLONAL EVOLUTION IN PRIMARY AND RELAPSED MANTLE CELL LYMPHOMAS

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Introduction: The genetic mechanisms underlying disease progression and therapy resistance in mantle cell lymphoma (MCL) remain largely unknown.

Methods: Whole-exome sequencing was thus performed in 27 MCL samples, representing consecutive biopsies obtained at diagnosis and/or relapse from 13 patients.

Results: Twenty-five genes were found to be recurrently mutated in these samples, including known (*ATM, MEF2B* and *MLL2*) and novel targets (*S1PR1* and *CARD11*). The latter genes were subsequently screened in addition to 173 MCL samples, and mutations were observed at a frequency of 8.5% and 5.5%, respectively. The genetic alterations identified in *S1PR1* were associated with reduced protein expression and were preferentially detected in relapse samples. Mutations observed in *CARD11* provided genetic evidence for activation of B-cell receptor (BCR)/NF-κB signalling pathways in a subset of MCLs. Furthermore, by analysing these mutants in MCL cell line and by retrospectively sequencing the MCL samples from patients treated with BTK inhibitors, we suggest that *CARD11* mutations might also underlie the development of resistance to some of the BCR/NF-kB signalling inhibitors including ibrutinib and lenalidomide. Genetic alterations that are specifically associated with the relapse samples were also identified and found to be heterogeneous, affecting genes in the MAPK, p53 and Wnt signalling pathways. Moreover, most of the primary and relapse tumours seem to arise independently from a common progenitor through acquisition of distinct mutations.

Conclusions: In summary, this study highlights the genetic heterogeneity of MCL, especially during relapse, and might provide insights into therapeutic strategies.

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PROGNOSTIC SIGNIFICANCE OF GENOMIC ALTERATIONS IN MANTLE CELL LYMPHOMA

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Introduction: Whole genome, exome and targeted sequencing have identified various genomic alterations in mantle cell lymphoma (MCL). We describe genomic alterations in MCL using a targeted sequencing platform and correlate these with clinical outcome.

Methods: Genomic DNA was isolated from formalin-fixed paraffin-embedded tissues and screened for mutations using the Memorial Sloan Kettering Cancer Center (MSKCC) HEMEPACT targeted signalling platform, including 585 biologically significant genes in hematologic malignancies. Significant non-synonymous variants were identified as mutations from the COSMIC database, amplifications of established oncogenes, homozygous deletions of known tumor suppressors, inactivating mutations, and in-frame mutations. Mutations were correlated with overall survival (OS) and proliferative index (PI).

Results: Twenty-three pretreatment specimens from newly diagnosed MCL patients treated at MSKCC from 1999 to 2006 were sequenced to high coverage, averaging >300× for DNA. Median age was 60 years (range 24–76). Most patients (96%) had advanced stage disease and 43% had an elevated Ki-67 (≥30%). Treatments included intensive therapy (4 cycles of R-CHOP + 2–3 cycles of (R)ICE + autologous stem cell transplant) (48%), radioimmunotherapy (^{131}I-tositumomab followed by 6 cycles of CHOP) (48%), or rituximab (3%).

In the 23 cases analysed, 78 unique genomic alterations were identified. ATM was the most frequent mutation seen in 48% of patients; however, presence of ATM mutation was not prognostically significant. Other known driver mutations in MCL were also found, such as p53 (17%) and CCND1 (13%). Mutations in p53 were significantly associated with inferior OS ($p = 0.023$) and an elevated PI ($p = 0.024$). Alterations in chromatin modifying genes (e.g. MLL2, SETD2 and WHSC1) were common, occurring in 30% of cases. Recurrent alterations in the Notch pathway (NOTCH1/FBXW7) were seen in 17% of cases. Alterations in BIRC3 (17%), a component of the alternative NF-κB pathway and apoptotic mediator, were identified. Previously undescribed in MCL, but known to be associated with colorectal cancer, recurrent mutations in the APC gene were identified in 13% of cases.

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Conclusions: This analysis describes the genomic landscape in MCL prior to frontline therapy using a comprehensive targeted sequencing platform. The study identifies potential targets for mechanism-based therapy. For example, patients with Notch mutations may benefit from notch inhibitors, gamma secretase inhibitors, or modulators of the NF-kB pathway. This is the first report describing genetic alterations in the APC gene in MCL. In the future, we plan to analyze more cases to elucidate the biologic and clinical significances of these genetic alterations with the aim of developing biologically targeted therapies in MCL.

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FGF SIGNALLING SUPPORTS MANTLE CELL LYMPHOMA SURVIVAL IN THE PRESENCE OF MESENCHYMAL STROMAL CELLS

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Introduction: Mantle cell lymphoma (MCL) represents an aggressive, incurable form of non-Hodgkin's lymphoma (NHL). The health complications associated with advanced age of MCL patients further restrict treatment with intense chemotherapy. The presence of *t*(11;14) is responsible for overexpression of cyclin-D1, the hallmark of MCL. Studies on primary MCL are now possible through the propagation of primary MCL in the presence of human mesenchymal stromal cells (hMSC). We hypothesized that tumour-initiating cells are responsible for MCL relapse and chemoresistance, and thus, identification of survival signals responsible for survival and maintenance of MCL-initiating cells (MCL-ICs) is essential for design of successful treatment strategies.

Methods: Isolates of primary MCL cells ($n = 20$) were co-cultured with hMSC, and content of MCL-ICs were analysed by flow cytometry based on marker expression profile; CD34-CD3-CD45+CD19-. Cytokine array was used to identify the soluble factors enriched by co-cultures, and the expression of these factors was confirmed by RT-PCR analysis. The signalling pathways employed by the newly identified factors were blocked in MCL cell lines (Z139, JVM2 and Mino) to demonstrate the role of identified signalling mediators in survival of MCL.

Results: Co-cultures of primary MCL isolates with hMSCs supported the growth of MCL cells with continued presence of MCL-ICs (CD34-CD3-CD45+CD19-) representing ~1% of MCL cells. MCL-IC induced tumour formation in nude mice. We found IL-6 triggered a FGF/FGFR when MCL cells were co-cultured with MSCs, consistent with use of an FGF autocrine stimulation. Patients with high FGFR levels in MCL tissues compared to normal lymphocytes and had a lower overall survival rate compared to those with low FGFR levels. Blocking FGF/FGFR induced death of MCL cells and also sensitized the cells to the killing effects of death receptor ligands.

Conclusion: Our results suggest that primary MCL uses an FGF autocrine loop for their survival in the presence of stromal cells, and targeting the FGF signalling pathway could be therapeutically beneficial in these patients.

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PRIMA-1MET INDUCES MYELOMA AND MANTLE CELL LYMPHOMA CELL DEATH, BY IMPAIRING ROS/GSH BALANCE.

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Introduction: *TP53* is the most frequently mutated gene in cancer, inactivation of p53 pathway is associated with resistance to therapy in numerous cancers, including multiple myeloma (MM) and mantle cell lymphoma (MCL). In MM, *TP53* mutations are rare at diagnosis and restricted to patients with a deletion of short arm of chromosome 17 (del17p), who have a shorter survival (Avet-Loiseau, 2010). In MCL, *TP53* mutations are frequent at diagnosis (25%, Slotta, 2012;Stefancikova, 2010) and are associated with adverse prognosis. In both pathologies, therapeutic approaches bypassing p53 resistance are needed.

To this end, we assessed efficacy of molecules described as 'p53 reactivating molecules'. Indeed, PRIMA-1Met (APR-246) (PRIMA) was isolated according to its ability to restore apoptosis in SAOS2-His-273 cells in a p53-dependent manner. PRIMA was shown to bind to p53 and to induce a functional reconformation of the mutant protein.

Methods: Using a wide number of myeloma cell line, primary myeloma cells and MCL cell lines, we investigated whether PRIMA could induce MM and MCL cell death.

Results: We showed that PRIMA induced cell death irrespective of *TP53* status (LD50), ranging from 4 μM to 200 μM, median value 37 μM. PRIMA failed to induce re-expression of p53 target genes, and cell death was not impaired upon p53 silencing, confirming that cell death mechanism was p53 independent. We showed that PRIMA induced expression of Noxa at mRNA level (3.8-fold, $p = 0.03$) in $OPM2^{Mut}$ cell line and silencing of Noxa, using siRNA, inhibited PRIMA-induced cell death by 89% ($p = 0.02$). Of major interest, we showed that PRIMA deeply depleted glutathione (GSH) from cells (mean of reduction −75% ± 10%) and induced reactive oxygen species (ROS). Moreover, we demonstrated that PRIMA dramatically synergized with BSO (L-buthionine sulfoximine), an irreversible inhibitor of γ-glutamylcysteine-synthase (which inhibited synthesis of GSH). Indeed, combination of suboptimal doses of both PRIMA and BSO (10 μM and 0.5 mM, respectively), induced cell death (>90% of cell death) in 25 of 27 cell lines ($p < 0.001$), irrespective of *TP53* status ($p = 0.8$). PRIMA-induced cell death in primary cells of patients with a median of 55% in 25 samples. The combination of PRIMA–BSO increased the induced cell death from 32% to 87% ($p = 0.03$, $n = 6$).

In MCL cell lines (4 wild-type *TP53* and 3 mutated and/or deleted *TP53*), we showed that PRIMA was also efficient alone and in combination with BSO. Interestingly, $TP53^{Abn}$ cells were more sensitive than TP53wt cells. PRIMA–BSO combination induced a mortality of 91% in MCL cell lines with a suboptimal dosing of PRIMA (10 μM) vs 2.4% with PRIMA alone ($p = 0.0006$), irrespective of *TP53* status.

Finally, we showed that in the myeloma $TP53^{Abn}$ JJN3-xenograft model (beige-SCID mice), PRIMA inhibited tumour growth and induced tumour regression in association with BSO ($p < 0.05$).

Conclusions: PRIMA has been evaluated in phase I trial, without major toxicity, and could be of a real interest, associated with BSO, for patients with p53 impaired pathway.

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CHRONIC LYMPHOCYTIC LEUKAEMIA B-CELLS ARE RESCUED FROM APOPTOSIS BY EXTRACELLULAR VESICLES FROM BONE MARROW MESENCHYMAL STROMAL CELLS

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Introduction: The interactions between chronic lymphocytic leukaemia (CLL) cells and the microenvironment (primarily composed of mesenchymal stromal cells—MSC) play an important role in promoting the increased survival of leukaemic B cells. Extracellular vesicles (EVs) produced by leukaemic cells and the microenvironment may be implicated in this cross-talk. EVs, including microparticles and exosomes, are small plasma membrane fragments with sizes ranging from 0.01 to 1 μm, and contain products specific to the original cell, such as microRNA, mRNA and proteins. Our objective is to assess the role of EVs in the cross-talk between malignant cells and their microenvironment.

Material and methods: Ultracentrifugation at 150 000× g during 1 h was applied to isolate EVs from supernatant of MSC culture. Protein concentration was measured by BCA kit and nanodrop. Different concentrations of EVs were added to CLL-B-cells to evaluate their impact on cell proliferation and survival. PKH67 labelling and qRTPCR were performed to prove the inclusion of EVs in CLL B-cells (18 samples were analysed).

Results: We first demonstrated that EVs from MSCs are able to enter in CLL B-cells. By flow cytometry with PKH67-labelled EVs, we observed that 44.2%, 93.8% and 100% of CLL B-cells had integrated fluorescent EVs after 1, 3 and

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24 h, respectively. Two highly expressed mRNAs (collagen and fibronectin) in MSC, also detected in MSC-derived EVs by qRTPCR, were increased in CLL-B cells after 24 h of incubation with EVs confirming EV-mediated mRNA transfer to target cells. Further analysis of apoptosis in CLL cells were assessed by flow cytometry using an Annexin/7AAD staining: addition of increasing concentrations of EVs showed a protective effect on CLL B-cells from cell death (mean increase of 11% of live cells, $n = 19$/p-value = 0.007).

Conclusion: We demonstrated, by two methods, that MSC-derived-EVs enter into CLL B-cells. These vesicles protect CLL cells from spontaneous apoptosis and affect mRNA expression involved in CLL cell functions. This study provides evidence of the critical role played by EVs in the interactions between leukaemic cells and their microenvironment.

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PROGNOSTIC SIGNIFICANCE OF TET AND IDH MRNA EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Introduction: Epigenetics plays a crucial role in cancer physiopathology. DNA hydroxymethylation is catalysed from methylated DNA by ten-eleven translocation (TET) enzymes requiring co-factors produced by isocitrate dehydrogenase (IDH) proteins. This modification could be a step in the demethylation process and could thus have an impact on gene expression. TET2 mutations have already been reported in several leukaemias. However, little is known about hydroxymethylation in B-chronic lymphocytic leukaemia (CLL).

Methods: Expression of TET1, 2, 3, IDH1 and 2 mRNA was assessed by qPCR on purified leukaemic B-cells from a cohort of 214 CLL patients with a median follow-up of 75 (6–380) months and compared with those of purified peripheral normal B-cells. The influence of CLL microenvironment on TET enzyme expression was investigated by culture of CLL cells ($n = 10$) in the presence or absence of bone marrow mesenchymal stromal cells (BMSC).

Results: TET1, 3 and IDH2 are underexpressed in leukaemic B-cells compared with healthy volunteers B-cells ($p = 0.0221$, 0.0013, <0.0001, respectively), while IDH1 is overexpressed ($p = 0.0037$). Expression of TET2 is similar in both groups. When we stratified patients according to low and high expressions, TET2 and IDH1 significantly predict treatment-free survival (TFS): patients with high TET2/IDH expression had a median TFS of 110 months, while patients with low expression presented a median TFS of 78 months ($p = 0.0071$/0.0123). Finally, we observed a decreased TET1 expression ($p = 0.0371$) and an increased TET3 ($p = 0.0273$) and IDH2 expression ($p = 0.0039$) in CLL cells after co-culture with BMSC. Further analysis in 14 CLL patients shows that ZAP70+ patients present higher hydroxymethylated DNA than ZAP70-patients.

Conclusions: This is the first report that DNA hydroxymethylation enzymes are deregulated in B-CLL compared to normal B cells: (i) TET2 and IDH1 overexpression have a good prognostic value; (ii) TET1, TET3 and IDH2 expressions are modulated by microenvironment interactions, resulting in CLL cells survival. Our observations suggest that DNA hydroxymethylation plays a major role in CLL physiopathology and supports the potential therapeutic benefit of agents targeting epigenetics in CLL patients.

HODGKIN LYMPHOMA

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OPTIMAL TREATMENT STRATEGIES FOR EARLY STAGE HODGKIN'S LYMPHOMA WITH B SYMPTOMS AND BULKY DISEASE AT PRESENTATION

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Purpose: Treatment of stage IIB bulky Hodgkin lymphoma in patients is controversial, with wide variation in treatment strategies employed by different institutions. We evaluated patients with IIB bulky disease treated with combined modality therapy at our institution by describing their long-term clinical outcomes.

Methods and Materials: We identified 149 consecutive patients with stage IIB bulky Hodgkin lymphoma treated between 1971 and 2012. Clinical, pathologic and treatment characteristics were extracted from medical records. Actuarial overall and disease-specific survival was calculated using the Kaplan–Meier method. Independent factors associated with these outcomes were identified using a multivariate Cox regression model. Outcomes were further compared against a subgroup of 126 patients with advanced stage disease treated between 1971 and 2009.

Results: Median overall survival (OS) time for patients with stage IIB bulky Hodgkin lymphoma was 322.4 months (vs 145.9 months for stages III/IV patients; $p = 0.005$). The 5-year OS rate for stage IIB bulky patients who received combined modality ABVD and radiation was 89.1%; the 5-year relapse-free survival rate was 79.1%. On multivariate analysis, age <40 years (hazard ratio [HR] 0.31, 95% CI 0.16–0.62, $p = 0.001$), receipt of ABVD (vs MOPP; HR 0.32, 95% CI 0.11–0.80, $p = 0.014$) and radiation dose $\geq$30.1 Gy (HR 0.26, 95% CI 0.11–0.67, $p = 0.007$) were associated with improved OS. Cardiac events ($n = 11$) and secondary malignancies ($n = 11$) all occurred in patients treated before 1995. A subgroup analysis demonstrated significantly improved outcomes in IIB bulky vs advanced stage patients (median OS: 322.4 vs 145.9 months, $p = 0.008$). The trend of improved outcomes in IIB bulky patients was especially evident in the modern era (>1995; $p = 0.004$)

Conclusion: In this cohort, patients with stage IIB bulky Hodgkin lymphoma demonstrated excellent outcomes after treatment with combined modality ABVD and radiation therapy Temporal trends in treatment strategies have changed substantially, with concomitant improvements in disease outcomes and long-term toxicities.

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INFRADIAPHRAGMATIC HODGKIN LYMPHOMA: LONG-TERM OUTCOME IMPROVED BY RADIOTHERAPY–CHEMIOTHERAPY VS CHEMIOTHERAPY ALONE

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Introduction: Infradiaphragmatic Hodgkin Lymphoma (IHD) accounts for 5–10% of adult cases of stages I–II Hodgkin lymphoma (Mauch, Hematol Oncol. 1983; Vilamor, Eur J Haematol, 1991). Because of small number of patients (pts) in the previous published series, there is no consensual standard treatment and prognostic factors for pts with an IHD. The strategy of treatment has been improved and standardized along the last decades in most clinical subsets of HL, while it remains heterogeneous in IHD with these pts being often excluded from clinical trial. Thus, we retrospectively collected demographic, clinical and biological data in a series of pts coming from 4 french institutions and analysed the prognosis impact of the therapy used.

Methods: The clinical, biological data at baseline, the details of treatment and outcome of pts with a first diagnosis of stages I–II of IHD were retrospectively collected in 4 departments of haematology. Pts with a positive HIV serology and those treated with radiotherapy alone were excluded. For all patients, clinical, biological and therapeutic data were collected.

Results: From 1975 to 2012, 107 pts with a median age of 48 years were included. Histologic subtypes were nodular sclerosis, mixed cellularity, lymphocyte predominance lymphocyte depleted and unknown in 62%, 24%, 6.4%, 1% and 6% of cases, respectively. Baseline staging was mainly based on a whole body CT scan, and 19 pts had a stage I (18%) and 88 pts a stage II (82%) disease. Fifty-seven pts (53%) had lombo-aortic adenopathy, 79 pts (74%) had inguinal adenopathy and 11 (10%) a splenic involvement. Most pts received ABVD (80 pts, 75%) or ABVD-like (19 pts, 18%) chemotherapy regimen with a median number of 6 courses (2–8). Radiotherapy was performed in 52 pts (49%). With a median follow-up of 49 months

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(2–312), 33 progression or relapse occurred (31%) and 19 pts died (18%), 9 from HL, 4 from second malignancies, 1 from infectious event, 1 from chemo-toxicity and 4 from cardiac or vascular events. Age (>45 vs <45 years), gender, B symptoms, bulk (>10 cm vs <10 cm), Ann Arbor stage, leukocytosis (>15 G/l vs <15 G/l), lymphopenia (<0.6 G/l vs >0.6 G/l), monocyte count (>1 G/l vs <1 G/l), type of chemotherapy regimen were not found to influence pts outcome. Inversely, a ratio of lymphocyte/monocyte <1, observed in 10% of the 73 tested pts, was related to a shorter PFS (5-year PFS: 100% vs 65%, $p = 0.04$) with a trend towards a shorter OS. Pts who received radiotherapy had also a better PFS (5-year PFS: 81% vs 65%, $p = 0.0008$) and OS (5-year OS: 90% vs 79% $p < 0.035$).
In multivariate analysis, only radiotherapy remained to be an independent factor to predict PFS (HR = 2.94 (95% CI 1.3–6.45), $p = 0.0073$).

Conclusion: This multicenter retrospective study shows that omission of radiotherapy in responding pts with an IHD is associated with a short PFS and OS and suggests that a combined modality therapy has to be proposed to these pts.

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CONSOLIDATION RADIATION AFTER ABVD IS EFFECTIVE TREATMENT FOR SELECTED PATIENTS WITH HODGKIN'S LYMPHOMA (HL) WITH INTERIM PET POSITIVE SCANS

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Introduction: Classical HL is associated with a high cure rate even for advanced stages, but it has become clear with advancements in imaging that those patients who have continuing PET positive disease after initial cycles of chemotherapy have a high risk of early relapse and poor outcome. On this basis, patients with interim PET (iPET) positive scans are now considered for early dose escalation in ongoing clinical trials. We present here the outcomes using radiotherapy alone as consolidation treatment at the end of standard ABVD chemotherapy.

Methods: The West of Scotland haemato-oncology network serves a population of 2.6 million, and the database records staging, treatment and outcome on all patients. We examined records on all HL patients reported as having positive iPET imaging since this was introduced in 2007. Patients who progressed on first-line chemotherapy and those proceeding to dose escalation were excluded as were those deemed PET negative on updated review of imaging. For the cohort with interim positive scans receiving radiotherapy alone at the end of standard ABVD, we recorded age, gender, stage, primary chemotherapy and whether there was an end of treatment PET scan performed. We then evaluated radiotherapy fields relative to original sites of disease and sites remaining PET positive on interim imaging. Finally, we recorded duration of follow-up to December 2014, progression-free survival (PFS) and overall survival (OS).

Results: From 2007 to 2013, there were 244 patients recorded as new diagnoses of HL. Following radiology review, 65 had positive iPET imaging of whom 15 received consolidation radiotherapy following standard ABVD chemotherapy. There were 7 men, 8 women; ages 17–71 y, median 33 y. Five had stage IIA disease and 10 were stages IIB–IV. Twelve had 6 cycles ABVD and 3 had dose reductions for comorbidity. Radiotherapy fields covered whole site of involved disease in 10 but in 4 were limited to small mediastinal fields because of prior therapy or in young women. One patient had two fields to cover separate sites at neck and groin. Follow-up ranged from 7 to 75 m, median 33 m. Only one patient has relapsed and that was outside radiotherapy field at 36 m. One patient died of colitis 4 m after end of treatment but was free of HL. PFS is 87%. OS is 93% (one non-HL death).

Conclusions: Gallamini published worldwide series of HL patients with iPET positive imaging in 2014 demonstrating only 28% PFS at 3 y compared to 95% in PET negative group. Studies using treatment intensification in the iPET positive group have improved PFS to as high as 65%. Our network has achieved 87% PFS without intensification by use of consolidation radiotherapy planned to more than just site of residual positive uptake. We believe consolidation radiotherapy offers effective risk adapted strategy in a group at high risk of local relapse.

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PENCIL BEAM SCANNING PROTON THERAPY FOR LYMPHOMA PATIENTS WITH MEDIASTINAL INVOLVEMENT: A DOSIMETRIC STUDY AND PRELIMINARY CLINICAL DATA

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Introduction: Radiotherapy (RT) is associated with risk of acute and late toxicities. Toxicity risk is related to RT dose and irradiated volume. Pencil beam scanning (PBS) is a new proton RT technique. The aim of the study is to confirm dosimetric advantages of PBS compared to standard RT technique for lymphoma patients (pts) and to evaluate acute toxicity and early control.

Methods: Overall, 24 pts (men 11 and women 13) with supradiaphragmatic lymphoma (HL 19 pts., DLBCL 3 pts, T-lymphoblastic NHL 1 pt and T-peripheral NHL 1 pt) were irradiated between May 2013 and February 2015. Fourteen pts were analysable, 8 pts were early after RT and 2 pts had RT without mediastinum. PBS was used in the first-line treatment in 12 pts, and 2 pts were reirradiated. Median age was 33 years. RT volume: involved field 7 pts, RT on residual disease 6 pts and involved site 1 pt. The range of total dose: 19.8–40 CGE (median 30 CGE). Comparative 3D-conformal photon plan was made for all evaluable pts. Dosimetric advantages of PBS over 3D-conformal photon RT are presented in Table 1.

Results: The dose to most organs was significantly lower compared to standard photon technique. Relevant sparing of lungs, spinal cord, cardiac structures and volume of body exposed to radiation was reached. The sparing of mammary glands and esophagus was individual. Of evaluable pts, 12 achieved CR, 1 pt (reirradiated) achieved a stable disease and 1 pt died from generalized CMV infection 5 months after RT. Acute RT toxicity was mild, in most pts: dysphagia gr. I, radiodermatitis gr. I/II, asymptomatic neutropenia gr. II in 1 pt and gr.III in 2 pts. Asymptomatic changes of lungs occurred on post-RT CT scan in 1 pt.

Conclusion: Proton RT using PBS technique offers promising and safe possibility for most pts indicated for mediastinal RT. PBS has a potential to decrease significantly the dose to important organs at risk compared to standard photon technique used in most lymphoma pts. Early local control of PBS is comparable to standard photon techniques.

Abstract 174 Table 1 Comparison of dosimetric parameters for organs at risk

Organ at risk (assessed parameter)	Photon (dose Gy)	Proton (dose Gy)	Absolute difference photon vs protons (dose Gy)	Relative difference photon vs. proton (%)
Lungs bilat. (D mean)	10.3	5.8	4.5	57
Left lung (D mean)	12.6	7.1	5.6	56
Right lung (D mean)	9.1	5.1	4.0	56
Spinal cord (Dmax in 2% of volume)	26.9	9.6	17.3	36
Left mamma (D mean)	3.1	2.2	0.9	70
Right mamma (D mean)	1.7	0.4	1.3	22
Left ventricle (D mean)	7.9	3.2	4.8	40
Right ventricle (D mean)	10.0	6.1	3.8	62
Left atrium (D mean)	19.3	11.0	8.3	57
Right atrium (D mean)	14.8	8.6	6.2	58
Valvula mitr. (D mean)	16.6	5.4	11.1	33
Valvula tric. (D mean)	12.2	4.5	7.7	37
Valvula aort. (D mean)	22.0	8.6	13.4	39
Body (D mean)	3.9	1.3	2.6	33
Esophagus (D mean)	22.5	18.0	4.5	80

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CARDIAC RADIATION DOSE AND PREDICTED CARDIAC MORTALITY IN THE UK NCRI RAPID TRIAL IN EARLY STAGE HODGKIN LYMPHOMA

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Background: The initial management of early stage Hodgkin lymphoma (HL) involves optimising the balance between maximising cure and minimising late effects of treatment on the incidence of second cancers and cardiovascular disease. Results of the RAPID trial showed that patients who were PET scan 'negative' after 3 cycles ABVD had a very good 3-year progression-free survival (PFS) without further treatment. Involved field radiotherapy (IFRT) improved 3-year PFS, but this gain was obtained at the expense of irradiating all patients, many of whom were already cured. In the current study, cardiac doses of radiation received by individual patients taking part in RAPID were calculated, and the associated excess risk of cardiac mortality associated with receiving this treatment estimated.

Methods: Using original treatment data, individualised cardiac dosimetry was performed for patients who had received IFRT within the RAPID trial. Cardiac doses were used to estimate absolute excess 15-year cardiac mortality using two different prediction methods: the relative seriality model and a novel method based on a relative risk per Gy mean whole heart dose (MWHD) and population-based cardiac mortality rates individualised for age and gender.

Results: Dosimetry was completed for the majority of patients in the trial ($n = 247$, 79%). The average MWHD was 4.2 Gy (range 0.01 to 24.00 Gy). The majority of individuals (68%) received a low MWHD (<5 Gy). For more than half of those who received IFRT (58% and 62%), the predicted excess cardiac mortality was <0.1% and for more than three-quarters (76% and 95%), it was <1%, regardless of the prediction model used. A minority of individuals (13% and 2%) had an estimated excess cardiac mortality of >2% at 15 years. The extent of mediastinal involvement was the main determinant of MWHD and estimated cardiac mortality risk.

Conclusions: A wide range of radiation dose to the heart was received by patients in the RAPID trial allocated to receive IFRT. In most cases, the MWHD was small and the estimated absolute excess risk of cardiac mortality low, but for the minority with extensive mediastinal disease, the MWHD was high and excess mortality at 15 years 2%. These risks are likely to be higher if the impact of anthracycline toxicity and co-existing cardiac risk factors are taken into account and with longer follow-up.

The results of this study show that an individualised approach to treatment of early stage HL can be used to avoid excess cardiac mortality associated with high cardiac doses of radiation. Assessment of this risk at diagnosis combined with consideration of the risk of other late effects, particularly second cancers, will allow individualisation of the decision as to whether radiotherapy should be part of initial management, whether advanced radiotherapy techniques should be considered or whether a chemotherapy-only approach recommended for those achieving PET negativity.

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RISK OF RELAPSE IN CLASSICAL HODGKIN LYMPHOMA AT EVENT-FREE TIME POINTS AND SURVIVAL COMPARISON TO THE GENERAL POPULATION IN BRITISH COLUMBIA.

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Introduction: Studies in cHL typically measure the time to event from diagnosis (dx). Most relapses occur within the first 2 y. Estimates of risk of relapse at subsequent time points would aid in patient (pt) counseling, surveillance and clinical trial design. We evaluated the risk of relapse at defined event-free survival (EFS) time points and compared the risk of death to an age- and gender-matched population in BC.

Methods: The BC Cancer Agency Lymphoid Cancer Database was screened to identify all pts aged ≥16–69 y with cHL between May 1989 and December 2012 treated with an ABVD/ABVD-like regimen. Limited stage (LIM) = IA/B or IIA; advanced stage (ADV) = III/IV or stage IIB ± bulky disease (≥10 cm). Radiotherapy (RT) eras were extended field (EF) May 1989–December 1996; involved field (IF) January 1997–January 2001; and involved nodal (IN) February 2001–present. EFS was defined as time from dx to relapse/progression, unplanned treatment or death from any cause. Risk of relapse in the following 5 y was measured from the time of dx (Year0) and at EFS time points: 1 y (Year1), 2 y (Year2), 3 y (Year3) and 5 y (Year5). Event decomposition was performed using a competing risk analysis. Expected (E) survival was determined from BC life tables with matching for age and gender. Relative survival was calculated using a conditional approach and expressed as a standardized mortality ratio of O : E deaths.

Results: One thousand four hundred two pts were identified: 749 males (53%), median age 32 y, ADV 949 (68%). The median follow-up was 7.8 y (0.1–25.4 y). For LIM pts, 5-y risk of relapse from Year0 was 6.5% and <2% from Year2 (Table 1). For ADV pts, 5-y risk of relapse from Year0 was 23.7% but <5% by Year3 and comparable to LIM pts ($P = 0.07$). For ADV pts, the risk of relapse was inferior in high risk IPS ($n = 141$) pts at Year0 ($P = 0.002$) but those remaining event free at 1 y had a similar risk of relapse to low-risk pts ($P = 0.42$). The risk of relapse was similar by age categories. For all pts, the 5-y OS was 91.5% and DSS was 93.6%. Although 5-y survival improved as pts remained free of relapse, the relative survival did not normalize regardless of age, stage, RT era or IPS.

Conclusions: The 5-y risk of relapse for ADV cHL is <5% for pts remaining event free at 3 y and is unaffected by age. Thus, robust older pts should be treated with curative intent. Although the relative survival improves with duration of EFS, it remains inferior to the general population.

Abstract 176 Table 1 Five-year risk of relapse in cHL pts at diagnosis (Year0) and subsequent EFS time points

	5-y risk of cHL relapse % calculated from initial time point				
Group	**Year0**	**Year1**	**Year2**	**Year3**	**Year5**
All patients ($n = 1402$)	18.1 (16.1–20.2)	10.0 (8.4–11.7)	5.6 (4.3–7.1)	3.5 (2.4–4.9)	2.5 (1.5–3.9)
LIM stage ($n = 453$)	6.5 (4.4–9.0)	4.5 (2.7–6.8)	1.9 (0.8–3.9)	2.3 (1.0–4.5)	1.6 (0.5–3.9)
ADV stage* ($n = 949$)	23.7 (21.0–26.5)	12.9 (10.7–15.4)	7.6 (5.7–9.8)	4.1 (2.7–6.1)	3.0 (1.6–4.9)
IPS low ($n = 719$)	21.4 (18.4–24.5)	11.9 (9.5–14.6)	7.1 (5.0–9.5)	4.1 (2.5–6.4)	3.2 (1.7–5.6)
IPS high ($n = 141$)	34.0 (26.2–42.0)	16.8 (10.2–24.8)	8.3 (3.6–15.5)	4.0 (1.0–10.4)	1.6 (0.1–7.5)
Age 16–24 y	16.4 (12.7–20.5)	7.7 (5.0–11.0)	3.7 (1.8–6.4)	2.5 (1.0–5.2)	2.9 (1.0–6.3)
Age 25–44 y	18.3 (15.5–21.3)	10.4 (8.1–13.1)	4.9 (3.3–7.1)	2.6 (1.3–4.5)	1.4 (0.5–3.1)
Age 45–60 y	17.8 (13.2–22.9)	11.4 (7.5–16.1)	7.0 (3.9–11.3)	5.5 (2.6–9.9)	3.8 (1.4–8.2)
Age 61–69 y	23.3 (15.7–31.8)	11.5 (5.8–19.4)	12.8 (6.0–22.2)	7.4 (2.3–16.8)	4.9 (0.8–14.8)

Risk of relapse is expressed as a percentage (95% confidence intervals).
*89/1402 ADV pts unable to be assigned to low or high groups.

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HEALTHCARE UTILIZATION IN THE AETHERA TRIAL: PHASE 3 STUDY OF BRENTUXIMAB VEDOTIN IN PATIENTS AT INCREASED RISK OF RESIDUAL HODGKIN LYMPHOMA POST ASCT

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Introduction: AETHERA is a randomized, double-blind, phase 3 study of brentuximab vedotin and best supportive care (BSC) versus placebo and BSC in Hodgkin lymphoma (HL) patients at increased risk of relapse or progression post-autologous stem cell transplant (ASCT). Early consolidation post-ASCT with brentuximab vedotin demonstrated improved progression-free survival (PFS) per independent review compared with placebo (median PFS 43 vs 24 months; HR = 0.57, $p = 0.001$). The most common treatment-emergent grade ≥3 adverse events (AEs) were neutropenia (29% brentuximab vedotin vs 10% placebo), peripheral sensory neuropathy (10% vs 1%), thrombocytopenia (4% vs 3%), peripheral motor neuropathy (6% vs 1%) and anaemia (4% vs 2%). Treatment discontinuation due to AEs occurred in 33% vs 6% of patients, and 53 patients died on study (17% vs 16%). This analysis evaluated healthcare resource utilization (HRU) among patients on the two treatment arms of the AETHERA trial.

Methods: HL patients aged ≥18 years at high risk of residual HL post-ASCT, defined as a history of refractory HL, relapse or progression <12 months after frontline therapy or extranodal involvement at the time of pre-ASCT relapse, were eligible. Patients were randomized to receive brentuximab vedotin 1.8 mg/kg or placebo on day 1 of each 21-day cycle, for up to 16 cycles or until disease progression. The total number of hospitalizations, outpatient visits and missed days of work/other activities for patients/caregivers, occurring from time of informed consent up to 24 months after the first study treatment, was summarized by treatment group in the intent-to-treat population.

Results: A total of 329 patients (median age 32 years [range 18–76]; 53% male) were randomized to receive brentuximab vedotin ($n = 165$) or placebo ($n = 164$). There were 68 (41%) vs 61 (37%) patients with ≥1 hospitalization on the brentuximab vedotin vs placebo arms, respectively, with a total of 176 vs 198 hospitalizations. The hospitalization rate per patient-year was 0.58 (95% CI: 0.49, 0.67) vs 0.65 (95% CI: 0.56, 0.74). The median duration of stay was 16 vs 26 days per patient. There were 119 (72%) vs 133 (81%) patients with ≥1 outpatient visit, with a total of 2687 vs 3803 visits. The outpatient visit rate per patient-year was 8.84 (95% CI: 8.51, 9.18) vs 12.43 (95% CI: 12.03, 12.82). The most common reasons for hospitalization and outpatient visits were AEs and disease-related symptoms. There were 85 (52%) vs 94 (57%) patients with ≥1 missed day of work/other activities, with a median number of 15 vs 26 missed days. There were 7 (4%) vs 24 (15%) caregivers with ≥1 missed day of work/other activities, with a median number of 7 vs 16 missed days.

Conclusions: Preliminary results suggest a trend towards lower HRU with brentuximab vedotin compared with placebo. These data prompt further investigation of the economic impact of early consolidation post-ASCT with brentuximab vedotin in HL.

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SAFETY AND EFFICACY OF BENDAMUSTINE AFTER THE FAILURE OF BRENTUXIMAB VEDOTIN IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA: EXPERIENCE ON 27 PATIENTS

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Introduction. The optimal management of patients with heavily pretreated Hodgkin's lymphoma is controversial. Brentuximab vedotin is an active single agent in this context. Also bendamustine can be regarded as a safe and effective alternative for patients relapsing after autologous transplantation and as an interesting cytoreductive strategy prior to allogeneic transplantation.

Methods. This observational, multicentre, retrospective study involved 27 heavily pretreated patients with relapsed or refractory Hodgkin's lymphoma who all received brentuximab vedotin as their last treatment and who showed a subsequent disease progression, refractoriness or early relapse. Patients' median age was 32 (range 15–79) years. The median number of prior chemotherapy regimens was 5 (range 3–14), with 85% of patients showing resistance to autologous stem cell transplantation and 7% to allogeneic transplantation. Bendamustine was administered as a single-agent therapy on an outpatient basis at the standard dose of 90 mg/m^2 (days +1 and +2 of a 28-day based cycle, up to 6 cycles). The primary study endpoint was the objective response rate, and the secondary endpoint was the safety of the bendamustine regimen.

Results. The overall response rate was 55.5%, with 10/27 (37.0%) patients obtaining a complete response. In comparison, the overall response rate previously observed with brentuximab vedotin in the same subset of patients was quite lower (18.5%). Among the 10 patients in complete response after bendamustine, only one was a complete responder to brentuximab, with 2 being partial responders and 7 showing stable/progressive disease. With a median duration of response of 8 months, all of these patients are maintaining a continuous response. The treatment was well tolerated, with rather infrequent adverse events and transient and manageable toxicities.

Conclusions. Albeit with the limits of an observational retrospective study, these data indicate that bendamustine shows its efficacy in patients already treated with brentuximab vedotin, regardless of their previously obtained response and without any significant toxicity.

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HODGKIN LYMPHOMA POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (HL-PTLD): A COMPARATIVE ANALYSIS OF PROGNOSIS AND SURVIVAL

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Background: HL-PTLD is a rare subtype of PTLD that carries an unknown prognosis. Further, little is known regarding the characteristics of HL-PTLD, and the optimal treatment is not well defined.

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Methods: Patients (pts) diagnosed with HL-PTLD from 1999 to 2011 were identified in the Scientific Registry of Transplant Recipients, a prospective database of solid organ transplant (SOT) recipients. Additionally, non-PTLD HL pts ($n = 13\,847$) were identified in SEER. We compared HL-PTLD and HL-SEER pts exactly matched on age, sex, and year (yr) of diagnosis.

Results: We identified 192 HL-PTLD pts. Compared with HL-SEER pts, HL-PTLD pts were older (38 vs 51 yrs, respectively, $p < 0.04$), more likely male (54% vs 73%, respectively, $p < 0.001$) and had more extranodal disease (3% vs 42%, respectively, $p < 0.001$). Median OS for the HL-PTLD cohort was 88 months and 5-yr OS was 56%. In the exactly matched cohort, 5-yr OS for HL-PTLD ($n = 179$) was significantly inferior compared with HL-SEER pts ($n = 1244$; 57% vs 80%, respectively, $p < 0.001$). Furthermore, the aHR of death for HL-PTLD compared with HL-SEER pts remained increased on multivariable analysis (MVA) controlling for age, sex, race, extranodal disease and yr of diagnosis (2.38, 95% CI 1.79–3.15). In addition, 10-year disease-specific survival (DSS) was inferior for HL-PTLD vs HL-SEER pts (76% vs 82%, respectively, $p < 0.001$). Treatment (Tx) data were available for 173 HL-PTLD pts. Seventy-five per cent had reduction in immune suppression (RIS) as part of Tx; 16% had RIS + radiation (RT), 14% RIS + rituximab, 60% RIS with chemotherapy (Ctx) and 24% of pts received Ctx without RIS. For Ctx, 20% of pts received ABVD or ABVD-like therapy and 13% had other HL-like Ctx regimens (e.g. Stanford V, MOPP and BEACOPP); 18% received CHOP and 24% had 'other' non-traditional HL Ctx. Factors associated with inferior OS for HL-PTLD pts on univariate analysis were advanced age (HR 1.30/decade, 95% CI 1.15–1.45), heart transplant (HR 1.63, 95% CI 1.02–2.61) and increased baseline creatinine (Cr; HR 1.78 per 0.1 gm/dL increase, 95% CI 1.29–2.44). On MVA, age (aHR 1.26/decade, 95% CI 1.10–1.45) and elevated Cr (1.68/0.1 gm/dL, 95% CI 1.15–2.46) remained significant. In terms of Tx, use of any Ctx was associated with improved OS (aHR 0.54, 95% CI 0.32–0.90). By type of Ctx, OS appeared inferior for pts who received CHOP or 'other' Ctx (HR 2.21, 95% CI 1.11–4.39 and 2.07, 95% CI 1.10–3.91, respectively). On MVA, there was no statistical difference for CHOP vs HL-specific regimens (aHR: 1.66, 95% CI 0.81–3.42); however, risk of death remained increased for pts who had 'other' cytotoxic Tx or if treated without Ctx (aHR 2.01, 95% CI 1.06–3.82 and 2.78, 95% CI 1.43–5.40, respectively). Tx with RIS, RT or Rtx had no impact on OS on MVA.

Conclusions: Compared with HL-SEER pts, HL-PTLD pts were older, more likely male and had more extranodal disease. Furthermore, HL-PTLD pts had significantly inferior OS and DSS. We also identified several clinical factors that identified HL-PTLD pts with markedly divergent outcomes. In addition, therapy without Ctx appeared insufficient, and moreover, treatment with HL-specific Ctx resulted in the most optimal outcomes.

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THE RELAPSE TIME AFTER AUTOLOGOUS TRANSPLANT OR REFRACTORINESS DO NOT IMPACT THE SURVIVAL OF HODGKIN'S LYMPHOMA TREATED WITH ALLOGENEIC TRANSPLANT

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Introduction: Relapsed/refractory Hodgkin's lymphoma (RR-HL) has few options of cure. We analysed the RR-HL patients referred to our centre to understand whether the timing of relapse after autologous transplant (autoSCT) or refractory disease may change the survival after allogeneic transplant (alloSCT).

Methods: The multivariate analysis included as covariates the disease pretransplant status (CR vs PR vs SD or PD), donor (HLA identical sibling, unrelated or haploidentical) and time of relapse after autoSCT/refractoriness (relapse <12 months after autoSCT or primary refractory vs relapse >12 months after autoSCT).

Of 221 patients referred to our centre with HL, 105 were RR-HL. Sixty-three (60%) RR-HL patients received alloSCT, 41 patients (40%) did not receive alloSCT for progressive disease (29), advanced age (3) or complete response after autoSCT after third-line chemotherapy (9 pts). Donors were HLA identical siblings in 38%, matched unrelated in 35% and haploidentical donors in 27% of patients. At alloSCT median age was 33 (range 17–60) years, and 43% of patients were in CR, 30% in PR and 27% in SD or PD. Eighty-three per cent of patients had relapsed <12 months from autoSCT or with primary refractory disease, 17% had relapsed >12 months after autoSCT.

Results: Median follow-up was 5.0 years (range 0.5–10.8). Overall survival (OS) was 60% at 3 years and 55% at 5 years of follow-up. In multivariate analysis, disease status before alloSCT significantly impacted OS ($p = 0.003$, HR = 1.6, CI 95% 1.2–2.2), whereas donor and timing or relapse/refractoriness did not change OS ($p = 0.164$, HR = 1.3, CI 95% 0.9–2.0, and $p = 0.587$, HR = 1.2, CI 95% 0.6–2.5, respectively). Progression-free survival (PFS) was 43% at 3 years and 43% at 5 years. Pretransplant disease status impacted PFS ($p < 0.001$, HR = 1.6, CI 95% 1.2–2.1), which was not influenced by donor ($p = 0.349$, HR = 0.8, CI 95% 0.6–1.2) and timing of relapse/refractoriness ($p = 0.912$, HR = 1.0, CI 95% 0.5–1.9). Relapse incidence was 38% at 3 years and 38% at 5 years of follow-up. Relapse was impacted by donor (0.033, reduced risk for MUD donors, HR = 0.5, CI 95% 0.3–0.9) and pretransplant disease status ($p = 0.003$, HR = 1.7, CI 95% 1.2–2.4), whereas timing of relapse/refractoriness did not change relapse incidence ($p = 0.591$, HR = 0.8, CI 95% 0.3–1.8). NRM was 10% at 100 days, 13% at 6 months, 18% at 1 year and reached a plateau of 20% at 2 years of follow-up. NRM was not impacted by the factors analysed (donor $p = 0.158$, pretransplant status $p = 0.134$ and refractoriness $p = 0.327$).

Conclusions: Long-term PFS and OS for RR-HL after alloSCT is 42% and 55%. Disease pretransplant status and not the time of relapse after autoSCT or refractory disease impact OS and PFS. All the efforts should be done to obtain the deepest response before alloSCT, irrespective from time of relapse after autoSCT or refractoriness, to achieve the best results from it.

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FIRST IN-PATIENT PROOF OF SAFETY AND EFFICACY OF A 4TH GENERATION CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS FOR THE TREATMENT OF R/R CD30-POSITIVE LYMPHOMA

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Introduction: Many lymphoma patients cannot be cured by standard chemoradiotherapy. CD30 is expressed in Hodgkin's lymphoma (HL), anaplastic large cell lymphoma (ALCL), diffuse large B-cell lymphoma and peripheral T/NK cell lymphoma. Brentuximab (SGN-35), an antibody-drug against CD30, has been approved by US Food and Drug Administration (FDA) for the treatment of relapsed or refractory classical HL and systemic ALCL. However, SGN-35 is not available or approved in many countries. Nevertheless, CD30 represents an attractive target for chimeric antigen receptor (CAR)-based immune cell therapy. This study reports the safety and efficacy of a 4th generation CAR T cell treatment for the management of relapsed and refractory CD30 positive lymphomas (www.clinicaltrials.gov; #NCT02274584).

Methods: Lymphoma patients with relapsed or progressive CD30 positive disease are recruited. T cells are transduced with lentiviral CAR containing anti-CD30-scFv and T cell signalling domains including CD28/CD137/CD27 and CD3zeta. The CAR is fused with an apoptosis-inducing gene, FKBP-caspase 9 (iCasp9), to establish a safety-improved CAR (4S-CAR). CAR T cells and cytokines in blood are detected by quantitative PCR and ELISA, respectively.

Results: A 22-year-old man, diagnosed with stage III HL (nodular sclerosis, NS) in December 2011, had been heavily treated with three lines of chemotherapy and autotransplantation. The patient relapsed in May 2014 and has been enrolled in this study. He received a conditioning regimen of three daily doses of fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 one week before CAR T infusion.

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The total cell number infused was 3.2×10^8, of which 5% were CAR-positive. There were no infusion-related toxicities. Thirty-five days and 2.5 months after infusion, CT scan showed resolution of multiple tumour nodules, which indicated partial remission. However, 5 months after infusion, disease slowly progressed based on CT scan. The CAR T cells peaked on day 45 accounting for >20% of circulating lymphocytes. Peak levels of interferon-γ and interleukin-6 were detected around day 40 coincided with peak CAR T detection.

Conclusions: We demonstrate for the first time the safety and efficacy of CD30 4S-CAR T cells in a heavily treated, relapsed late stage CD30 positive HL patient. Compared with leukaemia and other subtypes of lymphoma, HL has unique pathological characteristics. The NS subtype is the most common HL characterized by dense bands of collagen fibrosis and an overt immunosuppressive tumour niche. Such feature may result in the difficulty of CAR T cells to penetrate into the tumour mass. We are designing new treatment regimens to overcome this obstacle. Expansion of patient cohort and long-term follow-up are in progress.

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CHRONIC LYMPHOCYTIC LEUKAEMIA TREATMENT AND OUTCOME IN THE UK'S POPULATION-BASED HAEMATOLOGICAL MALIGNANCY RESEARCH NETWORK

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Methods: One thousand thirty patients newly diagnosed in 2005–2009 were followed until 2014. Demographic, prognostic, treatment and outcome were analysed using standard statistical methods; relative survival (RS) was estimated using UK national life tables.

Results: With a median age of 71 years (range 26–97), 62% of patients were male, 88% had an ECOG score of 0–1, 14% had B-symptoms, 76% were Binet stage A and 50% were in the low Rai group. With respect to initial treatment, 83% were managed by watch and waited for 6 months or more and 12% by chemotherapy (Table 1). The latter group were more likely to have B-symptoms (45%), advanced disease (Binet stage C = 46% and Rai high = 53%) and be tested (79%) for a del17p mutation; 25% of those tested had the deletion.

Median survival was 8.1 years; 1 and 5-year overall survival (OS) were 89% and 64%, respectively. Corresponding 1 and 5-year RS estimates were 95% and 81%. Age was strongly predictive of outcome; 5-year OS estimates were 91% in those <60 years ($n = 190$) and 58% in those ≥60 years ($n = 838$); corresponding 5-year RS estimates being 93% and 76%, respectively.

For the 311 patients treated with chemotherapy, initially ($n = 121$) or after observation ($n = 190$), 5-year OS and RS estimates were 49% and 62%, respectively (Table 1). Survival was poorest for patients treated with chlorambucil, but this group was older, and many of the deaths were not solely attributable to CLL, 5-year OS and RS being 27% and 40%, respectively. By contrast, 5-year OS and RS for those treated intensively were more closely aligned.

Median progression-free survival in patients treated with chemotherapy was 2.9 years, was shortest for those treated with chlorambucil (1.5) and longest for R-FC (4.4). Ninety-five (31%) of those treated with chemotherapy went on to receive second-line chemotherapy, median survival from this point being 2.7 years. No variations by regimen were detected.

Conclusions: Analysis of data from our unselected UK population-based cohort demonstrate that, outside trials, survival from CLL is generally very good; with a 5-year RS >80%. This is especially marked in younger patients, as well as those on watch and wait, where only 20% went on to chemotherapy. Older patients, generally treated with chlorambucil, had the worst survival; but among those treated more intensively, no survival differences were detected by chemotherapy regimen.

Abstract 182 Table 1 Disease management, age and outcome

	Total	Median age (years)	1-year survival (%)		5-year survival (%)	
			Overall	Relative	Overall	Relative
Total	1030	71.4	88.8	95.2	64.1	81.3
First-line management						
Watch-and-wait	853	70.9	94.8	99.5	69.5	86.9
Chemotherapy	121	69.8	88.5	92.0	55.5	71.9
Palliative approach	55	81.9	0.0	0.0	—	—
Chemotherapy[a]	311	70.2	87.2	90.9	49.2	62.2
Chlorambucil	110	78.2	78.7	83.5	26.8	39.7
Fludarabine cyclophosphamide (FC)	97	62.0	90.7	92.4	63.7	71.0
Fludarabine cyclophosphamide + rituximab (FCR)	59	64.3	92.9	95.2	68.1	72.2
Chlorambucil + rituximab (R-chlorambucil)	18	68.2	100	100	69.0	73.8

[a]Includes 121 patients treated at first line and 190 patients initially on watch and wait for 6 months or more. Age is at the start of chemotherapy, and survival is calculated from this point.

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RESPONSE TO RITUXIMAB IN B-CLL PATIENTS IS ADVERSELY IMPACTED BY FREQUENCY OF IL-10 COMPETENT B-CLL CELLS AND FCGR3A POLYMORPHISM. A STUDY OF FCGCLL/MW AND GOELAMS GROUPS

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Introduction: Rituximab has dramatically improved outcome of chronic lymphocytic leukaemia patients (CLL). Its *in vivo* mechanisms of action remain unknown, but some factors influencing response to rituximab have been described such as polymorphism of the low affinity immunoglobulin gamma Fc region receptor III-A (CD16a and *FCGR3A*). In murine model, regulatory B-cells (B10) have been described to inhibit lymphoma cells clearance induced by anti-CD20 monoclonal antibodies through IL-10 negative regulation of monocyte Fc-mediated functions. A sub-population of CLL B-cells has a capacity to secrete IL-10 and could therefore influence rituximab activity in CLL patients. The goal of this work was to evaluate the role of IL10 competent B-CLL cells and *FCGR3A* polymorphism on *in vivo* rituximab activity in CLL patients receiving a pre-phase of high dose of rituximab before conventional immune-chemotherapy.

Methods: Untreated CLL patients received a pre-phase of rituximab (D0: 500 mg, D1, 8, 15: 1000 mg) before conventional immuno-chemotherapy (6xFCR, Protocol CLL2010-FMP, NCI number: NCT01370772). IL-10 competent CLL cells, IL-10 plasma concentration and FcγRIIIa-158VF polymorphism were assessed before treatment. Rituximab pre-phase efficacy was defined by lymphodepletion >90% at D22, before the beginning of immuno-chemotherapy. Response to treatment was evaluated 3 months after the end of immuno-chemotherapy according to international criteria.

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Results: No significant correlation was found between 90% lymphodepletion and clinical parameters, such as age, sex, Binet stage, ECOG, IGHV mutation, cytogenetic abnormalities (del11q, del13q and trisomy 12) or β2-microglobulin. B10 CLL cells frequency before treatment correlated statistically with IL-10 plasma level ($p = 0.017$; $r = 0.397$). Univariate analysis showed a statistically significant correlation between B10 CLL cells frequencies and 90% lymphodepletion ($p = 0.004$). B10 CLL cell frequency was also found to correlate with clinical response assessed 3 months after [(complete response (CR) vs non-CR, $p = 0.04$). FcγRIIIa-158V/F polymorphism was significantly associated with 90% lymphodepletion ($p = 0.01$). This was also found for FcγRIIIa-158V carriers ($p < 0.05$). FcγRIIIa-158V/F polymorphism failed, however, to correlate with clinical response 3 months after FCR. In multivariate analysis B10 cell frequency ($p < 0.01$) and FcγRIIIa-158VF ($p < 0.05$) were the only prognostic factors associated with lymphocyte depletion induced by rituximab alone (AUC = 0.855; 95% CI: 0.732–0.978).

Conclusions: Our results strongly suggest that IL-10 competent B-CLL cells inhibit rituximab-mediated antibody-dependent cellular cytotoxicity by macrophages, this effect being also influenced by *FCGR3A* polymorphism.

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RESULTS OF A PHASE II RANDOMIZING INTENSIFIED RITUXIMAB PRE-PHASE FOLLOWED BY STANDARD FCR VS STANDARD FCR IN PREVIOUSLY UNTREATED PATIENTS WITH ACTIVE B-CLL

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Introduction: Rituximab dosing regimen is largely empirical in B-CLL patients. Pharmacokinetics data from REACH study, randomizing FCR and FC for relapsed/refractory $CD20^+$ B-CLL, showed a correlation between rituximab exposure evaluated by AUC and $C_{through}$ and clinical response. We proposed to intensify rituximab regimen before the beginning of FCR in untreated fit patients with active B-CLL.

Patients and Methods: A cohort of medically fit patients (cumulative illness rating scale (CIRS) score of up to 6), less than 65 years old, without 17p deletion, were enrolled between July 2012 and October 2013. Patients were stratified according to *IGVH* mutational status and 11q deletion. They were randomly assigned to receive either intensified pre-phase of rituximab: 500 mg total dose at D0 (250 mg D-1, 250 mg D0 if leucocyte > 25 G/L), 2000 mg total dose at D1, D8 and D15 followed by 6 cycles of FCR (F 40 mg/m^2 PO d1–3 and C 250 mg/m^2 PO d1–3; R: 500 mg/m^2 d1 q 28 days: R-dense arm) or 6 cycles of standard FCR. Adverse events (AEs) were assessed per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 3.0). The primary endpoint was the rate of complete response (CR + Cri) according to IWCLL 2008 criteria associated with undetectable minimal residual disease (uMRD; $<10^{-4}$ by 8 colours FC assay) in peripheral blood and bone marrow 3 months after the end of treatment.

Results: One hundred forty patients with active B-CLL were included in this randomized phase II trial. Three patients were excluded for analysis for consent withdrawal: ($n = 1$); haemolytic anaemia ($n = 1$) or AgHbS ($n = 1$). A total of 137 patients were analysed for response (ITT), 68 patients in R-dense arm, 69 in standard arm. Patients' characteristics were well balanced between the two arms. Toxicity of rituximab intensified pre-phase was reduced with only one patient experiencing IRR grade ≥3, six and 12 patients experiencing neutropenia grade ≥3 at D8 and D15, respectively. Lymphocyte count decreased as soon as D8 with 31%, 51% and 65% of patients with lymphocyte count lower than 5.0 G/L at D8, D15 and D22, respectively. Six of 55 patients evaluable for MRD after rituximab-intensified pre-phase had less than 1% of clonal cells in peripheral blood. Toxicity of FCR was not statistically different between the two arms with 31% and 26.5% of neutropenia grade ≥3 for R-dense arm and standard arm, respectively. ORR were 96% (CR + Cri: 56%, PR + PRn: 40%) and 93% (CR + Cri: 55%, PR + PRn: 38%) in R-dense arm and standard-arm, respectively. The rate of CR + Cri with uMRD in marrow and peripheral blood was 26.5% and 24.6% for R-dense arm and standard arm, respectively.

Conclusion: Intensified rituximab pre-phase is safe. Intensified rituximab pre-phase followed by FCR did not allow to increase CR + Cri with uMRD rate compared to standard FCR.

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A RANDOMIZED STUDY OF F(C)R IN FIT, ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) SHOWS HIGH RESPONSE RATES AND A DOSE INTENSITY EFFECT

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Introduction: Fludarabine (F), cyclophosphamide (C) and rituximab (R) gave superior progression free (PFS) and overall survival (OS) vs FC in the CLL8 Study. We aimed to assess the safety, tolerability and efficacy of FCR-based therapy in elderly patients (pts).

Methods: Previously untreated pts with progressive CLL aged ≥65 years were randomized to one of 3 therapy arms: (i) *FR5*: F 24 mg/m^2 po D1–5 + R (375 mg/m^2 cycle 1 and 500 mg/m^2 cycles 2–6) iv D1, (ii) *FCR3*: F 24 mg/m^2 po and C 150 mg/m^2 po D1–3 + R iv D1 or (iii) *FCR5*: F 24 mg/m^2 po + C 150 mg/m^2 po D 1–5 + R iv D1 all at 4 weekly intervals for an intended 6 cycles. Cycles could be delayed up to 2 weeks for grade 3+ toxicity.

Results: The eligible cohort was 116 pts with median age was 71 (range 65–82) years. Response and grade 3+ toxicity are shown. All 6 protocol cycles were completed in 69% but less on FCR5 44% vs FR5 89% and FCR3 76% ($p < 0.001$). FCR3 vs FR5 was not statistically significant (NSS). At 18 months, PFS was FR5 65%, FCR3 75% and FCR5 65% and OS 97%, 90% and 83%, respectively (NSS). Early cessation due to toxicity was more common with FCR5 ($p < 0.001$). Eleven over thirteen stopped early due to toxicity received ≥3 cycles of therapy (mean 3.5). Intention to treat full dose FCR5 CR + MRD rates were significantly higher (79%), but had higher CRi, toxicity and earlier cessation of therapy.

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Response 2 months post-Rx or at Rx end	Treatment arm			Total (*N* = 116)
	FR5 (%) (*N* = 37)	FCR3 (%) (*N* = 41)	FCR5 (%) (*N* = 38)	
Complete remission (CR) BM confirmed	10 (27)	18 (44)	17 (45)	45 (39%)
CR (bone marrow confirmed)	9 (24)	13 (32)	8 (21)	30 (26%)
CR-i (BM confirmed)	1 (3)	5 (12)	9 (24)	15 (13%)
Total MRD negative in PB	14 (38)	21 (51)	30 (79)	65 (56%)
Nodular partial remission (nPR)	11 (30)	13 (32)	3 (8)	27 (23%)
Partial remission (PR)	10 (27)	5 (12)	4 (11)	19 (16%)
Stable disease (SD)/ PD/early death	2 (6)	2 (4)	1 (3)	5 (5%)
Overall response rate (ORR)	35 (95)	39 (95)	37 (97)	111 (96%)
At least 1 grade 3+ AE	21 (57)	34 (83)	35 (92)	90 (78%)
Early cessation due to toxicity	2 (5.6)	1 (2.4)	13 (34)	16 (14%)

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Conclusions: Oral FCR therapy is generally safe and well tolerated in elderly CLL. Toxicity was mostly haematological and manageable. Response rates were very high. Full-dose FCR is highly effective, and while early cessation due to toxicity occurs, a dose intensity effect appears to exist.

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TARGETING MUTATED BRAF WITH VEMURAFENIB IS SAFE AND HIGHLY ACTIVE IN RELAPSED/REFRACTORY HAIRY CELL LEUKEMIA: A PHASE-2 ITALIAN CLINICAL TRIAL (HCL-PG01)

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Background: Purine analogues (PA) are effective in hairy cell leukaemia (HCL), but ~40% of patients relapse and respond progressively less well to PA, which can also cause cumulative myelotoxicity. Our discovery of the BRAF-V600E kinase-activating mutation as the genetic lesion underlying HCL opens the way to a targeted therapy with BRAF inhibitors.

Methods: We performed the first clinical trial with the BRAF inhibitor vemurafenib in refractory/relapsed HCL. This is a phase-2, academic, single-arm, Italian, multicentre study (EudraCT 2011-005487-13). Over 11 months, 8 centres enrolled 28 patients requiring treatment due to cytopenias, who were refractory to ($n = 6$) or early relapsed after ($n = 21$) a PA, or had severe toxicity from a PA ($n = 1$). Previous treatments also included interferon, rituximab and splenectomy in 13, 14 and 8 patients, respectively (Table).

Results: At the oral dose of 960 mg twice daily for a median of 16 weeks, vemurafenib was overall well tolerated. Most frequent drug-related adverse events were arthralgias, skin toxicities and pancreatitis, usually of grades 1–2. Vemurafenib was not myelotoxic. We observed no cutaneous squamous cell carcinomas/keratoachanthomas (as frequently reported in BRAF-V600E+ melanoma patients treated with vemurafenib), but 2 patients developed skin basaliomas and 1 patient a cutaneous superficial melanoma, all treated with simple excision.

In 26 evaluable patients, the overall response rate was 96%, 9/26 (34.6%) CRs and 16/26 (61.4%) PRs, obtained after a median of 8 weeks (Table). With a median follow-up of 18 months post-treatment, the median relapse-free and treatment-free survivals: (i) were not reached in CR patients (with 5 and 6 of them being relapse- and treatment-free, respectively, at 18 months); and (ii) were 7 and 18 months, respectively, in PR patients (Table). In all patients (including those in CR), immunohistochemistry showed residual HCL cells. The latter exhibited a persistent phospho-ERK expression at the end of treatment in 6/13 evaluable cases, which correlated with a shorter event-free survival (median of 3 months; $p = 0.004$) and suggests by-pass reactivation of MEK-ERK as a resistance mechanism. Upon retreatment with vemurafenib, 3/3 patients relapsing after CR responded again (1 CR, 1 PR and 1 PR currently at mid-retreatment), whereas 4/5 patients relapsing after PR had a minor response and only 1/5 a second PR.

Conclusions: A brief oral treatment with vemurafenib was safe and proved highly and rapidly active in heavily pre-treated HCL patients. Retreatment with vemurafenib was more effective in patients relapsing after CR than after PR.

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Pt.	Sex	Age	Previous line of therapy	Response to vemurafenib	Week of treatment until best response	Total	Relapse-free survival[c] (in months)[f]	Treatment-free survival (in months)
1	M	44	DCF, IFN, CDA, IFN, RTX, IFN	PR	8	20	6	7
2	M	76	DCF, DCF, CDA	CR	8	20	12	18
3	M	58	IFN, RTX, CDA	PR	12	20	3	8
4	F	52	Splenectomy, 2CDA][a], DCF + RTX	PR	16	16	8	8
5	F	45	CDA[a]	PR	16	16	21+	21+
6	F	81	DCF[b]	PR	12	16	15	21+
7	M	27	CDA[a], splenectomy	PR	8	16	10	11
8	M	77	Splenectomy, IFN, CDA, IFN; CDA, RTX, RTX	CR	8	8	5	9
9	M	57	IFN, IFN, DCF, CDA, CDA, IFN, DCF + RTX	MR	16	16	—	4
10	M	68	Splenectomy, CDA, DCF, CDA, IFN, CDA	PR[d]	12	12	12	18
11	M	49	DCF, CDA, CDA, RTX, IFN, CDA	CR	12	12	9	21+
12	M	57	CDA, CDA, CDA, DCF	PR	4	16	3	18+
13	M	71	DCF, 2CDA, RTX, DCF, RTX, CDA	CR	8	8	21+	21+
14	M	70	Splenectomy, IFN, CDA, RTX	PR	8	16	5	5
15	M	80	CDA + RTX, RTX, splenectomy, CDA	Not evaluable[e]				—
16	M	84	IFN, DCF, CDA, CDA, IFN	CR	4	8	18+	18+
17	M	50	CDA, CDA	PR	16	16	3	14+
18	M	43	CDA, RTX	PR	4	16	3	7+
19	M	52	CDA[a], IFN	PR	4	16	3	18+
20	M	51	CDA, CDA	PR	8	16	9	14
21	M	38	CDA, CDA	CR	4	8	21+	21+
22	M	67	IFN, CDA, CDA+RTX	CR	8	8	21+	21+
23	F	39	DCF[a]	CR	4	8	12	18
24	M	39	CDA	CR	5	8	19+	19+
25	M	57	IFN, CDA, CDA, CDA, IFN	PR[d]	8	16	16+	16+

(*Continues*)

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Abstract 186 Table (Continued)

Pt.	Sex	Age	Previous line of therapy	Response to vemurafenib	Week of treatment until best response	Total	Relapse-free survival[c] (in months)[f]	Treatment-free survival (in months)
26	M	68	Splenectomy, IFN, IFN, IFN, DCF, DCF, IFN, CDA, RTX, CDA, CDA, CDA	PR	10	14	8	16+
27	M	72	CDA, CDA+RTX	Not evaluable[e]				—
28	M	56	IFN, CDA[a], DCF, RTX, splenectomy	PR	8	14	3	19+
				Median	8	16	9	Not reached

DCF, pentostatin; IFN, interferon; 2CDA, cladribin; RTX, rituximab.
CR (complete response): normal blood counts (N ≥1500/mmc and PLT ≥100 000/mmc and Hb ≥11 g/dl), no splenomegaly and no HCL cells in the bone marrow biopsy by morphology.
PR (partial response): normal blood counts, ≥50% reduction of splenomegaly and ≥50% reduction of HCL infiltration in the bone marrow biopsy by immunohistochemistry.
MR (minor response): ≥50% improvement in any cytopenias or ≥25% reduction of splenomegaly or ≥50% reduction of HCL infiltration in the bone marrow biopsy.
[a]Primary refractory.
[b]Severe septic arthritis after DCF.
[c]N <1500/mmc and/or PLT <100 000/mmc and/or Hb <11 g/dl.
[d]With delayed platelet recovery.
[e]Off-study after ≤1 week of therapy for drug-unrelated acute myocardial infarction (pt. 15) or for consent withdrawal due to drug-related, reversible, grade 3 pancreatitis (pt. 27).
[f]From the end of treatment.

INDOLENT LYMPHOMAS

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THE CLINICAL COURSE OF PATIENTS WITH FOLLICULAR LYMPHOMA IN THE RITUXIMAB ERA

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Introduction: The clinical course of patients with follicular lymphoma (FL) was last described in 1995, before novel agents were introduced into standard care. The aim of this study was to describe the effect most recent therapeutic advances have had on the pattern of relapse in patients with FL.

Methods: Between 1997—the date when rituximab was introduced in our institution—and 2012, 235 patients (female, 53%; median age: 57 years—range: 24–89; stages III–IV: 68%) were diagnosed with grades 1–3a FL at St Bartholomew's hospital and constituted the study group. We analysed the response and relapse rates and the duration of remission from best response, in addition to the overall survival (OS). Survival analysis and duration of remission were performed by the Kaplan–Meier method, and the Cox regression test was used to test for significant associations.

Results: One hundred and sixteen patients were managed expectantly at diagnosis, whereas the remainder received immediate treatment (28 CHOP ∓ rituximab, 55 chlorambucil ∓ rituximab; 21 radiotherapy; 10 fludarabine based, 2 bendamustine based and 3 single-agent rituximab). After a median follow-up of 8 years (range: 1.0–17), 41 patients never required treatment. High-dose therapy with autologous stem cell rescue (HDT-ASCR) was performed to consolidate response in 11 patients in first remission, in 19 at second remission and 1 in third remission. One hundred and thirty-seven patients received rituximab at some point as part of their treatment (91 as part of the initial treatment and 46 as part of subsequent treatments), while 98 have never received rituximab. The 5-year and 10-year OS and for the whole group were 82% (95% CI: 76–86) and 66% (95% CI: 58–72), respectively. Response rates, the median duration of remission from best response, the median survival from best response and relapse rate are in the table below.

Conclusions: In contrast with the previous description of the clinical course in patients with FL in the pre-rituximab era, we did not observe a progressive substantial shortening of the response duration and survival with each subsequent relapse. This demonstrates that the introduction of new therapeutic options impacts not only on the outcome of patients but can also alter the clinical course of the disease.

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EVENT-FREE SURVIVAL AT 12 MONTHS AND SUBSEQUENT OVERALL SURVIVAL IN PATIENTS WITH FOLLICULAR LYMPHOMA MANAGED IN THE RITUXIMAB ERA

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Introduction: Recent advances in follicular lymphoma (FL) have resulted in a prolongation of overall survival (OS). In this study, we assessed if landmark time points of event-free survival (EFS) can inform subsequent OS in FL.

Methods: Nine hundred and twenty newly diagnosed patients with grades 1–3a FL enrolled in the University of Iowa/Mayo Clinic Lymphoma SPORE Molecular Epidemiology Resource (MER) from 2002 to 2012 were evaluated. EFS was defined as time from diagnosis to progression, relapse, retreatment or death due to any cause with EFS12 defined as EFS status 12 months after date of diagnosis. OS was compared to age and sex matched survival in the general US population using standardized mortality ratios (SMR), which is the ratio of observed deaths in the study population versus the number expected in the reference population, and 95% confidence intervals (CI). Replication was performed in a cohort of 412 patients with FL from two Lyon, France, hospital registries with OS compared to French population data.

Abstract 187 Table 1 Response rate, duration of remission, relapse rate and survival for each event

	Number of patients treated	Patients treated with rituximab (%)	Response rate (%)	Median duration of best response	Relapse rate (%)	Median survival from best response
Presentation	194	47	92	5.5 years	50	Not reached
1st relapse	80	51	80	7.5 years	42	Not reached
2nd relapse	26	19	69	Not reached	27	5.5 years

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Results: Median age at enrollment was 60 years (range 23–93) and 52% were male. The most common initial management approaches were observation ($n = 326$, 33%), rituximab monotherapy (RM, $n = 111$, 12%) and immunochemotherapy (IC, $n = 349$, 8%). At a median follow-up of 71 months (range 5–144), 83% achieved EFS12 and 130 patients (14%) died. At the time of initial diagnosis, FL patients had a modest decrease in OS compared to the age and sex matched general population (SMR = 1.14, 95% CI: 0.96–1.36, $p = 0.12$) with good outcome in patients managed with observation (SMR = 0.83, 95% CI: 0.60–1.14, $p = 0.24$) or RM (SMR = 0.64, 95% CI: 0.34–1.19, $p = 0.16$). Patients initially treated with IC had inferior survival (SMR = 1.83, 95% CI: 0.96–1.36, $p = 5.4 \times 10^{-6}$). Patients achieving EFS12 had excellent subsequent OS (SMR = 0.73, 95% CI: 0.57–0.94, $p = 0.014$) including those initially treated with IC (SMR = 0.73, 95% CI: 0.46–1.16, $p = 0.18$). Conversely, failing to achieve EFS12 was associated with poor OS (SMR = 3.72, 95% CI: 5.70–13.4, $p = 2.5 \times 10^{-21}$), most strongly in IC-treated patients (SMR = 17.63, 95% CI: 12.4–35.3, $p = 1.9 \times 10^{-57}$). Results replicated in the Lyon cohort with FL patients achieving EFS12 having subsequent SMR = 1.02, 95% CI: 0.62–1.66, $p = 0.95$ and FL patients failing to achieve EFS12 having subsequent SMR = 8.74, 95% CI: 5.70–13.4, $p = 2.9 \times 10^{-23}$.

Conclusions: Prognostic reassessment at 12 months post-diagnosis further defines those who are event free to have excellent prognosis, independent of initial prognostic indices or management. EFS12 has important implications for clinical practice, patient counselling and predicting long-term clinical trial outcome at an early time point after diagnosis.

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FOLLICULAR LYMPHOMA AND CLINICAL CHARACTERISTICS OF HISTOLOGIC TRANSFORMATION

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Background and objectives: Histologic transformation (HT) of follicular lymphoma (FL) is possible during the natural course of the tumour. HT rates vary in published series between 10% and 60%, probably due to methodological differences between reports, disparities within study populations, inconsistent HT definitions and varying diagnostic methods. The purpose of this study is to register the frequency of HT in the more aggressive lymphomas in the large series of patients with follicular lymphomas treated by the Spanish Lymphoma Oncology Group (GOTEL).

Materials and methods: Clinical characteristics of a large number of patients obtained prospectively were analysed to identify significant prognostic and therapeutic features. Data such as age, sex, stage of the disease (Ann Arbor), histologic grade, B symptoms, number of affected lymphatic chains, number of extralymphatic sites affected, bone marrow infiltration, performance status, presence of bulky disease and detection of hepatitis C virus as well as lactate dehydrogenase, b2 microglobulin, albumin and standard blood tests were obtained upon diagnosis. International prognostic indexes (IPI) and FLIPI (poner primero qué significa y la sigla en parenthesis) for FL were also recorded. Comparisons were established using a chi square.

Results: The study population included 1076 patients with a diagnosis of FL and a median follow-up of 54 months. The incidence of HT in our series was 3%, seen mainly in patients with diffuse large-cell B lymphoma. Median follow up was 54 months (range 0,1–678) in the general series and 58 (range 2–418) in the HT cohort ($p = 0.37$). Multiple clinical characteristics were analysed to determine their HT prediction value but were not significant. In terms of treatment modality, neither receiving anthracyclines nor observing influenced subsequent transformation. Twenty-three per cent of the general population has died during follow-up, whereas patients in the HT group have had a mortality rate of 49% ($p = 0.002$).

Conclusions: In our series, the incidence of HT is 3%. In our experience clinical factors or the type of treatment do not influence later histological transformation.

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RISK INCIDENCE OF FOLLICULAR LYMPHOMA TRANSFORMATION: MULTICENTRE RETROSPECTIVE ANALYSIS OF THE SPANISH GELTAMO GROUP

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Introduction: Follicular lymphoma (FL) may, over time, transform into an aggressive lymphoma, usually diffuse large B-cell lymphoma (DLBCL). Transformed follicular lymphomas (tFL) have a worse prognosis due to poorer response to treatment than primary DLBCL. The incidence of transformation is estimated in ~3% per year, although it varies largely between different studies (24–70% overall). These differences are mainly due to different criteria to define tFL, lack of evidence of tFL by biopsy, absence of clonality studies discarding secondary *de novo* NHL, studies performed in the pre-Rituximab era or different follow-up times among studies. With all this pitfalls, the actual incidence of transformation remains an open question. The aim of the present study is to analyse the incidence and prognostic impact of transformation in patients with FL in a large retrospective series of the Spanish group of Lymphomas (GELTAMO).

Patients and methods: A total of 1096 patients (grades I, II and IIIa) from 8 Spanish centres diagnosed of FL between 2000 and 2010 were included in the study. Data were obtained from the database of centres willing to participate in this study. True tFL (FL to DLBCL) were recorded. Composite FL + DLBCL, discordant tFL (FL in bone marrow and DLBCL in adenopathy or vice versa) and downgrading tFL (DLBCL at diagnosis and relapse of FL) were excluded from the preliminary analysis. This study was approved by the Salamanca University Hospital ethic committee.

Results: Seventy-one patients (median follow-up of 6 years) were transformed to DLBCL (6, 5%). Cumulative incidence of transformation at 5, 10 and 15 years was of 5%, 8% and 14%, respectively. Median time to transformation was 30 months, ranged 3–150. Considering survival from diagnosis of FL, tFL patients had a shorter OS than non-transformed (20% vs 68%, $p < 0.0001$). Most of the tFL patients (92%) have previously received treatment for FL, 56% of them with rituximab. Median number of treatment lines before transformation was 2 (1–6). Patients receiving rituximab as first-line therapy showed decreased time to transformation at 15 years (10% vs 19%, $p = 0.025$). Other factors influencing risk of tFL included no response to first-line therapy, age at diagnosis >60 years, FLIPI and Ann Arbor stage. Consolidation therapy with autologous transplant for tFL showed an increase in OS (67% vs 8%, $p = 0.001$). However, these results should be confirmed in prospective studies.

Conclusions: High-risk FLIPI, Ann Arbor stages III–IV, age >60 years, no use of Rituximab in first-line therapy and response to first line have shown to predispose to a higher risk of transformation. Autologous transplantation could have a benefit

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in terms of OS in transformed patients. Effort should be made in order to clarify criteria for transformation. Biological studies on tumoural samples will help to determine tFL pathogenesis in helping to design clinical trials with new molecules.

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* S. Alonso-Álvarez, M. Alcoceba and L. Magnano have equal contribution.
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COMPARISON OF LENALIDOMIDE AND RITUXIMAB WITH CHEMOIMMUNOTHERAPY IN PATIENTS WITH UNTREATED GRADES 1–2 FOLLICULAR LYMPHOMA TREATED AT THE MD ANDERSON CANCER CENTER

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Introduction: The optimal initial therapy of follicular lymphoma (FL) remains unclear. Induction therapies combining rituximab with chemotherapy are highly effective but at the cost of toxicities. Preclinical models suggest that targeting the immune microenvironment may be effective. We recently demonstrated that combining the immunomodulatory drug lenalidomide with rituximab (R^2) is well tolerated and highly effective in patients with untreated FL (Fowler 2014). The aim of this study was to compare the outcomes of patients receiving bendamustine plus rituximab (BR), rituximab, cyclophosphamide, adriamycin, vincristine and prednisone (R-CHOP) and R^2 treated at MD Anderson Cancer Center.

Methods: Utilizing the Department of Lymphoma's clinical outcomes database, we identified patients treated between 2004 and 2014 with FL. Patients were included if they received frontline BR, R-CHOP or R^2. Patients with concurrent DLBCL or grade 3 FL were excluded. Patient characteristics, diagnosis and outcomes following treatment were collected. Progression free (PFS), overall survival (OS) and follow-up were determined from date of initiation of therapy to date of last data census. Comparisons between survival curves were made using log-rank analysis. Univariate and multiple Cox regression analyses were performed to assess the association between PFS and patient characteristics.

Results: Three hundred fifty-six patients with untreated FL were identified. The baseline characteristics, follow-up and outcomes are summarized below (Table). Briefly, pts treated with R-CHOP had poorer risk features, including higher FLIPI scores, elevated LDH and β2m. Elevated LDH, β2m, performance status ≥1, B symptoms, high FLIPI and treatment with R-CHOP or BR without maintenance were associated with inferior PFS by univariate analysis. Multivariate analysis using the above factors demonstrated treatment with R^2 as the only factor associated with superior PFS [hazard ratio 0.46 (95% CI 0.23–0.91), $p = 0.026$).

Conclusion: Although patients treated with R-chemotherapy had more adverse risk features, results of the multivariate analysis suggest that treatment with a non-chemotherapy containing induction regimen (R2) was associated with superior PFS in patients with untreated FL. Ongoing randomized frontline studies comparing R2 vs R-chemotherapy are underway.

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	BR $n = 78$	R-CHOP $n = 184$	R^2 $n = 94$	p-Value
Median age (range), years	61 (29–85)	57 (23–81)	55 (28–84)	0.013[a]
Female (%)	39 (50%)	80 (43%)	43 (46%)	0.61
Stage				
2	0	0	1 (1%)	0.004[b]
3	17 (22%)	45 (24%)	38 (41%)	
4	61 (78%)	139 (76%)	53 (58%)	
B symptoms	12 (15%)	47 (25%)	14 (15%)	0.06[b]
Performance status				
Unknown	18 (22%)	15 (8%)	3 (3%)	<0.001[b]
0–1	58 (71%)	162 (88%)	93 (97%)	
≥2	5 (7%)	11 (6%)	0 (0%)	
Hemoglobin <120 g/L	13 (16%)	36 (19%)	3 (3%)	<0.001[b]
LDH elevated	12 (14%)	49 (26%)	4 (4%)	<0.001[b]
β2-Microglobulin elevated	37 (46%)	108 (57%)	26 (28%)	<0.001[b]
FLIPI				
Low (0–1)	12 (15%)	31 (20%)	37 (39%)	<0.001[b]
Int (2)	35 (43%)	64 (34%)	39 (41%)	
High (3–5)	34 (42%)	86 (46%)	18 (19%)	
GELF high tumor burden	65 (80%)	168 (89%)	67 (71%)	0.001[b]
Median follow-up (range) years	2.3 (0.2–5.9)	6.7 (0.3–15)	3.4 (0.4–6.4)	—
Maintenance therapy	33 (42%)	64 (35%)	—	0.26[b]
3-year PFS (95% CI)				
Without maintenance	63% (42–78)	60% (51–69)	87% (78–93)	0.001[c]
With maintenance	97% (80–100)	72% (59–82)		
3-year OS				
Without maintenance	85% (66–94)	92% (86–96)	97% (89–99)	0.001[c]
With maintenance	100% (—)	97% (88–98)		

[a]Rank-sum test.
[b]Chi-squared test.
[c]Log-rank test.

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EXTENDED DOSING LENALIDOMIDE WITH INTENSIFIED RITUXIMAB IN UNTREATED INDOLENT NHL

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Background: The treatment options for patients with indolent non-Hodgkin lymphoma (NHL) are expanding. Lenalidomide has been shown to have activity in relapsed NHL, and rituximab (R) is effective in combination with chemotherapy. Phase II studies have shown significant activity of the combination of lenalidomide and R (R2) in untreated indolent NHL. The optimal schedule and length of dosing are yet to be determined. The aim of this study was to evaluate the efficacy and safety of extended dosing of lenalidomide with early rituximab intensification in untreated indolent NHL.

Methods: Pts with measurable (>1.5 cm) untreated small lymphocytic lymphoma (SLL) and follicular lymphoma (FL) received 20 mg/day of lenalidomide on days 1–21 and rituximab 375 mg/m^2 on days 1, 8, 15 and 22 of cycle 1 and day 1 of each subsequent 28-day cycle for 12 cycles. Pts with SLL started at 10 mg of lenalidomide, with monthly dose escalation. Prophylactic growth factors were not used. Response was assessed every 3 cycles using 1999 International Working Group Criteria.

Results: Forty-five pts were enrolled in study and received treatment; 44 are evaluable for response. Histologies included: SLL ($n = 15$) and FL ($n = 30$). The median pt age was 59 (28–80) years, and 51% were male. Eighty per cent of FL pts had a FLIPI score of ≥2; 87% met GELF criteria for high tumour burden. All pts had advanced stage disease. Thirty-eight over forty-five (84%) pts completed all 12 cycles. The overall response (ORR) rate for all pts was 95% with 77% attaining a complete response (CR). Nine pts (20%) had a partial response and stable disease in 2 (5%). ORR and CR rates in FL were 100% and 97%, respectively. The best response was observed by cycle 6 in 83%, and 79% of FL and SLL patients, respectively. At a median follow-up of 37 (1–41) months, six pts experienced progression of disease, including one pt with FL. The projected 36-month PFS for SLL patients is 48% (95% CI 17–74%) and 97% (95%CI 78–100%) for FL.

The most common grade ≥3 non-hematologic toxicities were rash ($n = 6$, 13%), fatigue ($n = 6$, 13%), pain ($n = 4$, 9%) and diarrhoea ($n = 3$, 7%). Grade ≥3 neutropenia and thrombocytopenia occurred in 30 (67%) and 2 (5%) pts, respectively. Four patients (9%) experienced neutropenic fever, all recovered with supportive therapy. Two pts developed a second primary malignancy while on study, one pt with resectable colon cancer and one with smouldering myeloma. All pts remain alive at last follow-up.

Conclusion: Extended dosing of R2 with rituximab intensification results in prolonged disease control in indolent NHL. This approach is also associated with increased but manageable hematologic toxicity. Randomized phase III studies based upon this schedule versus chemotherapy are underway in untreated follicular lymphoma.

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IDELALISIB IN FOLLICULAR LYMPHOMA (FL): EFFICACY AND SAFETY FROM A PHASE 2 STUDY

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Introduction: There is a need for new treatment options in FL, particularly for heavily pretreated, high-risk patients refractory to anti-CD20 and chemotherapy. In a pivotal phase 2, open-label study (NCT01282424), the PI3Kδ inhibitor idelalisib showed antitumour activity and acceptable tolerability as monotherapy in indolent non-Hodgkin lymphoma (iNHL) refractory to rituximab (R) and an alkylating agent. Efficacy and safety of idelalisib in the FL patient subset was assessed in this *post hoc* analysis.

Methods: Patients with double refractory histologically confirmed iNHL received oral idelalisib 150 mg BID until disease progression (PD) or unacceptable tolerability. An independent review committee evaluated responses using standardized criteria; overall response rate (ORR) was the primary endpoint.

Results: Median age of FL patients (grade 1, 2 or 3a; $n = 72$) was 62 y at study entry, 54% had a high-risk FLIPI score, 22% had bulky disease and 17% had FL grade 3a. Patients had a median (range) of 4 (2–12) prior treatments; 86% were refractory to their last therapy (32/50 to bendamustine). Median (range) treatment duration was 6.5 (0.6–31.0) mo at data cut-off; 65 (90%) patients were off treatment (PD, $n = 38$; adverse events [AEs], $n = 15$; investigator decision, $n = 7$; death, $n = 5$). The ORR (95% CI) was 56% (43–67; $p < 0.001$), including 10 complete responses (CR) and 30 partial responses. Kaplan–Meier (KM)-estimated median (range) time to response was 2.6 (1.6–11.0) mo, median response duration was 11 mo overall and 27 mo in patients with CR. Progression-free survival was 11 mo, substantially longer vs the last regimen; 45% of patients were progression free at 48 weeks. KM-estimated overall survival (OS) at 1, 1.5 and 2 y was 87%, 74% and 68%, respectively; median OS was not reached. Lymph node size decreased during treatment by ≥50% SPD in 57%. The most common AEs (any grade/grade ≥3, %) were diarrhoea (51/14), cough (32/0), pyrexia (29/4), fatigue (28/0) and nausea (28/3). Additional grade ≥3 events included neutropenia (22%), transaminase elevation (14%), thrombocytopenia (6%), pneumonitis (4%) and anaemia (3%).

Conclusions: In a *post hoc* analysis of a phase 2 study, idelalisib demonstrated rapid, durable responses that were substantially longer than those with the previous regimen with acceptable safety in patients with highly refractory, relapsed FL with limited treatment options.

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IDELALISIB MONOTHERAPY RESULTS IN DURABLE RESPONSES IN PATIENTS WITH RELAPSED OR REFRACTORY WALDENSTROMS MACROGLOBULINEMIA (WM)

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Introduction: Idelalisib (Zydelig®), a selective oral inhibitor of PI3Kδ, demonstrated considerable anti-tumour activity in patients with relapsed/refractory iNHL in phase 1 (p1; Flinn, 2014) and refractory iNHL in phase 2 (p2; Gopal, 2014) trials. This analysis evaluates the outcomes in the subset with WM.

Methods: Eligible WM patients (pts) included those with relapsed/refractory disease (p1), or those with disease refractory to both rituximab and an alkylating agent (p2). Idelalisib dosages were 150 mg QD, and 50–200 mg BID (p1), and 150 mg PO BID (p2) and were administered continuously until disease progression. WM response was assessed by IgM levels and CT imaging (Owen, 2013).

Results: Enrolled pts (p1 $N = 9$; p2 $N = 10$) had a median age of 63 and 60 years (range 42–83) and 78% and 80% were male, respectively. Patients had received a median of 4 prior regimens in both groups. Overall response rate (ORR) was 5/9 (56%) and 8/10 (80%; Table). Median time to minor or first response was 2 months, and most responses continued to improve over 6 months or longer. Median DOR was 32.8 months (p1) and not yet reached (p2). Sixty-seven per cent have continued response at 2 years (p2). Median PFS was 33.3 months and 22.1 months, respectively. Interestingly, >3 g/dL improvements in haemoglobin were noted in 5/9 and 7/10 subjects, respectively, over 3–6 months time frame. Grade ≥3 adverse events included increased ALT/AST 5/9 and 1/10, and diarrhoea/colitis 1/9 and 3/10. One G3 ALT elevation and 1 G3 diarrhea resulted in study discontinuation.

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	Study 02 ($n = 9$)	Study 09 ($n = 10$)
ORR, n (%), (95% CI)	5 (56%) (21–86)	8 (80%) (44–98)
CR	0	0
PR	1 (11%)	7 (70%)
MR	4 (44%)	1 (10%)
SD	2 (22%)	1 (10%)
PD	1 (11%)	1 (10%)
NE	1 (11%)	0

Conclusions: These combined data suggest that single-agent idelalisib monotherapy is active in Waldenstroms macroglobulinemia. Durable responses were seen in the majority of patients. Marked improvements in haemoglobin level were also associated with response. The safety profile was acceptable and manageable, with no apparent disease-specific safety signals. Phase 3 clinical trials of idelalisib with combination therapy are in progress for patients with iNHL, including WM. Clinical trials: NCT00710528 (p1) and NCT01282424 (p2).

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CLINICAL OUTCOME IN THE PRIMA STUDY FOR YOUNG PATIENTS WITH FOLLICULAR LYMPHOMA AFTER FIRST-LINE IMMUNOCHEMOTHERAPY

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Introduction: Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma with a median age at diagnosis in the 6th decade. Results from the PRIMA trial have established immunochemotherapy followed by rituximab maintenance as the standard of care for previously untreated patients with high tumour burden FL. However, although younger age is usually considered as a favourable prognostic factor in FL, few data are currently available evaluating this strategy for the youngest subgroup of patients.

Methods: We compared treatment outcomes of patients aged <40 vs ≥40 y included in the prospective cohort of the PRIMA study. After an immunochemotherapy induction, responding patients were randomized for maintenance between observation and rituximab maintenance therapy during 2 y.

Results: At the time of registration, 107 patients (9.4%) were <40 y (median 34 y, ranging from 22 to 39 y) among the 1135 patients of the induction analysis population. The two groups were similar according to Ann Arbor stages, FLIPI score, performance status, B symptoms and bone marrow involvement. Overall response rate (ORR) at the end of induction was similar [94.4% with 71.0% complete responses (CR) vs 90.1% with 63.8% CR for patients <40 vs ≥40 y respectively]. Ninety-eight patients <40 y and 875 ≥40 y were randomized for maintenance. Among the 98 patients <40 y, 55 (56.1%) were randomized in the observation arm and 43 (43.9%) in the rituximab arm. At the end of maintenance, ORR was significantly lower for patients <40 y (59.2% with 55.1% CR vs 70.2% with 62.6% CR, $p = 0.026$). Progression-free survival from registration did not significantly differ between the two age groups. At 3 y, PFS was 61.1% (95% CI 51.0–69.8) for patients <40 y and 66.3% (95% CI 63.3–69.2) for patients ≥40 y. In a multivariate Cox model adjusted on age group, gender, beta2-microglobulin, induction regimen, response to induction regimen, FLIPI factors and maintenance regimen, age group is not statistically significant for PFS. According to maintenance regimen for patients <40 y, median PFS was 34.2 months in the observation arm and not reached in the rituximab arm ($p = 0.0076$). No significant difference was observed between the two age groups for overall survival in a multivariate analysis. Relapse rate was not significantly different (52.3% vs 44.8% for patients <40 vs ≥40 respectively, $p = 0.139$) but was found to be slightly higher during maintenance period for patients <40 y (58.9% vs 48.2%). For cases with histological documentation after progression/relapse, the rate of transformation was 24.1% (7/29) for patients <40 y and 18.5% (34/185) for patients ≥40 y.

Conclusions: Age <40 y did not seem to be associated with a more favourable outcome. Although rituximab maintenance therapy appeared to benefit equally PFS of younger patients, these data suggest that early failures and transformation may be more frequently observed in these patients.

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RITUXIMAB MAINTENANCE (MR) IMPROVES PFS AND OS IN ALL PATIENTS (PTS) WITH FOLLICULAR LYMPHOMA (FL) – INDIVIDUAL PATIENT DATA (IPD) META-ANALYSIS

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Background: The effect of MR was evaluated in randomized clinical trials (RCTs) with conflicting results regarding OS. In a previous systematic review and meta-analysis of summary (aggregate) data, we demonstrated an OS benefit of MR treatment in pts with relapsed or refractory FL and an improved progression-free survival (PFS) in both untreated and relapsed refractory FLs. However, pts' disease and treatment characteristics may interact with the effect of MR.

To improve identification of sub-groups of pts who may benefit from MR treatment and to evaluate factors that interact with the effect of MR, we conducted an international IPD (raw data) meta-analysis of RCTs of MR for pts with FL.

Methods: In June 2014, we updated an electronic search in Pubmed and the Cochrane Library, conference proceedings and database of clinical trials for RCTs comparing MR to observation or rituximab at progression. The investigators of 11 trials that fulfilled inclusion criteria were contacted, and a collaborative group was established. Seven study groups contributed data on individual pts. In a series of Cox regression analyses stratified by study, we examined the effect of sex, age, number of past inductions, FLIPI, type of chemotherapy, and response after induction and their interaction with treatment group. We combined the assessed log hazard ratios (HR) using a log-rank test for each study and combined the results using a fixed effect meta-analysis.

Results: We obtained data for 2323 patients randomized in 7 trials of MR compared to observation after induction therapy. The median follow-up ranged from 28 to 114 months. The median age was 57 years (23–85 years), and 41% were older than 60 years. Fifty per cent of pts were women, 97% had WHO performance status 0–1; 1195 (53%) pts received CHOP/RCHOP, 453 (20%) CVP/R CVP; 1755 (77%) received rituximab containing induction; 33% of pts received 1st induction, 67% >2nd.

Pts treated with MR had an improved PFS compared to observation (HR 0.57, 95% CI 0.51–0.64, $p < 0.0001$, Figure 1). Although female sex, low–int FLIPI groups, MR after first induction ever, CHOP/fludarabine chemotherapy and achievement of complete response (CR) after induction were associated with a lower risk of progression/death, there was no interaction between these variables and the effect of MR. MR effect remained statistically significant after stratifying for sex, age, number of previous inductions, FLIPI risk group and level of response.

Pts treated with MR had an improved OS compared to observation. MR statistically significantly improved OS after stratifying for patients and disease characteristics. A sensitivity analysis with all 10 eligible trials supported these results.

Conclusions: An improved PFS and OS with MR therapy for patients with FL was shown based on IPD. This effect was robust stratifying for important disease and patient's characteristics including treatment line and type of induction. Additional analyses are expected.

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COMPARISON OF SUBCUTANEOUS AND INTRAVENOUS RITUXIMAB IN THE MAINTENANCE SETTING: UPDATED SAFETY RESULTS OF THE PHASE III SABRINA STUDY IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Background: Subcutaneous rituximab (R^{SC}) offers improved patient (pt) convenience and healthcare resource savings versus intravenous rituximab (R^{IV}). The phase III SABRINA study (NCT01200758) investigated induction R^{SC} or R^{IV} in combination with chemotherapy followed by maintenance R^{SC} or R^{IV} in pts with follicular lymphoma (FL). With a median follow-up of 14 months, the R^{SC} and R^{IV} arms had comparable response rates and safety profiles, without any new safety signals for R^{SC}. However, longer follow-up is needed to define the safety of R^{SC} versus R^{IV} in the maintenance setting. We present an updated safety analysis of R^{SC} and R^{IV} for the maintenance phase of SABRINA (median follow-up time: 26 months).

Methods: Pts with treatment-naïve CD20+ grades 1–3a FL ($n = 410$) were randomized to receive R^{SC} or R^{IV} ($n = 205$/arm), stratified by FL international prognostic index score, chemotherapy type and region. During induction, all pts received R^{IV} 375 mg/m^2 in cycle 1; for cycles 2–8, pts received 3-weekly R^{SC} 1400 mg or R^{IV} 375 mg/m^2. Pts received ≤8 cycles of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) or 8 cycles of CVP (cyclophosphamide, vincristine and prednisone). During maintenance, pts received 8-weekly R^{SC} or R^{IV}. Non-serious AEs were reported for 28 days following the last dose of rituximab. Serious AEs (SAEs) were recorded for 1 year post-treatment or until the start of new anti-lymphoma treatment. SAEs considered possibly related to study treatment were recorded indefinitely.

Results: In total, 407 pts received ≥1 dose of rituximab (safety population). Six R^{SC} pts discontinued therapy after cycle 1 (R^{IV} in both arms) and were included in the R^{IV} safety population (R^{SC} $n = 197$; R^{IV} $n = 210$). Most pts started maintenance (R^{SC} 87%; R^{IV} 85%). During maintenance, the most common AEs were of the system organ class (SOC) infections and infestations (R^{SC} 41%; R^{IV} 42%); most were grade 1/2 upper respiratory tract infections, urinary tract infections or nasopharyngitis. Grade ≥3 infections were reported in 8% (R^{SC}) and 3% (R^{IV}) of pts. Other common AEs (≥15% pts) belonged to the SOCs general disorders and administration site conditions (R^{SC} 28%; R^{IV} 19%); gastrointestinal disorders (R^{SC} 24%; R^{IV} 19%); musculoskeletal and connective tissue disorders (R^{SC} 23%; R^{IV} 21%); respiratory, thoracic and mediastinal disorders (R^{SC} 18%; R^{IV} 17%); blood and lymphatic system disorders (17% each); and skin and subcutaneous tissue disorders (R^{SC} 16%; R^{IV} 15%). In total, 5% (R^{SC}) and 3% (R^{IV}) of pts discontinued maintenance due to AEs. At data cut-off, 27 pts (R^{SC} $n = 11$, 6%; R^{IV} $n = 16$, 8%) had died.

Conclusions: Maintenance R^{SC} and R^{IV} were well tolerated in treatment-naïve pts with FL, without any new safety signals for R^{SC}. Administration of R^{SC} over approximately 6 minutes has positive implications for pt convenience and healthcare resource savings, without compromising pt safety or efficacy.

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PHASE II STUDY FOR THE EVALUATION OF FEASIBILITY, ACTIVITY AND SAFETY OF BENDAMUSTINE AND OFATUMUMAB IN COMBINATION IN MARGINAL ZONE B-CELL LYMPHOMAS (MZL)

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Introduction: Ofatumumab, a fully human type I anti-CD20 monoclonal antibody, and bendamustine, a cytotoxic agent with structural similarities to alkylating agents and antimetabolites, have both single-agent clinical activity in various lymphoma subtypes and chronic lymphocytic leukaemia. Chlorambucil and bendamustine, either alone or in combination with rituximab, are the most commonly used agents in the first-line treatment of MZL. For patients (pts) with relapsed disease, responses to alkylating agents or rituximab are usually of short duration, and the optimal therapy remains to be determined. In this pilot phase II study (EudraCT No. 2011-003495-36), we aimed to evaluate the feasibility and preliminary clinical activity of ofatumumab in combination with bendamustine in previously treated MZL pts.

Methods: Prospective, multicentre, phase II trial in which pts with relapsed/refractory histologically proven MZL received ofatumumab 1000 mg iv on day 1 and bendamustine 90 mg/m^2 iv on days 1–2, every 4 weeks, for 6 cycles. The primary endpoint was overall (complete + partial) response rate, along with the revised criteria (Cheson et al 2007). Response was defined according to Matutes et al. 2008 for pts with splenic MZL and according to GELA scoring system (Copie-Bergman et al 2003) for pts with gastric lymphomas. Secondary endpoints were 2-year progression-free survival (PFS), time to next treatment and adverse events (AEs) evaluation.

Results: Sixteen pts were enrolled between March 2012 and April 2014, with 14 evaluable for response (one patient withdrew consent after cycle 1 and, in one patient, diagnosis was reviewed after cycle 2 as not consistent with MZL). Baseline characteristics were median age 63.5 years (range 46–78); F/M = 7/ 9; primary site: gastric 4, splenic 4, other 7; stage at study entry: I/II/III/IV = 2/2/1/10; number of prior systemic treatments: 1/2/>2 = 6/4/6.

Treatment was well tolerated. Grade >2 treatment-related AEs included: grade 3 fever ($n = 1$), thrombocytopenia (1), hyponatremia (1), neutropenia (1), troponine increase (1), pneumonia (1), febrile neutropenia (2), myocardial infarction (1) and grade 4 neutropenia (1) and lymphopenia (1). Twelve pts completed 6 treatment cycles. One patient was withdrawn after 5 cycles due to AE, one after 4 cycles due to physician's decision and the two pts not evaluable for response as above reported.

Among the 14 pts evaluable for the primary endpoint, the overall response rate was 93% with 7 complete remissions (50%), 6 partial remissions (43%) and 1 stable disease as best responses.

Conclusion: The combination of ofatumumab and bendamustine is feasible, well tolerated and resulted in significant clinical activity in previously treated MZL pts. Results on longer follow-up and time related endpoints will be presented at the meeting.

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HIGHER RISK OF RITUXIMAB INTOLERANCE IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA

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Introduction: Rituximab is a mainstay of therapy in Waldenström's macroglobulinemia (WM). Although rituximab is active as both monotherapy and in combination with other anti-neoplastic agents, discontinuation due to intolerance that develops after the first infusion is commonly encountered. Among patients with other B-cell malignancies, including FL and CLL, discontinuation of rituximab due to intolerance is rare (<0.5%) (Salles et al, Lancet 2010; Hallek et al, Lancet 2010; Rummel et al, Lancet 2013). Given its known impact on augmenting depth of response and progression-free survival (Treon et al, Br J Haematol 2011), the discontinuation of rituximab could potentially hinder treatment outcome. We therefore sought to describe the incidence and clinical characteristics associated with rituximab intolerance in WM patients.

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Patients and Methods: We analysed the outcome of patients with the consensus diagnosis of WM who were seen at our institution from 1997 to 2014 and identified those patients who received rituximab. We excluded patients who experienced first-cycle infusion related reactions (IRRs)and patients in whom rituximab was stopped due to severe hypogammaglobulinemia and/or associated recurring or life-threatening infections. Clinical and laboratory data were collected and tabulated, and results presented were using descriptive statistics.

Results: One thousand four hundred sixty-six patients with the consensus diagnosis of WM were identified, of whom 1183 (80.7%) received rituximab during the course of their follow-up. Among these patients, 85 (7.2%) patients developed intolerance to rituximab. Baseline characteristics for these patients did not significantly differ from non-rituximab intolerant patients. Rituximab intolerance occurred during the first line of treatment in 34% of patients, and intolerance occurred in patients receiving rituximab as monotherapy (48%), and in combination with alkylators (28%), proteasome inhibitors (15%), nucleoside analogues (6%) or other agents (3%). The most common reasons for stopping rituximab were anaphylactic reactions (26%), hives (17%), hypotension (17%), pruritus (10%) and rash (10%). Eleven per cent of intolerant patients presented during an IgM flare, and 64% were responding to rituximab-based therapy at the time of intolerance. Among rituximab intolerant patients, 22 (26%) were transitioned to ofatumumab, with good tolerance observed in 18 (82%) of these patients.

Conclusions: Rituximab intolerance is more common in WM patients in comparison to the incidence reported in other B-cell disorders. Intolerance to rituximab can be observed during the first course of therapy and when used as mono- or combination therapy. Most patients showed response to rituximab-based therapy at the time of intolerance. Transition to ofatumumab was feasible and well tolerated in the majority of rituximab-intolerant patients in whom a switchover was enacted.

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HISTOLOGICAL TRANSFORMATION IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA

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Introduction: Waldenström macroglobulinemia (WM) is a rare lymphoma characterized by the malignant growth of IgM-secreting lymphoplasmacytic lymphoma (LPL) cells in the bone marrow. Transformation to more aggressive histological lymphoma subtypes has been described in association with specific therapies (i.e. nucleoside analogues), but this phenomenon remains largely unaddressed. The objective of this study is to describe the characteristics and outcomes of patients with WM who experience histological transformation (HT).

Methods: We performed a retrospective study in which patients with a clinicopathological diagnosis of WM seen at our centre between 2000 and 2014 were identified. From these, clinical data from patients with a pathological diagnosis of HT were gathered and analyzed. Clinical characteristics are presented using descriptive statistics. The median time from WM to HT diagnosis and median survival from HT to last follow-up or death were estimated using the Kaplan–Meier method. Univariate survival comparisons were made using the log-rank test.

Results: A total of 20 patients who experienced HT were identified in a cohort of 1500 WM patients for a crude incidence of 1.3%. The median age at WM diagnosis was 61 years (range 40–73 years). The male-to-female ratio was 1.5, and 25% of patients had a family history of haematologic malignancy. The median age at HT diagnosis was 70 years (range 42–88 years). The median time from WM diagnosis to HT diagnosis was 4.4 years. All the patients experienced HT to diffuse large B-cell lymphoma (DLBCL). The median number of lines of therapy for WM prior to HT was 2 (range 0–5); 68% had received rituximab, 58% alkylating agents, 37% nucleoside analogues, 32% proteasome inhibitors and 20% of patients were treatment-naïve. Eighty-five per cent of patients were 60 years or older, ECOG >1 was seen in 38%, elevated LDH levels in 71%, extranodal involvement in 83% and stage III or IV in 63% at time of HT diagnosis. IPI score was >2 in 69% of patients. The most common extranodal sites involved by HT were bone marrow, bone, orbits and pleura. The median IgM levels were 1920 mg/dl (range 365–7000 mg/dl) with 67% of patients experiencing increasing IgM levels from baseline. The most common frontline regimens were R-CHOP (75% of the cases) and R-EPOCH (10%); 67% of patients experienced complete response (CR) to frontline treatment and 30% of patients underwent autologous stem cell transplantation (ASCT). At the time of this report, 60% of patients have died; the most common cause was DLBCL progression (90%). The median OS was 4 years. Patients who achieved CR to frontline treatment ($p = 0.001$) or underwent ASCT ($p = 0.03$) experienced a better outcome.

Conclusion: The incidence of HT in WM patients is approximately 1%. HT can occur in treatment-naïve patients and even after 20 years from WM diagnosis. Most of patients present with poor prognostic markers such as a high IPI score and a large majority with extranodal involvement. The use of chemoimmunotherapy and ASCT might be associated with improved outcomes.

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OUTCOMES IN KAPOSI SARCOMA HERPESVIRUS-ASSOCIATED MULTICENTRIC CASTLEMAN DISEASE PATIENTS TREATED WITH RITUXIMAB AND LIPOSOMAL DOXORUBICIN (R-DOX)

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Introduction: Rituximab is effective in Kaposi sarcoma (KS) herpesvirus-associated multicentric Castleman disease (KSHV-MCD). Concurrent KS is common and can worsen with rituximab. Liposomal doxorubicin targets CD20-negative KSHV-infected cells and may be useful in some patients (pts). Long-term outcomes in KSHV-MCD pts with concurrent KS are unknown.

Methods: Pts with symptomatic KSHV-MCD were treated in a prospective pilot study of rituximab 375 mg/m^2 and liposomal doxorubicin 20 mg/m^2 every 3 weeks (R-Dox) until clinical improvement, followed by antiviral therapy or additional KS therapy if indicated. KSHV-MCD responses were evaluated by NCI criteria, survival by Kaplan–Meier and log-rank methods. Baseline clinical factors were evaluated as predictors of overall survival (OS). Effect of R-Dox on change in clinical biomarkers is evaluated by Wilcoxon signed-rank test. Two-sided p-value < 0.05 is considered significant.

Results: Twenty-two HIV-infected pts enrolled, including 17 previously published pts. 10 (45%) had KS (including 1 pulmonary KS), 4 additional had KS in lymph node only, 8 (36%) T1 KS (high-risk KS based on lymphedema, extensive oral cavity involvement or visceral disease). Baseline characteristics can be found in the table below. Median number of cycles is 3 (2–9). At end of R-Dox, clinical benefit responses were complete 77%, partial 9%, stable disease 5% and progressive disease 9%. During R-Dox, 8 had improvement in cutaneous KS, only 1 pt had mild transient worsening. Hemoglobin, albumin, CRP, KSHV viral load (VL) and serum free light chains all improved with therapy ($p < 0.0001$). With median potential 69-month follow up, 5-year estimates were 70% (95% CI: 47–86%) free from KSHV-MCD progression; OS 79% (56–92%). Baseline T1 KS was associated with inferior OS (5-year OS: no T1 KS 91% vs T1 KS 56%; $p = 0.03$ overall). Baseline CD4 <100 cells/uL ($p = 0.33$), hemoglobin ($p = 0.29$), platelets (0.36), KSHV VL ($p = 0.93$), CRP ($p = 1.00$) and serum free light chains (K, p =0 .22; L, $p = 0.32$) were not associated with OS.

Conclusions: R-Dox is effective in KSHV-MCD, including many pts with concomitant KS. Baseline measures of KSHV-MCD activity or CD4 count were not associated with OS. Inferior survival was noted in the few pts with baseline T1 KS treated with R-Dox, and additional approaches are needed for this population.

Abstract 201 Table Baseline characteristics

Characteristic	Result
Age, med (range)	43 (27–55)
CD4 (cells/uL), med (range)	255 (21–858)
HIV viral load (VL) <200 copies/mL, n (%)	17 (77.3)

(*Continues*)

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Abstract 201 Table (Continued)

Characteristic	Result
Prior KSHV-MCD therapy, n (%)	16 (72.7)
CRP (mg/L), med (range)	81.5 (<4–210)
Hemoglobin (g/dL), med (range)	9.8 (6.8–13.2)
Platelets (K/uL), med (range)	118.5 (11–567)
Albumin (mg/dL), med (range)	2.9 (1.2–4.0)
KSHV VL (copies/10^6PMBC), med (range)	18 622 (0–8, 780, 488)
Serum Free Kappa (mg/dL), med (range)	7.4 (1.9–22.6)
Serum Free Lambda (mg/dL), med (range)	5.7 (1.8–19.8)

MANTLE CELL LYMPHOMAS

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ASSOCIATION BETWEEN QUALITY OF RESPONSE AND OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED MANTLE CELL LYM PHOMA (MCL) RECEIVING VR-CAP VS R-CHOP IN THE PHASE 3 LYM-3002 STUDY

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Introduction: The phase 3 LYM-3002 study (NCT00722137) compared bortezomib plus rituximab, cyclophosphamide, doxorubicin and prednisone (VR-CAP; $n = 243$) with R-CHOP ($n = 244$) in patients (pts) with newly diagnosed mantle cell lymphoma (MCL) for whom stem-cell transplantation was not an option. VR-CAP was associated with significantly longer median PFS (24.7 mos vs R-CHOP 14.4 mos; $p < 0.001$), a 4-year overall survival rate of 64% (vs 54%) and longer TTNT (45 vs 25 mos); response rates by IRC in the VR-CAP ($n = 229$)/R-CHOP ($n = 228$) arms were CR 46/35%, CR/CRu 53/42%, PR 9/18% and overall response rate 92/89%. This *post hoc* analysis evaluates the association between the improved outcomes seen with VR-CAP vs R-CHOP and the quality of responses achieved.

Methods: Pts aged ≥18 years with newly diagnosed, measurable stages II–IV MCL and ECOG PS 0–2 were randomized 1:1 (stratified by IPI score and disease stage) to 6–8 × 21-day cycles of VR-CAP (bortezomib 1.3 mg/m^2 IV days 1, 4, 8, and 11, rituximab 375 mg/m^2, cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, all IV day 1, and oral prednisone 100 mg/m^2 days 1–5) or R-CHOP (as VR-CAP, substituting bortezomib for vincristine 1.4 mg/m^2 [max 2 mg] IV day 1). Outcomes were evaluated by CR/CRu/PR achievement, MIPI risk status and max reduction from baseline in target lymph node size expressed as sum of the product of the diameter (SPD). Time-to-event outcomes were assessed via Kaplan–Meier estimation.

Results: In analysis of time-to-event outcomes within each response category, PFS by IRC and TTNT were longer (VR-CAP vs R-CHOP) in pts achieving CR/CRu and in pts achieving PR. DOR, PFS and TTNT appeared similar between VR-CAP pts achieving PR and R-CHOP pts achieving CR/CRu. Prolongation of DOR, PFS and TTNT with VR-CAP vs R-CHOP within the CR/CRu and PR response categories was mainly evident in pts with intermediate- and low-risk MIPI scores; the treatment effect appeared less pronounced in high-risk MIPI pts. Analysis of pts achieving CRu suggested an association with long-term outcomes closer to the PR category than to CR, suggesting that it may be more clinically relevant to consider CRu with PR than with CR. In each response category, more VR-CAP pts had an SPD nadir of 0 vs R-CHOP.

Conclusions: These data suggest that the duration and quality of response (both overall and within individual response categories) in the VR-CAP arm were superior vs R-CHOP. The difference was mainly present in the intermediate- and low-risk MIPI categories and appeared associated with percentage of pts achieving an SPD nadir of 0 in their target lymph nodes.

Abstract 202 Table 1

	VR-CAP	R-CHOP
Median DOR, mos	36.5	15.1
In pts achieving CR/Cru	42.1	18.5
In pts achieving PR	20.2	9.6
Median PFS within each response category, mos		
In pts achieving CR/Cru	40.9	19.8
In pts achieving PR	17.1	11.7
Median TTNT within each response category, mos		
In pts achieving CR/Cru	NE	26.6
In pts achieving PR	35.3	24.3
Achievement of SPD nadir of 0, %		
In pts achieving CR/Cru	72	59
In pts achieving CR	79	68
In pts achieving PR	48	28

CR, complete response; CRu, unconfirmed CR; DOR, duration of response; NE, not estimable; PFS, progression-free survival; PR, partial response; TTNT, time to next treatment.

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R-CHOP21 AND R-CYTARABINE (3 + 3 CYCLES) FOR PATIENTS WITH NEWLY DIAGNOSED MANTLE CELL LYMPHOMA NOT ELIGIBLE FOR HIGH-DOSE THERAPY: PRELIMINARY RESULTS AT 2 YEARS FOLLOW-UP

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Introduction: Mantle cell lymphoma (MCL) is an aggressive type of B-cell non-Hodgkin lymphoma. Therapy of the elderly or comorbid pts is largely based on R-CHOP or R-bendamustin induction with or without R maintenance. Czech lymphoma study group initiated the observational study as a non-intervention, multi-centre trial with the primary objectives to prospectively evaluate efficacy of alternating R-CHOP21 and R-HDAC (araC 1 or 2 g/m^2, 2 doses at 24 hours) in newly diagnosed MCL pts not eligible for high-dose therapy (HDT).

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Methods: Primary endpoints were response rate by PET-CT and minimal residual disease (MRD) assessment by qPCR after completion of induction. Secondary endpoints were PFS, OS and toxicity. The choice between 1 and 2 g/m^2 araC was left at investigator's discretion. Exclusion criteria included eligibility to HDT, ECOG ≥ 4 and CNS involvement.

Results: Sixty-three pts (M vs W ratio 1.9:1) with median age of 70.2 years were enrolled into the study between 16 June 2011 and 9 December 2014. Eighty-nine per cent and 79% of pts were presented with stage 3/4 disease and infiltration of BM. Of patients, 57.1%, 38.1% and 4.8% had high, intermediate and low risk disease according to MIPI, respectively. Thirty-five per cent of pts had B-symptoms. Of patients, 77.8% were diagnosed from the lymph node, 22.2% from trephine biopsy. Of patients, 69.4%, 24.5% and 6.1% presented with classical, pleomorphic and blastic variant MCL, respectively. Ki67 $\geq 30\%$ was recorded in 50% and deletion of TP53 gene in 31% pts. Bulky disease >5cm was in 35% of pts, spleen involvement in 50.8% of pts. Extra-nodal involvement other than BM was histologically confirmed in 12.7% of pts. R-CHOP was used in 88% of pts (R-COEP in 12%), 87% of pts received 2 g/m^2 araC (13% 1 g/m^2). Two patients did not finish the treatment due to toxicity. Fifty-three per cent and 26% pts had grade 3/4 haematologic and non-haematologic toxicity, respectively. After 6 cycles of therapy, 88.9% pts (56/64) achieved response by PET-CT (83.9% CR). Progression on therapy occurred in 4.8% of pts (3/63). Insufficient response after 2 cycles resulted in premature change of therapy in 3.2% of pts (2/63 pts). Ninety-one per cent of pts (51/56) received maintenance rituximab. So far, MRD was evaluated in 42 of 56 pts, who completed induction and achieved response: 57.2% pts (24/42 pts), 21.4% pts (9/42 pts) and 21.4% pts (9/42 pts) were MRD negative, MRD positive-not quantifiable and MRD positive-quantifiable, respectively. With the median follow-up of 23.5 months, there were 13 relapses and 8 deaths. Two-year PFS and OS were 68% and 87%. Statistically significant correlation with PFS was observed for Ki-67 > 30% ($p = 0.0028$), bulk >5cm ($p = 0.0095$), TP53 deletion ($p = 0.0395$) and MIPI ($p = 0.0486$). Trend towards shorter PFS was observed in pts with spleen involvement ($p = 0.083$).

Conclusion: Alternation of R-CHOP and R-HDAC in newly diagnosed elderly or comorbid MCL pts represents a promising, very effective and well-tolerated treatment approach that induces high ORR and MRD negativity.

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CLINICAL, METABOLIC AND MOLECULAR RESPONSES WITH SEQUENTIAL R-CHOP, HIGH-DOSE CYTARABINE, AND IODINE-131 TOSITUMOMAB-BASED TRANSPLANT IN MANTLE CELL LYMPHOMA

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Methods: Eligible patients (pts) were transplant eligible with untreated MCL, CS II–IV. Induction chemotherapy consisted of R-CHOP-14 × 4 and 2 cycles of rituximab and high-dose cytarabine (R-HiDAC, age <65 years: 3 g/m^2 q12 h ×4 doses; age ≥65 years: 2 g/m^2). Responding pts received consolidation with iodine-131 tositumomab-BEAM-ASCR. Interim PET scans were performed after 4 cycles of R-CHOP therapy. If positive (Deauville 4–5), PET was repeated after R-HiDAC. In a subset of pts ($n = 17$), MRD was assessed by an NGS-based method (Faham et al. Blood 2012).

Results: Twenty-five patients were enrolled from February 2012 to October 2013. Two pts were excluded from final analysis due to change in diagnosis. The median age was 58 years (range 46–69) with expected male predominance (70%). MIPI distribution: low 48%, intermediate 30% and high 22%. Proliferation index was low (<10%) in 4, intermediate (10–29%) in 8, high (≥30%) in 9 and missing in 2. Among 23 evaluable pts, 65% (15/23) achieved a negative PET scan after R-CHOP-14 × 4 cycles. After R-HiDAC, 87% (20/23) achieved a negative PET scan. ORR was 100% to induction chemotherapy, and all pts were eligible to proceed to HDT/ASCR (refusal 1). The ORR at end of treatment was 100%, with 21 pts in CR and 2 pts in PR. At 6 months (mo) post therapy, all pts were in CR. At median follow-up of 22 mo, 2 pts have relapsed (4 and 27 mo post-ASCT). Overall, the treatment was well tolerated. There were 21 SAEs, most commonly, fever and neutropenia, anaemia, thrombocytopenia and pulmonary embolus. A baseline clonotypic sequence was identified in 88% (15/17) of pts (1 calibration failure and 1 sample pending analysis). MRD status was negative in most pts at all time points, both during treatment (10/11, 91%) and at 6 mo post therapy (8/9, 89%). The two pts who relapsed neither had a positive post-treatment MRD predicting relapse nor had a positive PET or MRD assessment during treatment.

Conclusions: Sequential R-CHOP-14, R-HiDAC and RIT-BEAM followed by ASCR are highly effective regimens with high rates of early PET and MRD negativity. Overall, the treatment programme was well tolerated. Further information regarding MRD in these patients will be presented.

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MULTI-CENTRE PHASE II STUDY WITH LENALIDOMIDE PLUS RITUXIMAB AS INITIAL TREATMENT FOR MANTLE CELL LYMPHOMA: SURVIVAL UPDATE AND HEALTH-RELATED QUALITY-OF-LIFE ANALYSIS

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Introduction: Mantle cell lymphoma (MCL) remains incurable, and initial treatment is not standardized. The therapeutic goal is to extend survival and maintain quality of life whenever possible. We have reported the high efficacy associated with a frontline biologic doublet of lenalidomide (Len) plus rituximab (R) (ORR 84.2% with 52.6% CR). We now report the health-related quality-of-life (HRQoL) outcome analysis.

Methods: The study includes both induction and maintenance. During induction, Len is administered at 20 mg daily on days 1–21 of a 28-day cycle for a total of 12 cycles, with dose escalation to 25 mg daily if tolerated. During maintenance, Len is administered at 15 mg. Standard dose R is administered weekly ×4 during cycle 1, then once every other cycle. Treatment with both agents is continuous until POD. HRQoL was measured by FACT-LYM, which consists of FACT-G questionnaires and a 15-item lymphoma subscale (LYM), at baseline, every 3 months for 2 years and every 6 months thereafter. QoL analysis was performed on 32 patients who have completed at least 18 months of therapy. The FACT-LYM total score and trial outcome index (TOI) score were correlated using paired *t*-test to clinical features, treatment and response.

Results: Thirty-eight subjects were enrolled at 4 centres from July 2011 to April 2014. Median age was 65 years. MIPI scores were evenly distributed between low-, intermediate- and high-risk (34%, 34% and 32%, respectively). At a median follow-up of 28 months, 28 (78%) of the evaluable 36 patients remain on study without evidence of disease progression, including 27 who have completed induction and are in maintenance. Eight evaluable patients had disease progression—3 with primary refractory disease and 5 progressed following initial responses (2 CR with PFS of 18 and 39 months, 3 PRs with PFS at 14, 25 and 28 months, respectively). Median PFS and DOR have not been reached. The 2-year PFS rate is estimated at 84.7% (95% CI = 66.9%, 93.4%), with 2-year OS rate at 96.8% (95% CI = 73.1%, 99.5%). At baseline, the mean of FACT-LYM total score was 130.8 (SD 22.8), and modified trial outcome index (TOI) was 91.0 (SD 17.5). Both scores did not correlate with age, PS, MIPI or clinical response. Improvements in FACT-LYM total and TOI scores were seen at month 12 following induction, compared to baseline

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(139.1 ± 19 and 98.2 ± 14, $p = 0.04$ and $p = 0.06$, respectively). At month 18, the mean FACT-LYM scores remained stable compared to baseline or month 12.

Conclusions: This study provides the first demonstration that a chemotherapy-free, combination biologic approach is active with durable responses as initial therapy for mantle cell lymphoma. High efficacy induction therapy with Len and R generally improves HRQoL, while maintenance strategy with both agents appears feasible with preservation of HRQoL in the upfront setting. (ClinicalTrials.gov – NCT01472562.)

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SUBGROUP ANALYSIS OF THE PHASE II RANDOMIZED MCL-002 (SPRINT) STUDY OF LENALIDOMIDE VS INVESTIGATOR'S CHOICE IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA

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Introduction: Lenalidomide, an immunomodulator with antineoplastic and antiproliferative effects, showed clinically significant improved activity over investigator choice (IC) in relapsed/refractory (R/R) MCL. This preplanned MCL-002 analysis evaluated efficacy across patient subgroups receiving lenalidomide vs IC.

Methods: Patients received lenalidomide (25 mg/day PO on days 1–21/28 days) or single-agent IC therapy (chlorambucil, cytarabine, fludarabine, gemcitabine or rituximab). The primary endpoint was progression-free survival (PFS); prespecified exploratory analyses of PFS by subgroups were conducted.

Results: Two hundred fifty-four patients with R/R MCL (median 2 prior therapies) were randomized 2:1 to lenalidomide ($n = 170$) or IC ($n = 84$). Patients receiving lenalidomide showed a significant improvement in median PFS vs IC (8.7 vs 5.2 months; HR = 0.61, $p = 0.004$). Subgroup analysis of PFS by central review demonstrated statistically significant reductions in the risk of progression or death in favour of lenalidomide vs IC across most baseline demographic and disease characteristics including age ≥65 years, women, any disease stage at diagnosis, ECOG PS 0–1, both high or low tumour burden, Ki-67 <10%, normal/elevated LDH, WBC counts $<10 \times 10^9$/L, no bulky disease, high MIPI score, negative bone marrow and normal renal function. The following subgroups with discordant results on risk reduction were WBC $\geq 15 \times 10^9$/L and positive bone marrow; both were not statistically significant. This was partly explained by low patient numbers in the IC arm. In the remaining categories, risk reduction was not statistically significant.

Overall, factors associated with significantly better PFS by univariate Cox regression analysis, beside treatment group (HR = 0.619; $p = 0.004$), were non-elevated LDH, WBC $<10 \times 10^9$/L, low + intermediate MIPI, low tumour burden and Ki-67 ≤30%. Highly significant in the multivariate analysis were treatment group (HR = 0.384) and Ki-67 ≤30% (HR = 0.344).

Conclusions: Multivariate and subgroup analyses of the primary study endpoint PFS favoured lenalidomide over IC therapy in providing consistent clinical benefit in patients with R/R MCL irrespective of baseline demographics or disease characteristics.

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POOR OVERALL SURVIVAL OF PATIENTS WITH IBRUTINIB-RESISTANT MANTLE CELL LYMPHOMA

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Introduction: Despite unprecedented clinical activity in previously treated mantle cell lymphoma (MCL), primary and acquired resistance to ibrutinib is common. The outcomes and ideal management of patients with ibrutinib-refractory MCL are unclear.

Methods: We performed a retrospective cohort study of all patients with MCL that experienced disease progression while receiving ibrutinib across 13 international sites. Medical records were evaluated for clinical characteristics, therapies used pre and post ibrutinib, and pathology and radiology data. Time-to-event statistics were estimated using the Kaplan–Meier method. Cox proportional hazard regression was used to calculate hazard ratios and test statistical significance.

Results: A total of 106 subjects (80 men and 26 women) met the eligibility criteria. The median age at start of ibrutinib was 68 years (range 46–85), and the median number of prior therapies was 3 (range 0–10). The median time from prior therapy to ibrutinib was 2 months and 49% of patients were resistant to their last prior therapy. The MIPI scores at start of ibrutinib were 46% high, 31% intermediate and 23% low risk. The ORR was 54% (PR 43% and CR 11%). The median time on ibrutinib was 4 months (range 0–42). The median overall survival (OS) following cessation of ibrutinib was 2.9 months (95% CI 1.5–4.9 months), with 32.8% of patients surviving for at least 6 months and 17.2% of patients surviving for at least 1 year. The median Ki67 was 23% in those patients evaluated immediately prior and 77% in those patients evaluated immediately following ibrutinib. Of the 96 patients with data available, 66 underwent subsequent treatment with 5 achieving CR and 12 achieving PR. Twenty-nine subjects underwent a second subsequent treatment at a median of 2 months from the start of the first post ibrutinib treatment. Multivariate Cox regression analysis of MIPI prior to ibrutinib, best response to ibrutinib, duration of ibrutinib and subsequent treatment with alkylator, bendamustine, anthracycline, purine analog, cytarabine, methotrexate, rituximab, bortezomib and lenalidomide revealed that only MIPI prior to ibrutinib and duration of ibrutinib were associated with OS (HR 1.81, $p = 0.017$ and HR 0.94, $p = 0.037$, respectively). Of 4 patients that underwent allogeneic stem cell transplantation as part of therapy immediately following ibrutinib failure, all died (3 due to lymphoma and 1 due to toxicity).

Conclusions: Poor clinical outcomes were noted in majority of patients with primary or secondary ibrutinib resistance. Patients with a low-risk MIPI score prior to ibrutinib and a long duration of ibrutinib had longer survival following ibrutinib, but we could not identify treatments that clearly improved outcomes. Future trials should focus on preventing ibrutinib resistance and on treatment following ibrutinib.

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ATRIAL FIBRILLATION IN IBRUTINIB TREATED RELAPSED MANTLE CELL LYMPHOMA, CLINICAL AND LABORATORY ANALYSIS OF RISK FACTORS

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Introduction: Ibrutinib (ibr) has been a breakthrough therapy for many lymphoid malignancies. However, Ibr has also been shown to increase risk of atrial fibrillation (afib), as ibr clinical trials have reported afib incidence of 3.5–6.5% in pts receiving ibr, which is much higher compared to that of the general population (1%). Currently, there is a paucity of information regarding the development of afib during ibr use in the clinic.

Method: Data from 105 pts with relapsed mantle cell lymphoma (MCL) who were started on either ibr or ibr/rituxan (R) treatment protocols at MD Anderson Cancer Center were included. Pt demographics including clinical and cardiac risk factors as well as afib status were scored.

Results: In brief, 105 pts were treated for relapsed MCL with ibrutinib, shown in Table 1. Multivariate analysis showed that hypertension (HTN, defined as known history or currently on antihypertensive medication at the time of ibr initiation) and coronary artery disease (CAD, defined as positive stress test or positive cardiac catheterization prior to start of ibr) were significant risk factors for afib ($p = 0.019$ and 0.0085, respectively). Analysis of pts who developed afib during treatment showed that HTN and CAD conferred odds ratios of 5.05 and 4.54, respectively. These odds ratios were markedly higher than previously reported ORs for the general population (odds ratios: 1.35 and 1.4, respectively, *Ciaroni et. al.* Am Heart J 2000, *Benjamin et. al.* JAMA 1994). Onset of afib was also precipitous: pts who developed afib did so within the first year of treatment.

Conclusions: Our preliminary data suggests that ibr increases risk for afib, particularly among patients who already have HTN or CAD as pre-existing risk factors. Furthermore, the onset of development occurred within a year of beginning treatment, suggesting that periodic ECG monitoring should be considered for patients with known HTN or CAD within the first year of ibr therapy.

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	Total		
Total *N*	105		
Protocol I/IR	55/50		
Age (range) years	69 (35–89)		
# Treatment prior to ibr (range)	2 (1–9)		
2Men (%)	85 (81)		
	No afib before or after	**Afib prior to ibr**	**New afib after ibr**
N (%)	86 (82)	14 (13)	5 (5)
Age (range)	69 (35–88)	70 (58–86)	66 (63–72)
Men (%)	67 (78)	14 (100)	5 (100)
HTN (%)	38 (44)	13 (93)	4 (80)
CAD (%)	11 (13)	7 (50)	2 (40)
Smoking (%)	38 (44)	9 (64)	3 (60)
Δ Atrial size (range %)	3% (−37 to 90)	59% (−14 to 70)	27% (7–40)
Δ QTcF (range %)	−2% (−14 to 6)	−3% (−12 to 8)	1% (0–4)
Time afib (days)	N/A	N/A	144 (81–371)

N/A: not applicable.

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HIGH DOSE SEQUENTIAL CHEMOTHERAPY WITH RITUXIMAB AND ASCT AS FIRST-LINE THERAPY IN ADULT MCL PATIENTS: CLINICAL AND MOLECULAR RESPONSE OF THE MCL0208 TRIAL, A FIL STUDY

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Introduction: In 2008, the Fondazione Italiana Linfomi (FIL) designed the phase III trial MCL0208 to evaluate the efficacy and safety of lenalidomide as maintenance therapy in patients (pts) with MCL achieving at least a PR after an upfront intensive chemotherapy with rituximab (R) and ASCT (NCT02354313). Herein, we present the analysis of clinical and molecular response after the chemotherapy with rituximab (R) and ASCT, one of the secondary objectives of MCL0208 study.

Methods: Adult pts (<66 years) with advanced stage MCL without clinically significant comorbidities are enrolled. The primary end point of the study is the 2-year PFS from randomization. Pts receive 3 cycles of R-CHOP-21, followed by R-HDS that includes R-high-dose cyclophosphamide (R-HD-CTX; 4 g/m^2), 2 cycles of R-high-dose Ara-C (R-HD-Ara-C) (2 g/m^2 q12 × 3 days), followed by BEAM and ASCT. CD34+ cell harvest is performed after the first course of R-HD-Ara-C. A second harvest will be performed after the second course of R-HD-Ara-C, if prior harvest is PCR+. After ASCT, responding pts are randomized between maintenance with lenalidomide (15 mg days 1–21 every 28 days) or observation for 24 months. Minimal residual disease (MRD) is examined at diagnosis, after R-HDCT, before and after ASCT, during maintenance/observation and during follow-up every 6 months. The total number of patients to be enrolled is 300.

Results: From May 2010 to November 2014, 260 pts have been enrolled by 49 cancer centres. The median age was 57 years (IQR 51–61), predominantly male (80%) and the majority of pts presented with advanced stage (98%), poor ECOG-PS (24%), bulky disease (>5 cm; 33%), elevated LDH (31%), BM infiltration (76%) and intermediate-high MIPI (53%). Nine per cent had blastoid variant. Among the 260 enrolled pts, 187 completed R-HDS (72%).

Ultimately, 168 pts (65%) proceed to ASCT and 146 (56%) have been randomized between lenalidomide or observation. At the time of the present analysis according to Cheson (2007), of 202 pts evaluable for final response, 137 (68%) reached CR after RHDS and 156 (77%) after ASCT. Regarding MRD, a molecular marker

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was found in 87%. Before ASCT, molecular responses on PB and BM were 72% and 53% by nested PCR and 80% and 67% by RQ-PCR. After ASCT molecular responses on PB and BM were 79% and 50% by nested PCR and 86% and 73% by RQ-PCR. After a median follow-up of 19 months the 2-year PFS and OS were 77% and 88%, respectively. As expected with intensive regimens, there was an haematological toxicity, particularly CTC grades 3–4 neutropenia (38% of cycles) and thrombocytopenia (31% of cycles), but the infections were recorded only in 17% of patients and the treatment-related deaths (TRD) were 1.6%.

Conclusions: R-HDS with ASCT is a feasible regimen with limited toxicity in a multicentre setting and produces a high rate of durable responses. These promising results are supported by the high rate of molecular responses by RQ-PCR.

AGGRESSIVE B-CELL LYMPHOMA

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THE STANDARD, REVISED AND A SIMPLIFIED INTERNATIONAL PROGNOSTIC INDEX RELIABLY PREDICT OUTCOME IN PATIENTS WITH PET/CT-STAGED DLBCL TREATED WITH R-CHOP

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Introduction: The standard and revised international prognostic index (IPI and R-IPI, respectively) estimate prognosis in diffuse large B-cell lymphoma (DBLCL) using 5 factors: age, LDH, performance status, stage and number of extranodal sites. However, these indexes were developed before the introduction of staging 18F-FDG PET/CT, which assesses the number and sites of extranodal involvement in DLBCL more accurately than traditional staging investigations. By refining two components of the IPI, stage and number of extranodal sites, PET/CT may more accurately predict outcomes.

Methods: Adult patients with newly diagnosed, PET/CT-staged DLBCL treated with R-CHOP or R-CHOP-like chemotherapy were retrospectively identified from institutional databases in Aalborg ($n = 155$, 2007–2012), Copenhagen ($n = 202$, 2009–2012) and British Columbia ($n = 87$, 2011–2012). The original staging PET/CT reports were reviewed to determine stage and sites of extranodal involvement. The prognostic impact of IPI and R-IPI subgroups were evaluated using the Kaplan–Meier method and compared using the log-rank test. A multivariate analysis was used to evaluate the impact of the number of extranodal sites on PFS and OS, adjusted for age, LDH and performance status.

Results: A total of 444 patients were identified with median age of 65 years (range 16–90), 57% men, 51% elevated LDH, 17% performance status >1 and 41% B symptoms. Extranodal disease was identified in 297 (67%) patients, including 121 (27%) with >1 site involved. Ann Arbor stage was $n = 97$ (22%) stage I, $n = 69$ (15%) stage II, $n = 67$ (15%) stage III and $n = 211$ (48%) stage IV.

With a median follow-up of 2.4 years (range 5 months–6.5 years), the 3-year PFS and OS were 69% and 73%, respectively. The distribution and outcomes of the different IPI and R-IPI groups are shown in the table. Both indexes identified distinct 3-year PFS and OS for each of their categories. Patients with very good R-IPI experienced 3-year PFS and OS 100%.

In multivariate analyses, involvement of >2 extranodal sites was associated with worse PFS (HR 3.05, 95% CI 2.03–4.57, $p<0.001$) and OS (HR 2.26 (95% CI 1.41–3.63, $p = 0.001$). Therefore, IPI and R-IPI were recalculated using age >60 years, performance status >1, elevated LDH, >2 extranodal sites and exclusion of stage. This simplified index resulted in very similar 3-year PFS and OS across all subgroups.

Conclusion: In this cohort of PET/CT-staged patients with DLBCL treated with R-CHOP, the IPI and R-IPI identified distinct prognostic subgroups. Patients with very good R-IPI experience 3-year PFS and OS 100%. A simplified version of the IPI and R-IPI provides very similar prognostic estimates.

Abstract 210 Table

Risk group	Number of IPI factor	Number of patient (%)	3-year PFS % (95% CI)	3-year OS % (95% CI)
IPI				
Low	0, 1	140 (31)	87 (79, 95)	91 (85, 97)
Low–intermediate	2	118 (27)	72 (62, 87)	74 (64, 84)
High–intermediate	3	103 (23)	57 (45, 69)	64 (54, 74)
High	4, 5	83 (19)	50 (38, 62)	55 (43, 67)
Simplified IPI				
Low	0, 1	148 (33)	87 (81, 93)	91 (85, 97)
Low–intermediate	2	134 (30)	69 (59, 79)	72 (62, 82)
High–intermediate	3	103 (23)	57 (45, 69)	63 (51, 75)
High	4	59 (14)	47 (33, 61)	54 (40, 68)
R-IPI				
Very good	0	49 (11)	100 (88, 100)	100 (88, 100)
Good	1, 2	209 (47)	76 (68, 84)	79 (71, 87)
Poor	3, 4, 5	186 (42)	54 (46, 62)	60 (52, 68)
Simplified R-IPI				
Very good	0	51 (12)	100 (88, 100)	100 (88, 100)
Good	1, 2	231 (52)	74 (68, 80)	78 (72, 84)
Poor	3, 4	162 (36)	53 (43, 63)	59 (51, 67)

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FEMALE PATIENTS WITH DLBCL AND INVOLVEMENT OF THE REPRODUCTIVE ORGANS HAVE POOR OUTCOMES AND MARKEDLY INCREASED RISK OF CNS RELAPSE WITH R-CHOP(-LIKE) THERAPY

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Background: Diffuse large B-cell lymphomas (DLBCL) involving the reproductive organs are rare. While testicular involvement with DLBCL has been well described, little is known about the outcome of DLBCL involving the internal female reproductive organs (ovaries and uterus).

Methods: This is a retrospective study of patients with newly diagnosed DLBCL presenting to hospitals in Denmark, British Columbia Cancer Agency (Canada) and Peter MacCallum Cancer Centre (Australia) identified through searches of national/local registries. Inclusion criteria were staging that included PET/CT and treatment with R-CHOP/CHOEP ± CNS prophylaxis ± radiotherapy. Patients with known CNS involvement at diagnosis were excluded.

Results: Among 1389 patients, 69 (5%) had reproductive organ involvement. Testicular involvement was seen in 45 (6%) of men and female reproductive organ

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involvement in 24 (4%) of women (uterus $n = 13$, ovaries $n = 10$ and both $n = 1$). Among women, reproductive organ involvement was more frequently associated with stages III–IV disease and poor risk IPI score (Table). CNS prophylaxis was used in 36/45 men (IT alone 19, systemic 9 and both 8) and 8/24 women (IT alone 3, systemic 2 and both 3). The median follow-up was 41 months for women and 61 months for men. Involvement of female reproductive organs was associated with inferior overall survival (OS) in sex-stratified multivariate analysis (MVA) including IPI (HR 1.8, 95% CI 1.0–3.3). Testicular involvement was not adversely prognostic for OS (HR 1.2, 95% CI 0.7–2.2) in MVA. The 2-year risk for CNS events was higher for women with reproductive organ involvement (Table). The increased risk of CNS events was confirmed for uterine involvement (HR 17.6, 95% CI 6.1–50.7) in MVA with inclusion of CNS prophylaxis and high-risk disease according to the recently validated DSHNHL risk model (Savage 394a, ASH 2014) for CNS relapse (5 IPI risk factors + kidney/adrenal involvement) but not for ovarian involvement. Testicular involvement was associated with a trend for increased risk of CNS relapse in MVA (HR 2.1, 95% CI 0.7–5.7).

Conclusions: Although involvement of reproductive organs in women mainly occurred in the context of disseminated DLBCL, the number of CNS events among women with uterine DLBCL was strikingly high and screening for occult CNS disease at diagnosis and consideration of CNS-directed prophylaxis may be appropriate in these patients. The widespread use of CNS prophylaxis for patients with testicular lymphomas likely reduced their risk for CNS events.

Abstract 211 Table

	Women ($n = 24$)	Men ($n = 45$)	*p*-Value
Median age, years	65	73	0.04
Stages III–IV disease, %	88	34	<0.01
Poor-risk IPI (3, 4, 5), %	67	22	<0.01
DSHNHL CNS high risk, %	46	18	0.02
2-year PFS, %	50	84	0.01
2-year CNS relapse, %	28	3	0.03[a]

[a]Log rank, entire follow-up period.

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EVALUATION OF THE SITE OF CENTRAL NERVOUS SYSTEM (CNS) RELAPSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) BY THE CNS RISK MODEL

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Introduction: We recently validated the German high-grade non-Hodgkin's lymphoma study group (DSHNHL) CNS prognostic model that incorporates the 5 IPI factors and kidney/adrenal involvement to stratify diffuse large B-cell lymphoma (DLBCL) patients into 3 risk groups (grps): 0–1 factors—low risk (LR), 2–3 factors—intermediate risk (IR) and 4–6 factors—high risk (HR), with 2-y risks of CNS relapse of <1%, ~5% and >10%, respectively (Savage ASH 2014, 394a). However, the site of CNS relapse in these risk grps has not been reported, which would help to guide the optimal strategy for CNS prophylaxis in HR patients (pts).

Methods: The BC Cancer Agency lymphoid cancer database was used to identify all pts ≥16 y of age with *de novo* DLBCL between December 2000 and August 2014 treated with curative intent R-CHOP. Pts were excluded if they had HIV, CNS involvement at diagnosis, PMBCL or transformed lymphoma.

Results: One thousand eight hundred fifty-two pts were identified, including 1597 pts from the original validation cohort (Savage ASH 2014, 394a). One hundred twenty pts (6%) were excluded due to missing information. For the remaining 1732 pts, the median age was 66 y (range 16–94), 46% had an IPI ≥3 and 71 (4.1%) had kidney/adrenal involvement. The frequency of pts in the LR, IR and HR grps was 30% ($n = 519$), 46% ($n = 797$) and 24% ($n = 416$), respectively. Forty-eight pts (2.8%; HR grp 3.6%) received CNS prophylaxis (IT chemotherapy $n = 36$; high-dose methotrexate (HDMtx) $n = 12$).

With a median follow-up for living pts of 4.3 y, 84 pts developed CNS relapse. The 2-y risks of CNS relapse for LR, IR and HR grps were 1.3%, 4.6% and 12.6%, respectively. The median time to CNS relapse for all pts was 7.2 m (range 0.7–96) and was longer in the LR grp (17.5 m). Overall, 45/84 pts (53.6%) experienced an isolated CNS relapse and only 3/45 (6.7%) of these pts had a subsequent systemic relapse. The remainder had systemic disease at the time of CNS relapse with similar findings across the risk grps ($p = 0.60$). In pts with CNS relapse, 61% had isolated parenchymal (P), 26% isolated leptomeningeal (LM) and 12% concurrent P-LM. There was a greater proportion of pts in the HR grp with any LM involvement (47.5%; $p = 0.06$).

Conclusion: CNS relapse in DLBCL predominantly involves the brain parenchyma possibly reflecting the poor CNS penetration of the R-CHOP components, including rituximab. LM disease also frequently occurs in the HR grp, and CSF analysis at diagnosis is warranted. These data support further investigation of CNS prophylaxis in HR grps using HDMTx or other novel agents that can penetrate all CNS compartments.

Abstract 212 Table 1 CNS relapse characteristics according to CNS risk groups

Factors	All $N = 1732$ *n* (%)	Low-risk $N = 519$ *n* (%)	Intermediate $N = 797$ *n* (%)	High-risk $N = 416$ *n* (%)	*p*
2-yr CNS relapse risk	77 (5.3)	6 (1.3)	31 (4.6)	40 (12.6)	<0.01
Median time to CNS relapse (months)	7.2	17.5	7.3	6.3	<0.01
Early relapse (<1 year from diagnosis)[a]	61 (72.6)	4 (33.3)	22 (68.8)	35 (87.5)	<0.01
CNS prophylaxis					
All	48 (2.8)	13 (2.5)	20 (2.5)	15 (3.6)	0.07
IT chemotherapy	36 (75)	11 (84.6)	17 (85)	7 (46.7)	
HDMTx	12 (25)	2 (15.4)	3 (15)	8 (53.3)	
Type of CNS relapse[a]					
Isolated	45 (53.6)	8 (66.7)	16 (50)	21 (52.5)	0.60
Concurrent systemic	39 (46.4)	4 (33.3)	16 (50)	19 (48.7)	
Site of CNS relapse[a]					
Parenchymal	51 (60.7)	7 (58.3)	23 (71.9)	21 (52.5)	0.06
Leptomeningeal	32 (38.1)	4 (33.3)	9 (28.1)	19 (47.5)	
Isolated leptomeningeal	22 (26.2)	3 (25)	7 (21.9)	12 (30)	
Concurrent parenchymal	10 (11.9)	1 (8.3)	2 (6.3)	7 (17.5)	
Intraocular	1 (1.2)	1 (8.3)	0 (0)	0 (0)	

[a]Denominator is pts with CNS relapse ($n = 84$).

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CLINICAL AND TREATMENT-RELATED FEATURES DETERMINING THE RISK OF LATE RELAPSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: In a population-based setting and with currently available 1st line therapies, a complete remission (CR) can be obtained in approximately 60% of patients with diffuse large B-cell lymphoma (DLBCL). Of all patients who experience a relapse, a subset will develop it late, i.e. >5 years after achievement of CR. Whether there are clinically exploitable differences in the biology and behaviour of early vs late relapses remains to be determined. The aim of the present study was to analyse a large population-based DLBCL cohort in order to identify (i) the overall frequency of late relapses (LR); (ii) clinical and paraclinical parameters influencing the risk of LR; and (iii) the impact of introducing rituximab on the occurrence of LR.

Methods: The data sets of 7247 DLBCL patients diagnosed within the period of 1983–2014 were obtained from the population-based Danish Lymphoma Registry (LYFO). Only data from patients with a documented CR, defined according to the 1999 Cheson criteria, were utilized for the study. Early relapse (ER) was defined as biopsy-proven reoccurrence of disease ≤2 years after achievement of 1st CR. Correspondingly, the disease-free interval required to fulfil the definition of LR was 5 years or more after 1st CR. To better separate ER from LR events, an intermediate patient group with relapse occurring between 2 and 5 years from CR ($n = 184$) was excluded from the analysis.

Results: Of 4097 patients reported as having achieved a CR upon 1st line treatment, 3279 (80%) maintained a continuous CR, whereas 818 (20%) had a registered relapse. Among relapsed patients, 556 (68% of all relapses) had an ER, while 78 (10% of all relapses) relapsed ≥5 years after CR. Patients with LR presented with lower IPI ($p = 0.019$) and better performance score (WHO; $p = 0.004$) compared to ER patients. LDH elevation was found significantly more frequently in relapse patients (ER and LR alike) compared to non-relapse patients. Interestingly, focusing on treatment modalities, the use of radiotherapy was associated with a lower rate of ER ($p < 0.0001$), while it did not affect the rate of LR. On the other hand, the use of rituximab significantly lowered the occurrence of both ER and LR ($p < 0.0001$ and $p < 0.0001$, respectively). The choice of chemotherapy regimen did not impact on the risk of relapse (neither ER nor LR).

Conclusions: The risk of relapse after 5 years or more in CR did not appear to be predicted by specific clinical or paraclinical parameters at the time of diagnosis. In accordance with observations done by other groups, LR was more frequently associated with low-risk features and did not correlate with pre-therapeutic BM infiltration. An intriguing observation was that while the use of radiotherapy lowered the risk of ER, it did not seem to affect the rate of LR. Conversely, the use of Rituximab effectively reduced the risk of both early and late events suggesting a longer-lasting biological effect, possibly even at pre-malignant level.

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DIFFUSE LARGE B-CELL LYMPHOMA SURVIVAL BY SUBTYPE: A META-ANALYSIS OF GENE EXPRESSION PROFILING AND IMMUNOHISTOCHEMISTRY ALGORITHMS

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Introduction: Patients (pts) with diffuse large B-cell lymphoma (DLBCL) may have widely divergent outcomes despite having histologically similar tumors. Although gene expression profiling (GEP) and immunohistochemistry algorithms (IHC) have assigned pts to the favorable prognosis subtype germinal center B-cell-like (GCB), or the unfavorable activated B-cell-like (ABC) subtype, no standard exists for prognostic subtyping.

Methods: We performed a systematic review of studies in the MEDLINE database that directly compared overall survival (OS) and progression-free survival (PFS) for GCB and ABC/non-GCB subtypes generating 361 papers that were evaluated for inclusion. Inclusion in the meta-analysis was restricted to: (i) articles published in English 2007–2013; (ii) pts with *de novo* DLBCL, treated with a rituximab (R) + anthracycline-based chemotherapy regimen; (iii) direct comparison between GCB and ABC/non-GCB subtypes (Kaplan–Meier survival data with hazard ratio (HR), or data from which the HR could be calculated); (iv) no duplicated data. Engauge Digitizer was used to extract the data points from the OS plots, and these data points were used to fit parametric survival models. We compared Gompertz, Weibull and log-logistic models goodness-of-fit for all curves according to the Akaike information criterion and the Schwarz Bayesian criterion.

Results: We identified 24 articles for inclusion in meta-analyses that were conducted comparing survival outcomes for pts with GCB and ABC/non-GCB subtype by GEP and the Hans, Choi or Muris IHC algorithms. Pts with GCB DLBCL defined by GEP (OS hazard ratio [HR] 1.85; 95% confidence interval [CI], 1.5–2.4; PFS HR 1.80; 95% CI, 1.4–2.4) and the Muris algorithm (OS HR 2.12; 95% CI, 1.5–3.0; PFS HR 2.07; 95% CI, 1.3–3.4) had significantly better survival than pts with ABC/non-GCB DLBCL, but the Muris algorithm assigned more patients to the GCB subtype, in a ratio of 1.9:1. IHC subtyping using the Hans (OS HR 1.27; 95% CI, 0.9–1.6; PFS HR 1.49; 95% CI, 1.1–2.0) or Choi algorithms (OS HR 1.04; 95% CI, 0.7–1.6; PFS HR 1.12; 95% CI, 0.5–2.3) did not demonstrate significant differences in OS between GCB and non-GCB groups in meta-analyses. A Gompertz distribution demonstrated the best fit and generated 5-year OS estimates of 73% for GCB DLBCL and 52% for pts with ABC DLBCL by GEP.

Conclusions: GEP profiling to determine cell of origin produces subgroups with significantly different prognoses and is preferred for informing treatment decisions for pts with DLBCL.

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SPLEEN INVOLVEMENT IDENTIFIED ON BASELINE PET IMAGING INFLUENCES OUTCOME OF YOUNG PATIENTS WITH HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP14 BUT NOT R-ACVBP

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Introduction: Extranodal involvement is considered a poor prognostic factor in patients (pts) with diffuse large B-cell lymphoma (DLBCL). However, pts with a spleen involvement (SI) identified on the baseline CT were not found to have a worse outcome in the rituximab era (Cancer, 2012; 118: 4166). Due to its better sensitivity to identify extranodal sites, PET may improve SI detection in DLBCL patients. Thus, we explored the adding value of PET to detect the SI and its prognosis impact in pts prospectively enrolled in a phase II randomized trial testing 2 R-chemo regimens and a PET-driven consolidation strategy (NCT00498043).

Methods: Eligible pts for the present study had to be enrolled in the LNH07-3B trial and to have a baseline PET available for central review and SI evaluation. All pts were aged 18–59 y, with a previously untreated aaIPI 2–3 DLBCL and were randomly assigned to 4 cycles of either R-ACVBP14 or R-CHOP14 induction. Consolidation treatment was driven by centrally reviewed interim PET assessment during induction treatment as previously published (Blood 2011, 118: 37). Spleen was

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considered focally involved in case of hypermetabolic nodule or diffusely involved when the SUVmax of spleen was higher than the liver SUVmax. Pts with a reduction of SUVmax >70% between baseline PET and PET4 (DSUV0-4) were considered good responders.

Results: One hundred sixty-five pts with a median age of 45 y were included: Ninety-seven per cent had stage III/IV, 28% a bulky mass >10cm, 96% elevated LDH, 26% ECOG ≥ 2. A SI was found in 101 pts (61%) including 11 pts (11%) with a focal involvement, 47 (47%) with diffuse involvement and no splenomegaly, 43 pts (43%) with a spleen size>13 cm and focal or diffuse PET positivity. SI was not related to tumour bulk, Ann Arbor stage, elevated LDH or treatment arm but was more frequently associated to B symptoms (66% vs 31%, $p < 10\text{-}4$). In the whole cohort with a 45 months median follow-up, patients with a SI had a significantly shorter 4-y PFS (71% vs 85%, $p < 0.035$) and 4 y-OS (81% vs 93%, $p < 0.05$), while the outcome of pts with splenomegaly or normal size spleen was similar. However, the poor prognosis related to the SI was only observed in the R-CHOP arm (4-y PFS: 63% vs 94%, $p = 0.0006$ and 4-y OS: 75% vs 100%, $p = 0.005$, respectively) but not in the R-ACVBP arm ($p = 0.7$ and $p = 0.9$ for PFS and OS, respectively). In the R-CHOP arm, multivariate analysis showed that SI (HR = 0.08, $p < 0.02$) and DSUV0-4 (HR = 5.4, $p < 0.002$) had independent prognosis value for PFS and only DSUV0-4 (HR = 5.5, $p < 0.02$) retained prognosis value for OS. Thus, among pts with DSUV0–4 >70% of those who had no SI had a 100% 4-y PFS compared to a 68% 4-y PFS for those with a spleen positive PET ($p = 0.001$). Inversely among poor responder pts those with or without SI had similar outcome.

Conclusions: Spleen involvement identified on the basis of the baseline PET allows to recognize among young high-risk DLBCL patients treated with R-CHOP14 those with high risk of treatment failure. R-ACVBP overwhelms the prognosis impact of spleen involvement.

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EXCELLENT OUTCOME OF PATIENTS OVER 80 YEARS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH STANDARD THERAPY: A LARGE RETROSPECTIVE STUDY FROM 4 INSTITUTIONS

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Introduction: In contrast with improved outcome in young adults, lymphoma-related mortality in very elderly people (i.e. over 80 yrs) has not declined in the past three decades. This dismal prognosis is mainly due to decreased tolerance to standard curative and to the administration of non-curative less aggressive regimens, which are most frequently used in this population. Currently, there are no validated methods to prospectively identify elderly patients (pts) fit enough to receive optimal immunochemotherapy. DLBCL pts older than 80 yrs are not usually included in prospective clinical trials, and their management remains a matter of debate. However, a significant proportion of these pts may tolerate standard treatment if they do not present important comorbidities. Very few studies thus far were specifically focused on this particular population.

Methods: We performed a retrospective analysis using the local databases of 4 referral institutions in Switzerland and Northern Italy. We included all pts with newly diagnosed DLBCL treated from 1981 to 2013. Pts were treated with a variety of regimens, which were chosen by the treating physicians. Clinical characteristics at diagnosis and the presence of comorbidities were retrieved from the databases and clinical charts. Primary endpoints were overall survival (OS), progression-free survival (PFS) and cause specific survival (CSS).

Results: We identified 281 HIV-negative DLBCL pts. Clinical characteristics at diagnosis were as follows: median age 84 yrs (range 80–97), 114 (41%) men, 144 (51%) stages III–IV, 42 (37%) bulky disease >7 cm, 57 (20%) with B symptoms and 164 (58%) with ECOG PS 0–1. Charlson comorbidity index was 0 63 (29%), 1–2 89 pts (41%), 3–4 44 pts (20%) and >4 21 pts (10%). Eighty-five pts (30%) were treated before 2002. Pts were treated as follows: 14 (5%) no active treatment or only steroids, 28 (10%) radiation therapy or surgery alone, 73 (26%) chemotherapy without anthracycline, 166 (59%) chemotherapy regimens with anthracycline and 119 (50%) chemotherapy regimen with anthracycline and rituximab. At a median follow-up of 5.7 years (range 3.2–9.8), 5-year PFS was 26% (95% CI, 20–32%), 5-year OS was 31% (95% CI, 25–37%), and 5-year CSS was 42 % (95% CI, 35–49%) for the entire studied population. Five-year CSS was significantly higher in pts receiving at least some cycles of a standard regimen comprising anthracyclines and rituximab (57% vs 33%, $p < 0.001$). International prognostic index (IPI), LDH, PS and albumin level appeared to be prognostic also in this population.

Conclusions: To our knowledge, the present study is one of the largest analyses including this particular population ever presented. The accurate selection of pts >80 yrs able to tolerate RCHOP/RCHOP-like regimens is crucial. Fit pts without significant comorbidities should be treated with standard immunochemotherapy, which allows to achieve an excellent outcome.

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VINCRISTINE OMISSION, BUT NOT DOSE REDUCTION, IS ASSOCIATED WITH DECREASED SURVIVAL IN ELDERLY DLBCL PATIENTS

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Introduction: The elderly population is most vulnerable to chemotherapy toxicities due to age-related physiology and pre-existing comorbidities. Standard RCHOP-like chemotherapy for DLBCL includes vincristine (VCR). Pre-existing/developing neuropathy or GI dysmotility leads to frequent modifications in VCR dosing, yet there is few data on the outcome impact of such modifications.

Methods: This IRB-approved retrospective analysis included all pts ≥70 years old with pathologically confirmed aggressive NHL treated at MSKCC with (± R) CHOP-like from 1999 to 2009. VCR dosing was categorized as: standard dosing 1.4 mg/m^2 with 2 mg cap (Stnd), no VCR given with any cycle (NV), partial VCR (doses omitted for ≥3 cycles; PV) and any dose reduction of VCR (RV). Outcomes included OS, PFS and incidence of toxicities with focus on GI and/or neurological toxicities associated with VCR. Kaplan–Meier analysis was used to estimate OS and PFS. Candidate factors on UVA were incorporated into an MVA for each endpoint. Fisher's exact test was used to analyse toxicities and VCR groups.

Results: Three hundred twenty-five pts were analysed. Baseline demographics included: median age 76 (range, 69–94) years; men 47%; DLBCL 90%, other NHL 10%; Hans Model GC subtype 30%, non-GC 16%, undetermined 54%; advanced stage IIX/III/IV 65%, KPS ≤ 70 28%; aaIPI 2/3 47%, rituximab given 82%; and 6 + cycles of therapy 60%. Comorbidities predisposing to VCR toxicities were present in 40% of pts. VCR tx categories: Stnd 69%, RV 21%, NV 6% and PV 5%. Dose reductions of other agents >2 cycles occurred in 7%. Median f/u for the entire group was 65.3 mos.

Median PFS was 68.4 mos (95% CI 55.3–83.8) and median OS was 88.5 mos (95% CI 75.7–102.1). Variables included in UVA but $p > 0.05$ for PFS and OS: gender, histology, cell of origin and rituximab use. Age, aaIPI and NV vs Stnd were significant for PFS and OS in MVA. UVA and MVA for OS are in Table 1. Modifications in VCR dosing were correlated with baseline comorbidities. Use of VCR was

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correlated with more grade ≥3 non-haematological toxicities ($p < 0.001$) and GI/neurological toxicities ($p = 0.047$).

Conclusions: In pts where VCR was completed excluded, OS and PFS were significantly reduced. Pts that did not receive VCR often had the highest comorbidities, a confounding fact that may also have impacted survival and VCR omission. Dose reductions (PV and RV) in VCR, however, were not associated with survival impact and appear safe to enact given the increased risk for developing toxicities.

Abstract 217 Table 1 UVA and MVA for OS with significant factors ($p < 0.05$)

	UVA	MVA
	HR, *p*-value	
Age at dx	1.09, <0.001	1.07, <0.001
Stages I/II vs III/IV	1.37, 0.06	0.67, 0.17
aaIPI 0 vs 2/3	0.4, <0.001	0.33, 0.002
1 vs 2/3	0.61, <0.001	0.57, 0.02
Full vs modified tx	1.48, 0.06	1.00, 0.99
NV vs stnd	3.13 <0.001	2.73, <0.001
PV vs stnd	1.89, 0.046	1.06, 0.87
RV vs stnd	1.41, 0.08	0.86, 0.50
Dose reduction of other agents	2.25, 0.003	1.43, 0.25

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OUTCOME ANALYSIS OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN HIV-INFECTED AND IMMUNOCOMPETENT (IC) PATIENTS: THE SWISS HIV COHORT STUDY (SHCS)

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Introduction: Despite the introduction of highly active anti-retroviral therapy (HAART), lymphomas are still an important complication of HIV infection, occurring at higher frequency than in IC individuals. DLBCL represents the most common AIDS-related lymphoma (ARL). The prognostic factor and outcome of a population of HIV-related DLBCL were compared to those of DLBCL in IC patients (pts).

Methods: A population of 58 HIV-related DLBCL pts from the SHCS diagnosed and treated from 2004 to 2011 was retrospectively analysed and compared to 326 consecutive IC pts with a diagnosis of DLBCL in the same period whose clinical information was 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 (yrs), median overall survival (OS) was 10 yrs (IQR:3-nr); 5-yr OS was 68% (95% CI:63–73%) in IC pts and 63% (95% CI: 49–75%) in HIV pts, respectively, with no significant difference ($p = 0.220$). Age older than 60 yrs, presence of B-symptoms at diagnosis, ECOG performance status (PS) > 1, advanced Ann Arbor stage, high lactate dehydrogenase (LDH) serum level, more than one extranodal site of disease, high risk according to International Prognostic Index (IPI) and treatment with immunochemotherapy (RCHOP/RCHOP-like regimens) had a significant impact on OS in the whole population. In multivariate analysis, presence of B-symptoms and high risk according to IPI retained statistical significance. In the subset of HIV-infected pts, the risk according to ARL-IPI had a significant impact on OS.

To more reliably compare the two populations whose median age at diagnosis was significantly different (Table 1), the analysis was focused on the 113 pts (37 HIV-infected and 76 IC) uniformly treated with RCHOP/RCHOP-like regimens and younger than 55 yrs. After median follow-up of 5.5 yrs, 12 (16%) IC pts and 8 (22%) HIV-infected pts died. Median OS was not reached, and no significant difference was observed in the two subsets in OS ($p = 0.262$). Nevertheless, a higher proportion of early deaths was observed in the HIV-infected pts despite of the younger age at diagnosis of lymphoma. Indeed, 2-yr OS was 95% (95% CI:87–98%) in IC pts and 77% (95% CI:60–88%) in HIV-infected pts, probably due to treatment and lymphoma-related events.

Conclusions: In the rituximab and HAART era, HIV-infected DLBCL pts have a long-term survival similar to IC pts when treated with curative intent.

Abstract 218 Table 1 Clinical characteristics at diagnosis

Feature	HIV-negative pts (%)	HIV-positive pts (%)	*P*-value
Median age years (range)	67 (22–89)	49 (17–76)	<0.0001
Sex male	168/326 (52)	50/58 (86)	<0.0001
B-symptoms present	88/324 (27)	31/58 (53)	<0.0001
ECOG PS 2–4	54/322 (17)	23/58 (40)	<0.0001
Ann Arbor stages III–IV	193/326 (59)	44/56 (79)	0.006
LDH serum level >UNL	167/315 (53)	29/51 (57)	n.s.
Bone marrow involved	45/326 (14)	5/53 (9)	n.s.
Extranodal disease >1 site	138/326 (42)	25/57 (44)	n.s.
IPI risk int–high/high	153/322 (48)	25/52 (48)	n.s.
Type of chemotherapy			
Rituximab including	326/326 (100)	52/56 (93)	<0.0001
Anthracycline including	326/326 (100)	48/56 (86)	
RCHOP/RCHOP-like	326/326 (100)	48/56 (86)	
HIV-specific			
CD4 count cells/ul; median (IQR)	NA	301 (97–733)	
HIV viral load copies/ml; median (IQR)	NA	48 (0–491000)	
History of AIDS prior diagnosis of AIDS	NA	18/58 (32)	
HIV-score ($n = 53$) median (IQR)	NA	2 (1–3)	
Concurrent cART	NA	43/56 (77)	
ARL-IPI risk ($n = 45$)			
Low (0–6)		17 (38)	
Intermediate (7–10)	NA	20 (44)	
High (11–15)		8 (18)	

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SECONDARY CNS LYMPHOMA IN THE RITUXIMAB-ERA: DATA ON THE FIRST 100 PATIENTS FROM A PROSPECTIVE REGISTRY

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Introduction: Secondary central nervous system (CNS) involvement is rare in malignant lymphoma, and its optimal management is yet to be defined. In a prospective German registry, data on patients with secondary CNS lymphoma (SCNSL) is collected.

Methods: Patients with secondary CNS involvement of indolent or aggressive systemic lymphoma, both at first diagnosis (cohort 1) and at relapse (cohort 2) are eligible.

Results: Since July 2011, 110 patients have been included, 100 of whom have been analysed. The median age was 62 years (range 26–83). Fourteen patients had CNS involvement at first diagnosis (cohort I) and 86 had CNS involvement at relapse (cohort II). Twenty-nine patients in cohort II had active systemic disease at the time of CNS relapse. The histology at first diagnosis was aggressive B-cell lymphoma in 73 patients, mantle-cell lymphoma in 5, T-cell lymphoma in 2 and indolent B-cell lymphoma in 20. Primary therapy in cohort II was R-CHOP in 47 patients, CHOP in 7, R-CHOP/CHOP combined with other systemic chemotherapy in 17 and other systemic chemotherapy in 12 (3 patients did not receive any anti-lymphoma therapy). Median time from first diagnosis to CNS relapse in cohort II was 14.1 months (range 2–201). CNS lymphoma localisation was brain parenchyma in 56 patients, meninges in 27, spinal cord in 2 and combination of these in 15. Therapy for CNS involvement was thus far reported for 97 patients and was systemic chemotherapy alone in 45 patients (46%), systemic plus intrathecal (i.th.) chemotherapy in 46 (47%), i.th. chemotherapy alone in 4 (4%) and radiotherapy alone in 1 (1%); one patient did not receive any therapy for CNS involvement. Liposomal cytarabine (Depocyte®) was the most frequent i.th. chemotherapy (in 38 of 50 patients). Systemic therapy was high-dose methotrexate – based on 81 patients (84%) and high-dose cytarabine-based in 47 (48%). High-dose chemotherapy, mostly thiotepa-based, followed by autologous stem-cell transplantation was given to 36 patients (37%).

Conclusions: This is the largest prospective cohort ever reported on SCNSL. In the rituximab era, CNS relapses of malignant lymphoma are usually localised in brain parenchyma and not accompanied by systemic relapse. Systemic CNS-penetrating chemotherapy is frequently used in these patients; however, a reporting bias cannot be excluded. Data on outcome will be presented at the meeting.

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DOSE-DENSE CHEMOIMMUNOTHERAPY AND EARLY CNS PROPHYLAXIS FOR HIGH-RISK DLBCL. – INTERIM RESULTS FROM A NORDIC PHASE II STUDY

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Introduction: Survival of patients with high-risk diffuse large B-cell lymphoma (DLBCL) is suboptimal, and the risk of early central nervous system (CNS) progression is high. Here we present preliminary results from a Nordic phase II study, where systemic CNS prophylaxis with high-dose methotrexate (HD-Mtx) was given in the beginning of therapy and CNS targeted therapy further intensified by adding intrathecally (IT) administered liposomal AraC.

Methods: Inclusion criteria were age 18–65 years, primary DLBCL or grade 3B follicular lymphoma without clinical or radiological signs of CNS disease and cytology negative cerebrospinal fluid (CSF), age adjusted IPI 2–3, WHO performance score 0–3 and/or site specific risk factors for CNS recurrence. Treatment consisted of two courses of HD-Mtx in combination with R-CHOP14, four courses of R-CHOEP14 and one course of R-HD-AraC Liposomal AraC was administered IT in courses 1, 3 and 5, but it was omitted from the protocol during a part of the recruitment period due to production deficiencies. Primary endpoints were FFS, including progression, non-adherence to protocol and death from any cause at 3 years and CNS progression rate at 18 months. Secondary aims were to identify biological risk factors and elucidate if CSF cytology−/flow cytometry (FC)+ cases carry an increased risk of CNS relapse.

Results: The study was closed for recruitment December 2014. Of the accrued 143 patients, 125 had a complete set of treatment data. Median age was 56 years (range 20–64). The majority presented with DLBCL (98%), advanced stage (93%), elevated LDH (90%), more than one extranodal site (70%) and B-symptoms (65%). Seven CSF samples (6%) were FC+. One hundred and sixteen patients (93%) received full treatment. Liposomal AraC was given to 71 and radiotherapy to 35 patients. Grade 4 infections were observed in 11%, grades 3–4 mucositis and gastrointestinal toxicity in 20% and 28%, respectively, and grade 3 arachnoiditis in 1.7% of the patients. Three toxic deaths were reported. At the end of treatment, CR/CRu, PR and PD rates were 76%, 18% and 3.3 %, respectively. Of the 106 patients with PET-CT carried out, 81 (76%) achieved metabolic CR (Deauville score (DS) 1–3). After a median follow-up time of 15 months (0.5–46 months), 15 patients had relapsed; two in CNS and eight died. One-year FFS and OS rates were 86% and 96%, respectively. There was no correlation between gender, age, molecular subtype (GCB versus non-GCB), high Ki67 score (≥70%), number of extranodal sites (>1) and patient outcome. However, PET positivity after treatment (DS 4–5; $p = 0.008$), BCL2 ($p = 0.053$) and CD5 ($p = 0.015$) expression were associated with high risk of progression.

Conclusions: Safety profile and interim outcome results indicate highly satisfactory response rates, low number of CNS relapses and acceptable toxicity despite intensive therapy. PET response and biological factors identify patients with high risk of progression.

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SYSTEMIC HIGH-DOSE METHOTREXATE (HD-MTX) CONSOLIDATION IN POOR-RISK DIFFUSE LARGE B-CELL LYMPHOMA IS ASSOCIATED WITH IMPROVED SURVIVAL IN GERMINAL CENTRE B-CELL PHENOTYPE

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Introduction: While the majority of patients with DLBCL can expect to be cured with R-CHOP chemotherapy, the outcome for those with R-IPI ≥ 3 is poor with approximately 50% eventually succumbing to progressive lymphoma. Dose-intense regimens have failed to improve outcomes for the majority mainly due to toxicity. Since 2007, our centre has been consolidating poor risk patients with 2 cycles of systemic R-HD-MTX for the purpose of CNS prophylaxis.

Aims: The purpose of this study was to examine the effect of R-HD-MTX following completion of R-CHOP on overall survival (OS), progression-free survival (PFS) and CNS relapse in patients with R-IPI ≥3 DLBCL.

Methods: Patients with DLBCL and R-IPI ≥ 3 in remission after R-CHOP were included in the study. Patients receiving HD-MTX were compared with an historical cohort that received otherwise similar therapy. Cell of origin was performed by IHC according to Choi in 54 cases. Survival correlates were analysed by Cox regression using SPSS.

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Results: One hundred four patients with newly diagnosed DLBCL and an R-IPI ≥ 3 completed R-CHOP-like chemotherapy. Forty-two patients received 2 cycles of HD-MTX 3 g/m^2 with rituximab. Patients receiving HD-MTX were younger (median age 67 v. 72 yrs), though the proportion over 60 yrs (HD-MTX 76% v. standard 85%), proportion with advanced stage (98% v. 87%) and raised LDH (86% v. 74%) were similar.

At a median follow-up of surviving patients at 2.6 years, patients who received HD-MTX had improved 2-year OS (84% v. 66%, HR 0.47, $P = 0.06$) and PFS (81% v. 56%, HR 0.46, $P = 0.02$). This was attributable to a reduction in the 2-year systemic relapse rate (19% v. 32% (HR 0.49, $P = 0.07$) and CNS relapse rate 3% v. 15% (HR 0.42, $P = 0.15$). The benefit of HD-MTX was only seen in GCB phenotype (PFS 88% v. 50%, (HR 0.25, $P = 0.04$) cf ABC phenotype 63% v. 57% (HR 0.83 $P = 0.92$). The regimen was well tolerated with no severe toxicity.

Conclusions: The use of consolidative R-HD-MTX is associated with improved OS and PFS in patients with poor-risk DLBCL; however the benefit is only seen in GCB-DLBCL. The low rates of toxicity observed may be due to sequential rather than concurrent administration of HD-MTX. Further prospective studies are warranted to validate this approach in poor-risk patients with DLBCL.

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THE ADDITION OF RITUXIMAB TO CODOX-M & IVAC IN FIRST-LINE THERAPY OF POOR RISK BURKITT LYMPHOMA (IPI 3–5) YIELDS AN EXCELLENT OUTCOME: A PHASE 2 UK NCRI/LLR TRIAL (LLR 04058)

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Introduction: A previous report from our group (Mead et al Ann Oncol. 2002 Aug; 13(8):1264-74) helped to establish a modified CODOX-M and IVAC schedule as one of the standard of care options in adult Burkitt Lymphoma (BL) therapy. Though Rituximab is routinely used in DLBCL and CD20 expression is strong in BL, when we commenced this study in 2009, there were no published reports of the addition of Rituximab to CODOX-M and IVAC in BL. Currently, there has still only been one publication of the addition of Rituximab to CODOX-M and IVAC (Evens et al Ann Oncol.(2013) 24 3076-3081), and this substituted a liposomal anthracycline and used a non-standard (500 mg/m^2) dosage of rituximab. Therefore, a firm evidence base for the routine addition of standard dose rituximab to CODOX & IVAC has not previously been established.

Methods: Thirty-seven BL patients from 21 UK sites were treated between September 2009 and March 2013 on the same protocol as poor-risk DLBCL patients (reported separately). Though not routinely used in BL, the IPI criteria were used to define poor-risk status in both DLBCL and BL patients. All patients were high risk as defined by Mead et al (Blood. 2008 Sep 15;112(6):2248-60). One patient was ineligible (excluded from all analyses) and 1 progressed before treatment began and was withdrawn; they were removed from analyses of compliance, response and toxicity. Median age was 38 years (20–64). IPI scores were 3; $n = 19$ (52.8%) and 4; $n = 17$ (47.2%). The performance status (PS) was 0; $n = 11$ (30.6%), 1; $n = 9$ (25.0%), 2; $n = 7$ (19.4%) and 3; $n = 9$ (25.0%). Six patients had proven CNS disease. All patients were scheduled for the modified CODOX-M and IVAC regimen including CNS directed therapy but with the addition of 8 doses of rituximab. The primary endpoint of the study was progression-free survival (PFS); we aimed to show an increase from 45% to 65% at 2 years. Secondary endpoints included toxicity, CR rate and overall survival (OS).

Results: Thirty (85.7%) received 4 cycles of treatment. Although all patients experienced grades 3–4 toxicities, there was only 1 (1.8%) treatment related death (PS 3). Observed responses were CR/Cru; $n = 28$ (77.8%), PR; $n = 2$ (5.7%), PD/relapse; $n = 1$ (2.9%) and not assessable or missing in 4 (11.4%). The median follow-up is 39.3 months, and 10 patients have progressed or died. At 2 years, the PFS estimate is 72.2% (95% CI: 54.5–84.0), and currently, no PFS events have been seen beyond this point. The OS rate at 2 years is 80.1% (62.7–90.0).

Conclusion: The R-CODOX-M and R-IVAC regimen can be delivered to patients with poor-risk BL in a multi-centre setting. Haematological toxicity is in-line with expectation from previous studies of this regimen when given without rituximab. The significant proportion (25%) of PS 3 patients suggests that this cohort is highly representative of routine clinical practice. We conclude that rituximab can be safely added to CODOX-M and IVAC without an unacceptable increase in toxicity and that this may improve outcome.

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A PHARMACOKINETIC-PHARMACODYNAMIC MODEL ALLOWING TO OPTIMIZE RITUXIMAB DOSING REGIMEN ACCORDING TO BASELINE TUMOUR BURDEN IN DLBCL PATIENTS. A LYSA GROUP STUDY

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Objectives: Rituximab, a chimeric anti-CD20 monoclonal antibody, has profoundly improved the treatment of B-cell malignancies. High variability in clinical response to rituximab is partly explained by pharmacokinetic (PK) variability. An inverse correlation between rituximab concentrations and tumour burden was observed in the pivotal study. This study aimed at describing the impact of metabolic tumour volume (MTV_0) on rituximab PK and concentration–effect relationship in patients with diffuse large B-cell lymphoma (DLBCL) to propose a dose adjustment strategy according to baseline tumour volume.

Methods: Data of 108 patients with DLBCL from two prospective multicentre studies were evaluated for tumour volume and rituximab PK. Patients with localized stage ($n = 19$) were included in GOELAMS 0203 trial (NCI number: NCT00841945), whereas advanced stage ($n = 89$) were included in GELA 073B trial (NCT00498043). All patients received rituximab (375 mg/m^2) associated with anthracycline-based chemotherapy administered every 14 days (CHOP14 or R-ACVBP). Baseline tumour volume was evaluated by PET (metabolic tumour volume: MTV_0). Rituximab concentrations were measured before and after each rituximab infusion, on day 5 of each cycle, and 2 to 3 weeks after the fourth cycle. Rituximab PK was assessed using population compartmental modelling. Logistic regression was applied to assess the influence of area under the concentration curve (AUC) and MTV_0 on clinical response determined by PET after cycle 4. Cut-off values associated with clinical response were determined by ROC curve analysis.

Results: A 2-compartment model with combined residual error was shown to adequately describe rituximab pharmacokinetics. The final PK model estimations of mean (IIV) clearance (CL) and central (V1) and peripheral (V2) distribution volumes were 0.0232 L/h (48.2%), 3.96 L (28.7%) and 5.32 L (27.4%), respectively. V1 and V2 significantly increased by 2- and 9-fold between extreme MTV_0 values of 0.8 and 4340 cm^3, respectively. The increase in MTV_0 was associated with lower AUC ($R^2 = 0.51$, $p < 0.0001$) and a longer elimination half-life ($R^2 = 0.58$,

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$p < 0.0001$). A high AUC in cycle 1 ($AUC_1 > 9667.31$ mg.h/L) was predictive of a better clinical response ($p < 0.001$). According to the model, the recommended 375 mg/m^2 dose of rituximab is suitable for patients with MTV_0 of 200 cm^3 but not for higher MTV_0 values. This model would allow to calculate a recommended dose for individual MTV_0.

Conclusion: This study is the first to describe the tumour volume effect on rituximab pharmacokinetics in DLBCL patients using a population approach. An increase in MTV_0 led to a decrease in rituximab AUC and was associated with worst clinical response. This work may allow to optimize rituximab dosing according to baseline tumour volume in the future.

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PANOBINOSTAT FOR PATIENTS WITH RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: A PHASE II STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Introduction: Treatment of patients with relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) represents an unmet medical need. Panobinostat (PAN) is a HDAC inhibitor that showed encouraging therapeutic activity in HL, CTCL and other NHLs. On this basis, we performed a prospective, multicentre, phase II single arm study to evaluate safety and efficacy of single agent PAN as salvage therapy for R/R DLBCL.

Methods: Adult patients with R/R DLBCL who already performed high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) or were not eligible for ASCT were included. The treatment plan included 6 induction courses with PAN monotherapy followed by other 6 courses of consolidation; patients achieving complete response (CR), partial response (PR) or stable disease (SD) underwent maintenance until disease progression, intolerability or withdrawal of consent. In each 28-day course, PAN was administered orally at the dosage of 40 mg/day three times every week with dose adjustment according to toxicity up to 30 mg three times every other week. The primary objective was to evaluate overall response (OR) according to the Cheson 1999 criteria.

Results: Thirty-five patients were enrolled between June 2011 and March 2014. Clinical characteristics were median age 73 (range 65–75) years, stage IV in 18 (55%), B-symptoms in 9 (28%), increased LDH in 24 (69%), high–intermediate or high International Prognostic Index (IPI) in 18 (51%). Patients received a median of 2 prior lines of therapy (range 1–4). At the end of induction phase, 6 responses (17%) were observed, including 4 CR (11%). Median TTR was 2.6 months (range 1.8–12). Four patients prosecuted PAN treatment up to month 30, 2 up to month 35 and 1 is still in treatment at month 36. Median PFS was 3 months; calculated 12, 24 and 36 months PFS were 26%, 13% and 13%, respectively. In univariate analysis, favourable IPI score and cutaneous involvement at enrollment showed a trend towards a higher ORR ($p = 0.007$ and 0.061, respectively). No toxic deaths were reported; 25 patients died: 22 due to lymphoma, 2 due to infectious complications while in progression and one for allogeneic transplant related complications, performed after progression. Patients' median OS was 7 months; calculated 12, 24, and 36 months OS were 33%, 21% and 21%, respectively. Grades 3–4 thrombocytopenia and neutropenia were the most common toxicities (in 29 (83%) and 12 (34%) patients, respectively), while grades 3–4 extra-haematological toxicity included diarrhoea in 4 (12%), infectious complications in 1 (3%) and supraventricular arrhythmia in 2 patients (6%).

Conclusions: PAN might be remarkably active in some patients with R/R DLBCL, showing durable CR. Feasibility was impaired by relevant haematological toxicity, mainly frequent and dose-limiting grades 3–4 thrombocytopenia. Identification of biological markers related with PAN response could hopefully be useful to better address the use of PAN in peculiar subsets of patients.

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PROGNOSTIC IMPACT OF IMMUNOHISTOLOGICAL PROFILING IN PRIMARY CNS LYMPHOMA

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Introduction: Despite improvements in the treatment of primary CNS lymphoma (PCNSL), the response to chemotherapy remains heterogeneous and overall prognosis poor. Thus, identification of predictive and prognostic biomarkers for risk-stratified treatment decisions is highly desirable. We investigated the prognostic significance of B cell differentiation status and common B cell differentiation markers in 119 PCNSL patients homogenously treated with high-dose methotrexate (HDMTX)-based chemotherapy.

Methods: Protein expression of BCL-2, BCL-6, CD10 and MUM-1/IRF-4 were evaluated by immunohistochemistry, and the association with survival was analysed.

Results: The median follow-up of all patients was 67.5 months. The median progression-free survival (PFS) was 10.61 (95% CI 4.23–17.00) months; the median overall survival (OS) was 28.85 (95% CI 17.96–39.73) months. Eighty-nine tumours expressed BCL-2 (92.7%), 24 (20.5%) CD10, 60 (54.1%) BCL-6 and 87 (79.0%) MUM-1/IRF-4. On the basis of the Hans algorithm, 80 (73.4%) tumours were classified to the non-GCB group suggesting a post germinal centre origin of PCNSL. BCL-6 expression (cut-off point 30%), but none of the other markers, was associated with shorter PFS ($P = 0.047$) and OS ($P = 0.035$). After adjustment for MSKCC score on multivariate analysis, BCL-6 expression was associated with shorter PFS (HR 1.95, 95% CI 1.22–3.12, $P = 0.005$) but not OS (HR 1.85, 95% CI 0.71–4.80, $P = 0.21$). Classification according to Hans algorithm and expression status of single B cell markers BCL-2, CD10 and MUM-1/IRF-4 did not correlate with prognosis.

Conclusion: If validated in an independent cohort, BCL-6 is of clinical relevance as an unfavourable prognostic biomarker in PCNSL.

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ADDITION OF RITUXIMAB TO THE TREATMENT OF NEWLY DIAGNOSED PRIMARY CNS LYMPHOMA IS ASSOCIATED WITH IMPROVED OUTCOME: A FRENCH LOC NETWORK STUDY

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Background. Methotrexate and cytarabine are the main drugs used for the treatment of primary CNS lymphoma (PCNSL), but the optimal treatment schedule is unknown, leading to heterogeneous protocols in daily practice. To date, no comparative clinical trial has evaluated the impact of the addition of rituximab to chemotherapy on the outcome of PCNSL. The aim of this study was to analyse the characteristics, treatments and outcomes of patients with PCNSL treated or not with rituximab in first line.

Methods. Patients with PCNSL were prospectively registered in the database of the French LOC network *(Lymphomes oculo-cérébraux)* between 2011 and 2013. A treatment-oriented analysis was performed on the patients registered on 1 July 2014.

Results. Among the 516 patients registered in the LOC database, 437 patients (227 men and 210 women) with full treatment data were retrieved. Median age was 65.8 years (range: 19–87 years). First-line treatments were methotrexate (MTX), procarbazine–vincristine–cytarabine (MPV-A) (43%), other MTX with HD cytarabine-based regimens (33%), methotrexate, BCNU, VP16, methylprednisolone (MBVP) (9%), HD MTX without cytarabine (9%) and others (6%). Rituximab was administered to 53% of patients (R+ group), whereas 47% (R− group) did not receive rituximab. The median age was higher in the R− group compared to the R+ group (70.9 vs 59.9 years, $p < 0.001$), and the proportion of patients with Karnofsky lower than 70 was higher in the R− group (50% vs 39%, $p = 0.02$). A higher proportion of patients treated with rituximab received a post-remission consolidative treatment with radiation therapy or intensive chemotherapy with stem cell support (R−/R+: 6%/37%). Median PFS was 16.9 months in the R+ group vs 6.7 months in the R− group ($p < 0.001$). The 2-year overall survival (OS) was 35% in the R− group vs 64% in the R+ group ($p < 0.001$). With an adjustment by multivariate analysis performed on the whole cohort, both rituximab exposure (HR 0.64, $p = 0.028$) and consolidation treatment (HR 0.408, $p < 0.001$) were independently associated with a longer PFS. Similarly, consolidation treatment (HR 0.298, $p < 0.001$), rituximab exposure (HR 0.525, $p = 0.007$) and a Karnofsky score of 70 or above (HR 0.537, $p = 0.09$) were associated with a longer OS.

Conclusions. Rituximab in addition to methotrexate-based chemotherapy regimens is associated with an improved PFS and OS in newly diagnosed PCNSL. Further delineation of the role of consolidative treatment is pending.

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BAM INDUCTION FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: A MULTICENTRE STUDY FROM THE SPANISH GROUP GEL-TAMO

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Introduction: Primary central nervous system lymphoma (PCNSL) is a rare and aggressive disease with poor prognosis. To date, there is no standardized treatment of PCNSL. Concerns regarding neurocognitive toxicity of whole-brain radiotherapy (WBRT) have motivated development of alternative, dose-intensive chemotherapeutic strategies as consolidation, such as autologous stem-cell transplant (ASCT). In this study, we evaluated the efficacy and toxicity of chemotherapy induction followed by high-dose consolidation with ASCT in patients with newly diagnosed PCNSL.

Methods: GEL-TAMO developed a protocol for first-line therapy of patients with PCNSL, consisting of an induction therapy with 2 cycles of BAM (BCNU 100 mg/m^2 day 1, cytarabine 3 g/m^2 days 9, 25 and 41 and methotrexate 2 g/m^2 days 8, 24 and 40). Patients who achieved at least partial response (PR) received an intensification with high-dose chemotherapy (BCNU 400 mg/m^2 day 6 and thiotepa 5 mg/Kg days 5 and 4) followed by ASCT, whereas BAM refractory patients received WBRT (45 Gy) salvage or palliative treatment.

Results: Sixty-three patients (median age 59 years, range 29–70) were treated with this protocol between July 2008 and November 2012. All but 2 patients had a diffuse large B-cell lymphoma. BAM regimen was well tolerated, being the most common grades 3–4 toxicities: haematological (leucopenia in 43% of patients and thrombocytopenia in 21%), infectious (22%) and hepatic (5%). Thirty-two out of 63 patients (51%) completed the 2 BAM cycles, achieving complete remission of (CR) 20 patients (32%) and 8 (13%) PR. Reasons for not completing the 2 planned BAM courses were progressive disease ($n = 24$) and toxicity ($n = 7$). Nineteen patients received WBRT, resulting in 5 and 11 patients achieving CR and PR, respectively. Regarding WBRT toxicity, 1 patient showed mild cognitive impairment, 3 leukoencephalopathy (1 mild and 2 severe) and 2 hydrocephalus. Finally, 24 patients (38%) underwent ASCT (18 after BAM and 6 after WBRT). Reasons for not performing the transplant were progressive disease ($n = 20$), comorbidities ($n = 7$), death ($n = 6$), mobilization failure ($n = 2$) and physician decision ($n = 4$). After a median follow-up of 42 months (4–67), 27 patients are alive (78% in CR), 36 patients have died (81% due to lymphoma progression, 11% infectious complications and 8% other causes) and 1 patient have developed a secondary myelodysplastic syndrome (RAEB-2). The estimated 3-year progression-free survival and overall survival were 36% and 46%, respectively, for the whole series, and 68% and 85%, respectively, for transplanted patients.

Conclusions: BAM induction regimen followed by high-dose therapy and ASCT had an adequate toxicity profile. However, although results after ASCT seem to be good, only 50% of patients completed the 2 planned BAM cycles, mainly due to lymphoma progression. Consequently, more effective induction regimens are needed for these patients.

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HIGH EFFICIENCY AND TOLERANCE OF TEMOZOLOMIDE IN RELAPSE/REFRACTORY PRIMARY VITREO-RETINEAL LYMPHOMA: A MULTICENTRIC STUDY FROM THE LOC NETWORK

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Introduction: Primary vitreo-retinal lymphoma (PVRL), alias PIOL (primary intraocular lymphoma), is a rare subset of non-Hodgkin lymphoma characterized by a high level of relapse, especially in brain, and a short survival. There is no consensus on treatment procedures, even in first line, and prospective comparative studies do not exist. Classical attitudes in first line are systemic chemotherapy (SC) like high-dose methotrexate (Mtx), radiotherapy or intraocular injection of Mtx, but publication on R/R PVRL is exceptional. New treatments are necessary, especially with a good tolerance profile. As Temozolomide (Te) has some efficiency in primary central nervous system lymphoma, we used this drug in R/R PVRL and elderly patients.

Methods: Inclusion criteria were R/R PVRL and/or PVRL not eligible for IV chemotherapy. Diagnosis was established by cytology, phenotyping, cytokine level (IL10/IL6) and molecular analysis of vitrectomy material. Treatment consisted in Te at 150 mg/m^2/day, orally, 5 days per month, without corticosteroid use. In absence of any response at 2 months, dosage was increased to 200 mg/m^2/day. A complete response was defined as a normalization of eye exam and cytokine level.

Results: Eighteen patients were included, 5 men and 13 women, mean age was 75 years (35–90). All but four received SC with at least high-dose Mtx or high-dose cytarabine before Te, 2 were in first line, 8 in second line, 5 in third, 2 in fourth and 1 in fifth. Two patients have previously been treated by autologous stem cell transplantation (ASCT) conditioned by thiotepa, cyclophosphamide and busulfan. The 2 patients treated in first line where more than 80 years old. Median duration of treatment was 5 months. The median follow-up (fu) is 21 months. Overall response rate is 78%, with 12 CR (67%) and 2 PR (11%). At the last fu, 8 patients are still in CR, with a median DFS of 14 months. The 2 patients treated after ASCT are still in CR at 2 and 75 months. The two old patients treated in first line are in CR at last fu. One patient with cerebral localization is in persistent ocular and cerebral CR. Two patients experienced ocular response (1CR and 1PR), whereas they were refractory to lenalidomide. Median OS is not reached. Only 2 patients experienced haematological grade 3/4 toxicity. There was no treatment related to death. Three patients were treated a second time by Te after relapse, obtaining 2 new CR, one of 4 months and one persistent at 12 months, and one new short PR before progression.

Conclusion: This work represents the biggest study with a homogeneous treatment in R/R or elderly PVRL. Temozolomide appears as a safe and efficient treatment of R/R PVRL or in first line in elderly patients, even after high-dose chemotherapy or lenalidomide. Longer follow-up, prospective and larger studies are necessary to confirm these data.

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TEMSIROLIMUS FOR PRIMARY CNS LYMPHOMA

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Background: Salvage treatment is poorly defined in primary CNS lymphoma (PCNSL). High-dose chemotherapy followed by stem-cell transplantation (HD-SCT) is an option only for younger and otherwise healthy patients. In this multicentre phase II study (NCT00942747), temsirolimus (TEM) monotherapy was tested in patients with relapsed or refractory PCNSL not eligible for HD-SCT. Additionally, TEM penetration into cerebrospinal fluid (CSF) was evaluated.

Methods: Immunocompetent adults with histologically confirmed PCNSL after failure to high-dose methotrexate-based chemotherapy who were not eligible for HD-ASCT or failed it were included. The first cohort of 6 patients received 25 mg TEM, all following 75 mg TEM i.v. weekly. Primary endpoint was overall response rate (ORR); secondary endpoints were toxicity, progression-free survival (PFS) and CSF penetration of TEM and its metabolite sirolimus (SIR).

Results: Thirty-seven eligible patients with a median age of 69 years (range 22–83) and a median ECOG of 2 (range 0–3) were included. The median number of previous treatment regimens was 2 (range 1–5) and the median time from the last previous treatment to study treatment was 5 months (range 0.7–14). Complete and partial responses were achieved in 11 patients, each (30%) for a total response rate of 60%. Stable disease was observed in 6 patients (16%), 4 (11%) progressed and 5 (13%) died on therapy. All but one response were seen in the 75 mg dose group. One patient is still on treatment. Median PFS was 2.6 months (95% CI, 1.3–3). In 6 patients, PFS was >6 months (6.3–27.5). Most frequent CTC $\geq$ III° toxicity was hyperglycemia in 10 patients, thrombocytopenia in 8, infection in 6 (pneumonia in 4), anaemia in 4 and rash in 3. Fourteen blood/CSF pairs were collected in 9 patients (25 mg cohort: 10 pairs in 5 patients; 75 mg cohort 4 pairs in 4 patients). Mean maximum blood concentration was 292 ng/ml for TEM and 37.2 ng/ml for SIR in the 25 mg cohort and 484 ng/ml (TEM) and 91.1 (SIR) in the 75 mg cohort. No drug was detected in CSF (lower limit of detection 1 ng/ml) in either cohort.

Conclusions: Single-agent TEM at a weekly dose of 75 mg showed activity in a cohort of intensively pretreated and elderly patients with relapsed/refractory PCNSL. This, however, was at costs of notable toxicity.

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LONG-TERM OUTCOME OF 490 PATIENTS WITH EARLY STAGE EXTRA-NODAL MARGINAL ZONE LYMPHOMA

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Introduction: Localized early stage extra-nodal marginal zone lymphoma (MZL) presents with heterogeneous organ involvement and is treated with various modalities, including resection, radiotherapy and, infrequently, systemic therapy. We report the long-term outcome of a large cohort of extra-nodal MZL and assess the impact of patient and disease characteristics, organ site and treatment strategy on disease control and survival.

Methods: We identified 490 consecutive patients with stage IE or IIE MZL referred between 1992 and 2012 to Memorial Sloan Kettering Cancer Center. Pathology was confirmed by haematopathologists at our institution. Patient and disease factors and treatment types were analyzed for association with relapse-free survival (RFS), overall survival (OS) and cumulative incidence of relapse.

Results: Median follow-up was 4.8 years. Median patient age was 60 years and 57% were women. Ann Arbor stage was IE in 89%. Most common sites were stomach (32%), orbit (14%), lung (12%), skin (12%) and parotid (5%). Radiotherapy alone (RT) was the initial treatment in 50% of patients, followed by surgical resection (30%), observation (9%), immunotherapy (4%) and chemotherapy (2%).

Five-year OS and RFS were 90% and 64%, respectively; 10-year OS and RFS were 73% and 45%. Disease-specific death was 1.3% at 5 years and 1.8% at 10 years. Cumulative incidence of progression/relapse was 29% by 5 years and 39% by 10 years. Among the 384 patients with complete response (CR), 99 patients experienced relapse. On multivariable analysis, initial treatment type and primary disease site were independently associated with RFS and relapse (all $p < 0.005$). All disease sites (HR > 2.0, $p \leq 0.01$) except for thyroid ($p = 0.8$) had worse RFS relative to stomach. Compared with RT, chemotherapy or immunotherapy had worse RFS (HR 2.2, $p = 0.004$), while surgery was no different ($p = 0.52$). After RT, only 11 patients experienced in-field failure, with a 5-year cumulative incidence of 2.4%. Most common location of relapse after CR was distant; relapses were also observed in paired untreated organs, such as the orbit, salivary gland and breast. Crude rate of transformation to pathologically confirmed large-cell lymphoma was 2% (11 patients). Second tumours in irradiated sites developed in 3 patients: 2 of these were breast ductal carcinoma *in situ* cured with surgical resection.

Conclusions: Overall and cause-specific survival are excellent in early stage extra-nodal MZL. Treatment with RT or surgery was associated with longer RFS and

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reduced the need for salvage. Relapses are common after initial remission and most frequently occur in distant sites. Transformation to large-cell lymphomas is rare. Stomach cases are less likely to relapse than other anatomic primary sites, perhaps in part because the entire organ is irradiated, versus other sites that are either bilateral or where only part of the organ is treated, such as the skin and lung. This study supports the use of local therapies to treat stage IE and IIE MZL.

T-CELL LYMPHOMA

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CLINICAL CHARACTERISTICS AND PATTERNS OF CARE OF PATIENTS (PTS) WITH PERIPHERAL T-CELL LYMPHOMA (PTCLS) ACCORDING TO AGE AT TIME OF DIAGNOSIS: A T-CELL PROJECT SNAPSHOT

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Background: Due to their rarity, a satisfactory understanding of the full clinical and biological characteristics of PTCLs is lacking, no reliably effective treatment is available, and pts outcome remains very poor. Since pts age is a key factor in choosing initial therapy, we analysed pts aged ≥70 yrs comparing disease features, treatments and outcome of this subset to their younger counterparts. A parallel analysis is presented by the COMPLETE US network.

Patients and methods: The T-cell project is a prospective registry collecting data on baseline characteristics, details of therapy delivered and outcome data in pts with mature, aggressive PTCLs. Pts were grouped into three age categories (≤60, >60–<70, and ≥70 yrs): chi-squared test and *t*-test were used to compare the groups, log-rank test and Cox regression models were used for overall survival (OS).

Results: From 2006 to 2014, 1308 pts were registered from 73 sites worldwide. Complete baseline data were available for 819 pts (≤60: $n = 462$, >60–<70: $n = 168$, ≥70: $n = 189$), 754 of which had also therapy information. Median follow-up was 48 mos. There were no differences in gender or B-symptoms at presentation; however, elderly pts were more likely to suffer from disease-related symptoms (77%, $P = 0.01$) and to present with an ECOG performance status >1 (35%, $P = 0.002$). No difference in disease extent was noted, but pts ≥70 yrs more frequently had bone marrow involvement (28%, $P = 0.01$). A different distribution in histologic subtypes was observed in different age groups. Intent of therapy was curative in 96% of younger vs 87% of older pts ($P < 0.0001$). Anthracycline- and etoposide-containing regimens were adopted in 70% and 8% of pts ≥70, respectively, and consolidative stem cell transplantation (HSCT) in 10% and 1% of younger and older pts, respectively. Overall responses were higher in younger pts [74% (≤60), 69% (>60–<70), 57% (≥70 yrs), $P < 0.0001$]; 2-yr and 5-yr OS were 60% and 51% (≤60), 55% and 38% (>60–<70), 40% and 24% (≥70 yrs), respectively ($P < 0.0001$). Cox modelling suggests age (HR 1.12; 95% CI: 1.04–1.20, $P = 0.004$) and stage III/IV disease (HR 3.0; 95% CI: 1.46–6.22, $P = 0.003$) are predictors of inferior OS. HSCT emerged as a predictor of better OS (HR 0.50, 95% CI: 0.29–0.85, $P = 0.01$).

Conclusions: The T-cell project data document that PTCLs pts ≥70 yrs exhibit a poorer outcome than younger, and are more likely to receive non-curative intent therapy. Optimal treatment for this subset is still a relevant unmet need, and more efforts in defining better strategies are urgent.

Abstract 231 Table

	Histology subtype		
	≤60 yrs N (%) 462 (100%)	**>60–<70 yrs N (%) 168 (100%)**	**≥70 yrs N (%) 189 (100%)**
PTCL, NOS	148 (32)	70 (42)	89 (47)
AITL	56 (12)	31 (18)	52 (28)
ALCL, ALK–	77 (17)	5 (15)	17 (9)
ALCL, ALK+	53 (12)	24 (2)	3 (2)
NKTCL	65 (14)	16 (10)	12 (6)
Others	63 (13)	22 (13)	16 (8)
	First-line treatment		
	N (%) 426 (100%)	**N (%) 156 (100%)**	**N (%) 172 (100%)**
CHOP/CHOP-like	220 (52)	84 (54)	92 (54)
CHOEP/CHOEP-like	39 (9)	20 (13)	5 (3)
Gemcitabine-based	0	1 (<1)	4 (2)
Platinum-based	13 (3)	4 (3)	2 (1)
Ifosfamide-based	27 (6)	2 (1)	1 (<1)
Other	127 (30)	45 (29)	68 (40)

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CHARACTERISTICS AND PATTERNS OF CARE OF PATIENTS (PTS) ≥70 YEARS WITH T-CELL NON-HODGKIN LYMPHOMA (TCL) IN THE UNITED STATES (US)

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Background: Age-related differences in pts with TCL are not well described. We compared pts ≥70 years to their younger counterparts using data from COMPLETE. A similar analysis will be submitted by the T-cell project.

Patients and methods: COMPLETE is a prospective US registry of TCL pts designed to collect pt characteristics, treatments and outcomes. We divided pts into three categories (≤60, >60–<70 and ≥70 years). Descriptive statistics (chi-square and *t*-test) were used for comparisons. Log-rank test and Cox regression models were used for overall survival (OS).

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Results: From 2010 to 2014, 499 pts were enrolled from 55 US sites. Data on disease/clinical characteristics were available for 357 patients (≤60: $n = 192$, >60–<70: $n = 77$, ≥70: $n = 88$), while treatment data were available on 288 pts. Registry median follow-up (FU) is 1.9 years. Pts ≥70 years were more likely to have underlying liver (4.5%, $P = 0.02$) or cardiac disease (35%, $P < 0.0001$). Advanced stage disease and B symptoms were noted in a similar incidence, but younger pts were more likely to have elevated LDH ($P = 0.006$).

Table 1 summarizes differences in histologic distribution; as expected, ALK+ ALCL is rare in elderly pts. Nodal and extra-nodal involvement occurred in a similar frequency, but older pts were more likely to have skin involvement (47% vs 26%, $P = 0.02$). Younger pts were more likely to have an EBV-associated disease [44% (≤60), 31% (≥70), $P = 0.07$]. Intent of therapy was curative in 94% of younger versus 76% of older pts ($P = 0.001$). Pts ≥70 years were more likely to receive radiation alone (RT) or best supportive care (BSC) alone ($P = 0.004$ and $P = 0.02$, respectively). Younger pts were more likely to undergo stem cell transplantation (HSCT) as consolidation (24% vs 4%; $P = 0.001$). None of the pts ≥70 years received CNS prophylaxis, while 4.7% of pts ≤60 did ($P = 0.04$). Chemotherapy selections based on age are listed in Table 1. Median survival was 26.6 months in pts ≥70 years; median survival for the other groups has not been reached. Cox modelling suggests stage III/IV disease as predictor of inferior OS (HR 3.7; 95% CI: 1.75–7.70, $P = 0.0006$) and HSCT as a predictor of better OS (HR 0.17, 95% CI: 0.06–0.49, $P = 0.0008$).

Abstract 232 Table 1 Age-based chemotherapy selections

	≤60 years *N* (%) 192 (100%)	>60–<70 years *N* (%) 77 (100%)	≥70 years *N* (%) 88 (100%)
PTCL-NOS	54 (28)	32 (42)	34 (39)
ALCL	42 (22)	8 (10)	12 (14)
ALK–	23 (55)	4 (50)	11 (92)
ALK +	19 (45)	4 (50)	1 (8)
NK-T cell	28 (15)	7 (9)	5 (6)
AITL	18 (9)	20 (26)	16 (18)
Other	50 (26)	10 (13)	21 (24)
	First-line treatment		
	≤60 years	**>60–<70 years**	**≥70 years**
	158 (100)	63 (100)	61 (100)
CHOP /CHOP-like	44 (28)	22 (35)	20 (33)
CHOEP/CHOEP-like	34 (22)	11 (18)	9 (15)
Gemcitabine-based	5 (3)	1 (2)	6 (10)
Platinum-based	9 (6)	2 (3)	1 (2)
Ifosfamide-based	14 (9)	2 (3)	0 (0)
Other	52 (33)	25 (40)	25 (41)

Conclusions: TCL pts aged ≥70 years in the USA are more likely to receive non-curative intent therapy, while younger pts are more likely to receive HSCT. HSCT was an independent predictor of better OS. The overall poor outcomes strongly support the need for better treatment options.

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A LARGE PROSPECTIVE MULTICENTER STUDY OF PERIPHERAL T-CELL LYMPHOMA IN THAILAND: CLINICAL, HISTOPATHOLOGY, TREATMENT OUTCOMES AND SURVIVAL

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Introduction: Peripheral T-cell lymphoma (PTCL) is comprised of a heterogeneous group of tumours with various histological subtypes and prognoses. This study aims to characterize clinical characteristics, histopathology, treatment outcomes and survival of PTCL patients in Thailand.

Methods: Adult patients diagnosed as PTCL according to WHO classification 2008 were reviewed from 2002 to 2014 at the thirteen major medical centres in Thailand. The pathological consensus was made among pathologist panel. The patient characteristics, histopathology, prognostic scores, treatment options, outcomes and prognostic factors were analysed.

Results: Three hundred and thirty-two patients were reviewed. The histological subtypes included PTCL, NOS (35.5%), nasal NKTCL (23.8%), ALCL (14%), AITL (12%), SPTCL (8.4%), CTCL (3.6%) and others (2.7%). The median age was 48 years (range 14–91 years) and 61% were men. The majority (73%) of the patients was under 60 years old and had a good ECOG performance score (75.6%). Extranodal involvement presented in 72.6%. The common sites of involvement were bone marrow (34.4%), sinonasal (22.8%), liver (12%) and lung/pleura (10.4%). B symptoms occurred in 54.2% and elevated LDH level in 59.3%. Fifty-eight per cent of the cases had advanced Ann Arbor stage. Fifty-four per cent of the patients were classified as low to low–intermediate IPI and 57% as PIT score 0–1. The patients were treated with first-line chemotherapy in 81% and radiotherapy in 17.5%. CHOP regimen was commonly used in 78.4%. Of the 216 patients evaluated, the overall response rate was 72.7% with 61.6% complete remission. Nevertheless, 26.5% of the cases had progressive disease. Seven patients (2.1%) underwent stem cell transplantation. With a median follow-up time of 17–months, the median overall survival (OS) was 23.3 months, and the 5-year OS was 37%. One hundred and ninety-three patients died in this cohort of which 50% of them were related to progression of disease. Nevertheless, 104 patients are still alive without lymphoma. Regarding Cox regression analyses, extranodal lesions, advanced Ann Arbor stage, B symptoms, poor ECOG performance score and high LDH level were independent prognostic predictors for survival in this study ($p < 0.00$). Furthermore, both IPI and PIT significantly predicted survival in our patients ($p < 0.00$). SPTCL had the most favourable prognosis among PTCL subtypes (5-year OS 64%).

Conclusions: PTCL predominantly affected middle-aged males. The three common subtypes were PTCL, NOS, nasal NKTCL and ALCL. Most of the patients presented with extranodal lesions with low prognostic scores. CHOP regimen was commonly used and gave a high response rate. The IPI and PIT significantly predicted survival outcome. SPTCL had the most favourable prognosis.

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A PROPOSAL OF A NEW STAGING SYSTEM FOR EXTRANODAL NATURAL KILLER T-CELL LYMPHOMA, NASAL-TYPE

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Introduction: Extranodal NK/T-cell lymphoma, nasal type (ENKTL), is a rare and highly aggressive disease. The Ann Arbor staging system (ASS) failed to predict survival differences among different stages in ENKTL. The aim of this study was to establish a new prognostic staging system for ENKTL.

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Methods: Patients in the cancer centre of Sun Yat-sen University (1997–2006) with a diagnosis of untreated ENKTL were entered into a multivariate analysis to develop a new prognostic staging system (NSS): stage I: lesions confined within nasal cavity or nasopharynx without local invasiveness (paranasal sinuses or bony or skin invasion); stage II: localized disease with local invasiveness; stage III: localized disease with regional lymph node involvement (cervical lymph nodes); and stage IV: disseminated disease (lymph nodes on both sides of diaphragm and multiple extranodal sites). The NSS were validated in two multicentre studies in China and an international study.

Results: In the multicentre retrospective study conducted in 18 centres in China, 722 patients were analysed. The results showed that the distribution of NSS compared with ASS from stages I to IV were 24.1%, 34.9%, 18.3% and 22.7% vs 59.1%, 19.0%, 6.9% and 15.0%, and the 5-year OS rate of stages I to IV were 56.0%, 48.3%, 33.8% and 26.1% vs 50.7%, 39.1%, 10.8% and 28.0%. For the multicentre prospective study, 233 newly diagnosed ENKTL patients treated with L-asparaginase-based regimens were enrolled and also showed a balanced distribution of 17.2%, 39.9%, 19.3% and 23.6% vs 53.6%, 25.3%, 6.9% and 14.2% from stages I to IV and superior 5-year OS rate of 82.4%, 72.9%, 67.1% and 53.7% vs 75.2%, 65.6%, 46.9% and 73.8% from stages I to IV using the NSS compared with ASS. In the international study, patients showed more balanced distribution of 29.6%, 19.0%, 15.7%, 35.6% vs 48.1%, 23.1%, 3.7%, 25.0% from stages I to IV using NSS compared with ASS, the NSS also showed a better survival discrimination than ASS with the proportion of stages I to IV were 62.3%, 29.5%, 24.2% and 0.0% vs 48.5%, 28.5%, 0.3% and 0.0%.

Conclusions: The new staging system for ENKTL with better survival discrimination compared with Ann Arbor staging system could be useful to clinicians and for the design of clinical trials in the future.

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A CLINICOPATHOLOGICAL STUDY OF LENNERT LYMPHOMA AND POSSIBLE PROGNOSTIC FACTORS

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Introduction: The lymphoepithelioid variant of peripheral T-cell lymphoma (Lennert lymphoma, LEL) is a rare disease. However, clinicopathological studies regarding LEL are scarce, although a few studies have described the diagnostic criteria for LEL. In this study, we analysed the clinicopathological features of LEL and sought to identify potential prognostic factors.

Methods: We examined 29 patients with LEL who were diagnosed between 2004 and 2013, in Kurume University, using the World Health Organization diagnostic criteria. Patients who exhibited morphologically expanded meshworks of follicular dendritic cells (FDC) with high endothelial venules were excluded, in order to exclude cases of angioimmunoblastic T-cell lymphoma.

Results: Among the 29 patients, 14 men (48%) and 15 women (52%) were included with a median age of 74 years (range, 40–90 years). Eleven of 28 patients (39%) exhibited B symptoms. Extranodal involvement was observed in 9 of 25 patients (36%), 19 of 25 patients (76%) had Ann Arbor stage III or IV and 15 of 25 patients (60%) had a high–intermediate or high risk according to the international prognostic index. Twenty-six of the 29 patients (90%) received chemotherapy, and 12 of these patients (46%) achieved complete remission. Twelve patients died during the follow-up period (median, 22 months; range, 1–71 months).

Regarding the morphological findings in the 29 patients, the tumour cell size for all patients was small to medium, with slightly irregular nuclei. Twenty-five patients (86%) exhibited clusters of epithelioid cells, 9 patients (31%) exhibited polymorphic infiltrates, 5 patients (17%) exhibited vascular proliferation and 2 patients (7%) exhibited clear cells. Regarding the immunohistochemical findings, all patients were CD3-positive, 20 patients (69%) were CD4-positive, 5 patients (17%) were CD4/CD8-double positive and 4 patients (14%) were CD8-positive. Four patients (14%) were TIA-1 positive and Granzyme B negative, 17 patients (59%) were PD-1 positive, and 16 patients (55%) were CXCL13 positive. Three patients (10%) exhibited FDC meshworks without high endothelial venules, and 10 patients (34%) exhibited EBER-positive lymphocytes.

Log-rank testing of the Kaplan–Meier survival curves revealed a significantly worsened prognosis for patients with FDC meshworks ($p = 0.0020$), CXCL13 positivity ($p = 0.0015$) and PD-1 positivity ($p = 0.0157$). Univariate analyses also revealed that FDC meshworks ($p = 0.0292$), CXCL13 positivity ($p = 0.0010$) and PD-1 positivity ($p = 0.0141$) were significant prognostic factors. Furthermore, multivariate analysis confirmed that CXCL13 positivity ($p = 0.030$) was an independent prognostic factor.

Conclusions: The results revealed that FDC meshworks and the expression of follicular helper T-cell markers, particularly CXCL13, were associated with a poor prognosis for LEL. Although this was a small retrospective study, the results indicate that these markers may be useful for identifying patients with LEL who will experience unfavourable outcomes.

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SUBCUTANEOUS PANNICULITIS-LIKE T-CELL LYMPHOMA IN THAILAND: CLINICAL OUTCOMES, TREATMENTS, AND PROGNOSTIC FACTORS

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Introduction: Subcutaneous panniculitis-like T-cell lymphoma (SPTL) is a rare type of non-Hodgkin lymphoma (NHL). The natural history, optimal treatment strategy and prognostic factors associated with this malignancy are not well defined.

Methods: Using web-based registry system, we prospectively collected clinical information of newly diagnosed lymphoma patients from major medical centres situated in various regions of Thailand. Clinical data and treatment outcomes of patients with SPTL were retrieved and analysed.

Results: Thirty-seven patients with SPTL were identified in the registry. SPTL accounted for 1% of all NHL (37/3718) and 8.7% of mature T/NK-cell NHL (37/423). The median age at diagnosis was 27 years (range, 16–63), with female predominance (2:1). Bone marrow involvement was reported in 16% of patients. Systemic B symptoms and elevated serum LDH were common and seen in 54% and 84% of patients, respectively. Thirty-four patients received treatments: CHOP/CHOP-like ($n = 24$), cyclosporine ± prednisone ($n = 5$), CVP ($n = 3$), IVE ($n = 1$) and prednisone alone ($n = 1$). Three patients underwent autologous transplantation. The rates of objective responses and survival in each treatment group were summarized in the table. The best outcomes were seen in the patients who received cyclosporine. Patients in the cyclosporine or CHOP/CHOP-like treatment group had significantly better complete responses (CR), overall responses (CR + PR), overall survival (OS) and progression-free survival (PFS) when compared with those who received other treatments ($p < 0.05$). After a median follow-up of 55 months, the 5-year OS and 5-year PFS of all patients were 76.3% and 51.2%, respectively. A multivariate Cox-regression analysis showed poor ECOG performance status to be an independent adverse prognostic factor for OS and PFS.

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Abstract 236 Table

Treatment	n	CR (%)	CR + PR (%)	5-yr OS (%)	5yr-PFS (%)
CHOP/CHOP-like (CHOP [23] and CHOEP [1])	24	16 (66.7)	20 (83.3)	82.4%	52.8
Cyclosporine ± prednisone	5	4 (80)	5 (100)	100	80
CVP	3	1 (33.3)	1 (33.3)	50	33.3
Others (IVE [1] and prednisone [1])	2	0	0	0	0

Conclusions: Compared with Western countries, Thailand seems to have a higher rate of SPTL. SPTL occurs more commonly in young women, and the disease has a relatively favourable clinical course. Poor performance status at diagnosis is an independent prognostic factor associated with inferior survival. Our result suggests that cyclosporine is an active drug and may provide benefits equivalent to doxorubicin-based chemotherapy.

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IMMUNOPHENOTYPIC HETEROGENEITY OF T-LGLL: CLINICAL AND BIOLOGICAL IMPLICATIONS

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Introduction: T-large granular lymphocytes leukaemia is a rare disease characterized by an abnormal expansion of large granular lymphocytes (LGLs). T-LGLs typically exhibit a terminally differentiated cytotoxic T-cell phenotype (CD3+/CD8+/CD4−/abTCR+). Together with the most common CD8+ T-LGLL, rare forms of CD4+/CD8−/+dim LGL proliferation (CD4+ T-LGLL) have been described. In addition, LGLs variably express CD57, CD16, CD56 and NK receptors (NKR), originating different immunophenotype combinations. The disease generally follows an indolent clinical course with neutropenia representing the major feature along the natural history of the disease (40% of patients presenting with severe neutropenia). Recently, hotspot STAT3 and STAT5b mutations have been described in T-LGLL patients supporting the idea that mutations could lead to a cytokine-independent STAT activation. STAT3 mutations were described in 30–40% of T-LGLL patients, while STAT5b were found only in very few cases, these latters being more frequently detectable in patients with aggressive clinical course.

The aim of this study was to correlate immunophenotypes with relevant biological and clinical features, namely STAT mutations and severe neutropenia in a series of 101 patients with T-LGLL.

Methods: The immunophenotypic characterization of patients with T-LGLL was obtained by flow cytometer analysis. T-LGLs were purified from PBMCs by FACSAria cell sorter or by microbeads system. For the screening of STAT mutations, all the exons covering the activation gene region, where all of the mutations are located, were analysed. Sanger sequencing was performed on DNA of LGLs and of remaining autologous PBMCs. The presence of D661Y and Y640F STAT3 mutations undetectable by direct sequencing was further analysed by a DNA amplification refractory mutation system (ARMS-PCR). Data are expressed as mean ± median standard error (SEM), and statistical analysis and correlations were performed by Student *t*-test and χ^2 test, respectively.

Results: Our results show that CD8+ T-LGLL patients with CD16+/CD56−/CD57± immunophenotype ($n = 54$) were characterized by a high frequency of expression of NK receptors (KIRs and NKG2) and a significant association with STAT3 mutations ($p < 0.001$) and neutropenia ($p < 0.001$). Furthermore, the rare CD8+/CD56+/CD16−/CD57− immunophenotype ($n = 1$ patient) was associated with aggressive clinical behaviour and STAT5b mutations. Interestingly, in this patient, different subclones characterized by different phenotypes and STAT5b mutations could be identified. Expression of CD4+ by LGL was typically associated with a very indolent clinical course.

Conclusions: In conclusion, we provided further evidence of the heterogeneous pattern of immunophenotypes accounting for LGL leukaemia, suggesting that discrete LGL phenotypes might be predictive of different biological and clinical features of disease.

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BREAST IMPLANT ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA: A NEW DISTINCT CLINICOPATHOLOGIC ENTITY

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Introduction: ALK negative anaplastic large cell lymphoma associated with breast implant (iALCL) has been recently recognized as a distinct entity. From 2010 to 2014, 43 830 lymphomas were registered in the 'French network' of which 297 breast lymphomas (<1%). As expected, the most frequent were B-cell lymphomas (92%) and T-cell lymphomas (only 8%). Interestingly, the most frequent T-cell lymphoma was ALK-negative ALCL, and all of these 19 cases were associated with breast implants.

Methods: Clinicopathologic features of 19 iALCL cases and their outcome have been retrospectively analysed.

Results: Two clinical presentations were observed: (i) effusion (seroma)-associated lymphoma with fibrous capsule surrounding implant, and less frequently, (ii) tumour mass-associated lymphoma. These two clinical presentations correlated with distinct histopathologic features. In patients with a seroma, the proliferation of anaplastic cells was confined to the fibrous capsule ('*in situ* iALCL'), while patients presenting with a tumour mass showed more heterogeneous proliferations infiltrating surrounding tissues ('infiltrative iALCL'). The latter consists of either sheets or clusters of large anaplastic cells often accompanied by eosinophils. In some cases, the presence of numerous Reed–Sternberg-like cells, in a background rich in eosinophils, was highly suggestive of Hodgkin lymphoma. In two cases, the two morphologic patterns (i.e. '*in situ* iALCL' and 'infiltrative iALCL') were observed, suggesting that '*in situ* iALCL' may evolve with time to an 'infiltrative ALCL'. Malignant cells were strongly positive for CD30 and showed also a variable staining for EMA, while they did not express ALK. All cases had a T-cell phenotype with variable T-cell antigen loss and expressed cytotoxic molecules. T-cell receptor genes (TCR) were clonally rearranged (10/10). The median age of the patients was 61.5 y (range 42–90 y). The median time elapsed between placement of breast implants and diagnosis of iALCL was 8 y (range 1–14). Of note, all commercially available breast implants were found, most were textured and ruptured. Majority of patients presented with seroma (i.e. accumulation of fluid around the breast implant). Implant removal was performed in 14/15 patients. Additional treatment based on chemotherapy ($n = 9/15$) or/and radiation ($n = 2/15$) was given to 10/15 patients. The overall survival for '*in situ*' and 'infiltrative' iALCL was 100% and 80%, respectively.

Conclusions: Although a median follow-up of 20 months, our results showed that patients with an '*in situ* iALCL' have an indolent clinical course and generally remain free of disease after removal of implant and capsulectomy. However, patients presenting with a tumour mass associated with an 'infiltrative ALCL' may have a more aggressive clinical course that may require chemotherapy/radiation therapy in addition to removal of implant and capsulectomy.

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MOLECULAR HETEROGENEITY OF ALK-NEGATIVE ANAPLASTIC LARGE CELL LYMPHOMA

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Introduction: Anaplastic large cell lymphomas (ALCLs) are CD30-positive T-cell lymphomas that may be ALK-positive or ALK-negative by current WHO criteria. While ALK-positive ALCLs consistently have *ALK* rearrangements, the molecular pathogenesis of ALK-negative ALCLs is poorly understood. We previously identified recurrent rearrangements of the *DUSP22* and *TP63* loci in ALK-negative ALCLs and sought to study the frequency, morphology, phenotype and clinical outcomes associated with these rearrangements.

Methods: Paraffin sections of systemic ALCLs were examined for *DUSP22* and *TP63* rearrangements by FISH. The primary clinical endpoint was overall survival (OS). Morphologic features were scored in a blinded fashion. Phenotype was assessed by immunohistochemistry and RNA-ISH for the chemokine receptor gene *CCR8*. T-cell receptor (TCR) gene rearrangements were assessed by NGS of mate-pair DNA libraries and PCR.

Results: Among 73 ALK-negative ALCLs, 30% had *DUSP22* rearrangements and 8% had *TP63* rearrangements. These were mutually exclusive, and neither was present in 32 ALK-positive ALCLs. ALCLs with *DUSP22* rearrangements had a 5-year OS rate of 90%, similar to ALK-positive ALCLs (85%) and superior to ALCLs with *TP63* rearrangements (17%) or 'triple-negative' ALCLs lacking all 3 rearrangements (42%; $p = 0.0001$). ALCLs with *DUSP22* rearrangements showed sheet-like growth of hallmark cells with increased 'doughnut' cells ($p = 0.039$) and fewer pleomorphic cells ($p = 0.042$). These features correlated with the presence of *DUSP22* rearrangements in an independent validation cohort ($p < 0.0001$). ALCLs with *DUSP22* rearrangements generally lacked expression of cytotoxic markers (e.g. TIA-1, $p < 0.0001$) and showed increased expression of *CCR8* ($p = 0.0008$). ALCLs with *DUSP22* rearrangements all had clonal TCR gene rearrangements (100%); the frequency of clonal rearrangements was lowest in triple-negative ALCLs (53%; $p = 0.029$).

Conclusions: ALK-negative ALCLs demonstrate pathologic, molecular and clinical heterogeneity. *DUSP22* rearrangements occur in 30% of cases and are associated with classic ALCL morphology, lack of cytotoxic marker expression and excellent outcomes. The role of *CCR8* in T-cell trafficking to skin and other sites and the observation that *DUSP22* rearrangements also occur in 28% of primary cutaneous ALCLs suggest that systemic and primary cutaneous cases with this rearrangement share some biologic features. ALCLs with *TP63* rearrangements are less common and remain poorly understood. Their very poor prognosis indicates a need for further study of their clinicopathologic features, biology and potential for targeted therapies. FISH for both rearrangements can be performed easily in the clinical setting.

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NPM-ALK ANTIBODY TITER IN ALK POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): LONG-TERM FOLLOW-UP, THE FRENCH PEDIATRIC EXPERIENCE

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Introduction: ALK-positive ALCLs are associated with an NPM-ALK fusion protein resulting from the *t*(2;5) (p23;q35) translocation. This chimeric protein is expressed by tumour cells and generates immunity to ALK, implicated in the control of ALK positive ALCLs. A correlation was shown between high anti-ALK antibody titer at diagnosis, low clinical stage and decreased relapse risk. Three groups of patients can be identified according to the antibody titer: low, 0–1/750, medium, 1/2025–1/60750 and high, ≥1/60750. The combination of minimal disseminated disease (MDD) and anti-ALK titer will be considered in future ALCL trials for treatment stratification. Little is known about long-term evaluation of this immune reaction to ALK.

We present here ALK antibody titers results of a long follow-up of French paediatric ALCLs.

Methods: Sera of patients included in clinical trials from 1991 were frozen at diagnosis and/or during the follow-up after informed consent. ALK antibody detection and titers are measured with an indirect immunoperoxidase technique provided by K. Pulford (UK). COS-1 cells are transiently transfected with a *pcDNA3-NPM-ALK*. Cytocentrifuge preparations are made from cells harvested after 24 to 48 h of culture. Those are incubated with 1/50 and 1/100 plasma dilutions for antibody detection and between 1/250 and 1/60 750 for titer determination (6 levels). An (HRP)–conjugated rabbit antihuman immunoglobulin IgG is used as secondary antibody, and the analysis is performed under optical microscopy examination. The highest dilution of the serum samples at which staining of the NPM-ALK transfectants is still observed is taken as the antibody's titer.

Results: Fifty-two sera of 44 patients are already tested: 4 at diagnosis of which 2 with 4 other follow-up points and 48 during follow-up (median interval between diagnosis or relapse and sample collection: 4 years (0–21)).

Only one patient has no detectable antibody. According to the 3 groups previously described, a low titer is found in 28 sera (54%), an intermediate level in 23 (44%) and a high level in only one patient.

The results are also examined according to the time of sample collection since diagnosis or relapse:

- Sixteen sera between 2 and 5 years after diagnosis/relapse: 1 without detectable antibodies, 9 ≤1/750, 5 from 1/2250 to 1/60 750 and 1 ≥1/60750.
- Ten between 5 and 10 years: none without detectable antibodies, 4 ≤1/750, 6 from 1/2250 to 1/60 750 and none ≥1/60750.
- Thirteen more than 10 years after diagnosis, up to 21 years: none with antibodies levels ≤1/50, 10 ≤1/750, 3 at 1/2250 or 1/6750 and none ≥1/60 750.

Conclusions: The interesting point is that anti-ALK immunity might remain up to 20 years after diagnosis, with relatively high level of ALK antibodies in some patients. These results will need to be explored further and correlated to treatment, events and minimal disseminated disease (work in progress).

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ALK BINDING SITES OF ANTI-ALK-ANTIBODIES FROM PATIENTS WITH ANAPLASTIC LARGE CELL LYMPHOMA ANALYSED BY PEPTIDE ARRAY

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Introduction: Patients (pts) with ALK-positive anaplastic large cell lymphomas (ALCL) mount a humoral immune response against the oncoantigen NPM-ALK. ALK-antibody titers before therapy inversely correlate with relapse risk.

Binding epitopes of the ALK-antibodies within NPM-ALK are not known. Differences in the strength of anti-ALK antibody response might be explained by binding of ALK-antibodies to different epitopes within the NPM-ALK protein. We used overlapping peptide microarray technology to describe epitopes within the NPM-ALK protein that are detected by pts serum/plasma.

Methods: Peptide arrays (Peps for LifeScience, Heidelberg) consisted of 20 mer peptides with an overlap of 16 amino acids covering the whole NPM-ALK protein sequence.

Pre-treatment serum/plasma from 129 ALK-positive ALCL pts included in the trials NHL-BFM95 and ALCL99 between 1996 and 2011 who had an anti-ALK antibody titer >0 were analysed. Plasma from 20 healthy young adults and one ALK-negative ALCL pt served as negative controls. The ALK1 monoclonal antibody was used as positive control. After incubation of serum/plasma on the peptide arrays, secondary anti-human-IgGCy3 antibodies were used for detection. Slides were scanned with

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GenePix 4000B (Axon Instruments). Genpix result files were analysed using an integrated analytical method developed and described by Imholte et al. (J Immunol Methods 2013).

Results: Sera from ALCL-pts and controls did not detect epitopes of NPM or the NPM-ALK fusion site. ALK1 bound to 4 overlapping peptides corresponding to protein sequences distal of the kinase domain of ALK. Twenty-five per cent of the pts sera but not control sera detected these peptides as well. Sera of pts with ALK-antibody titers ≤1/750 detected mainly three epitopes distal to the ALK kinase domain. The number of detected epitopes increased in sera of patients with ALK-antibody titers >1/750.

Conclusion: Our results suggest that the humoral anti-ALK immune response is directed against the embryonal tumour-associated antigen ALK rather than epitopes from the tumour-specific NPM-ALK fusion site or NPM. Few epitopes within the ALK-portion distal to the kinase domain are detected by autoantibodies of pts with low and high ALK-antibody titers. The strength of the ALK-autoantibody titer is reflected by an increase in number of detected ALK epitopes.

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CLINICAL IMPACT OF INDUCTION TREATMENT AND OPTIMAL TIMING OF RADIOTHERAPY FOR THE LIMITED-STAGE NK/T-CELL LYMPHOMA

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Introduction: For the treatment of limited-stage (stage I or II) natural killer (NK)/T-cell lymphoma, combined treatment modality including radiotherapy (RT) is widely recommended. This study investigated the therapeutic effect of induction chemotherapy, the optimal timing of RT and the clinical outcome of different therapeutic modalities in patients with limited-stage NK/T-cell lymphoma.

Methods: We retrospectively analysed 145 patients with newly diagnosed, limited-stage NK/T-cell lymphoma between October 1998 and June 2013. The patients were categorized into 3 groups according to primary therapeutic modalities, i.e. (i) sequential chemotherapy followed by radiotherapy (SCRT), (ii) concurrent chemo-radiotherapy (CCRT) followed by non-anthracycline-based chemotherapy (CCRT/CT), and (iii) chemotherapy alone (CT).

Results: The median age was 52 years (range 17–85) with male predominant (66.2%). One hundred five (72.4%) patients received RT during primary treatment. In brief, 57 (39.3%) and 48 (33.1%) patients were treated by SCRT and CCRT/CT, respectively, and the remaining 40 (27.6%) patients were managed by CT alone. Overall response (OR) rate was 83.4% including 73.4% of complete response (CR). Patients who are treated with SCRT or CCRT/CT achieved markedly high CR rate (79.2%) compared to CT alone group (57.9%) ($P = 0.003$). Within RT-containing group, the CR rate was not different between SCRT and CCRT/CT regardless of chemotherapy regimen (83.6% and 73.9%, respectively, $P = 0.472$). With median follow up of 72.7 months, the 5-year progression-free survival (PFS) was 47.3%, and overall survival was 64.9%. The 5-year PFS of patients with RT containing protocol and with CT only group were 55.2% and 26.8%, respectively ($P < 0.001$). Among RT applied patients, the 5-year PFS of SCRT and CCRT/CT were statistically comparable as 63% and 46.2%, respectively ($P = 0.066$). Interestingly, 7 patients in CCRT/CT group could not proceed to the planned following chemotherapy due to disease progression just after CCRT ($n = 4$) and decreased performance status ($n = 3$). The disease relapse at outside of RT fields was higher in CCRT/CT group ($P = 0.029$). NKPI and RT were independent prognostic factors influencing survival in multivariate analysis

Conclusions: Based on this analysis, we were able to emphasize the role of RT for treating limited-stage NK/T cell lymphoma, again. However, the optimal timing of RT in early treatment course should be determined carefully in order to complete the planned treatment schedule or prevent systemic relapse. Moreover, RT at upper aero-digestive tract could decrease the therapeutic compliance for next following chemotherapy.

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POLYETHYLENE GLYCOL CONJUGATED ASPARAGINASE-CHOP IN ADULT NEWLY DIAGNOSED EXTRANODAL NK/T-CELL LYMPHOMA: A MULTI-CENTRE PROSPECTIVE PHASE II STUDY

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Introduction: L-asparaginase (L-ASP) combined with chemotherapy significantly improved efficacy and long-term survival of patients with adult extranodal natural killer NK/T-cell lymphoma. Administration of L-ASP is limited by hypersensitivity reactions mediated by anti-asparaginase antibodies. Native *Escherichia coli* L-asparaginase was conjugated to polyethylene glycol to formulate polyethylene glycol-conjugated asparaginase (PEG-ASP) with decreased immunogenicity and increased circulating half-life. The efficacy and safety of PEG-ASP in adult extranodal natural killer NK/T-cell lymphoma are unclear. In this study, we investigated the efficacy and toxicity of PEG-ASP combined with cyclophosphamide, doxorubicin, vincristine and prednisone (PEG-L-CHOP regimen).

Methods: The study was a prospective, multi-centre, open clinical trial. Between January 2012 and June 2013, Patients with adult newly diagnosed extranodal NK/T-cell lymphoma and an ECOG performance status of 0 to 2 were eligible for enrollment. Treatment included 6 cycles of PEG-L-CHOP regimen: PEG-ASP 2500 IU/m^2 on days 2 (maximal dose 3750 IU), 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 (maximal dose 2 mg) and prednisone 60 mg/m^2 on days 1 through 5 of a 21-day cycle. Radiotherapy was scheduled after 2–4 cycles of PEG-L-CHOP regimen, depending on stage and primary anatomic site. The primary endpoint was complete response (CR) rate.

Results: A total of 33 eligible patients (from 6 centres in China) were enrolled. There were 19 male and 14 female with a median age of 39 years (range 19–64 years). The primary lesions were located in upper aerodigestive tract NK/T-cell lymphoma (UNKTL) in 30 patients (90.9%). According to the Ann Arbor staging system, 21 patients (63.6%) had stages I–II disease. B symptoms were observed in 12 patients (36.4%). International Prognostic Index score was 1 or lower in 22 patients (66.7%). All patients (100%) underwent chemotherapy. Thirty-three patients completed 170 cycles of chemotherapy, the median cycle of 6 (range 1–6 cycle). Sixteen patients (48%) combined with radical radiotherapy. The overall response rate was 96.9% (31/33) with 75.8% (25/33) complete responses and 21.2% (7/33) partial responses. OS at 1, 2, 3 years were 100%, 90.61% and 80.54%, respectively. The major adverse event was bone marrow suppression in 25 patients (75.8%) with 21 (63.6%) grades 3–4 neutropenia. Decrease of fibrinogen level in plasma was in 15 patients (45.5%). The other adverse events included liver dysfunction and digestive tract toxicities. All patients were without the occurrence of allergic reaction. No treatment-related mortality or severe complications were recorded.

Conclusion: PEG-L-CHOP for adult extranodal natural killer NK/T-cell lymphoma is effective and safe. The major advantage of PEG-ASP is less allergic reaction. The second is more prolonged effect and convenience. Each cycle of treatment need only 1 time, with good compliance of patients.

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ENCOURAGING EXPERIENCE IN THE TREATMENT OF NASAL TYPE EXTRA-NODAL NK/T-CELL LYMPHOMA IN A NON-ASIAN POPULATION

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Introduction: Extranodal NK/T-cell lymphoma, nasal type (EN-NK/TCL-NT) is rare in the Western world while prevalent in the Far East. Previous analyses of Caucasian patients with EN-NK/TCL-NT showed poor prognosis, markedly worse than outcomes of patients with localized disease reported from the Far East.

Methods: We retrospectively collected data of consecutive EN-NK/TCL-NT patients (pts) diagnosed and treated at our centre from January 1996 to the end of 2014. Forty-three pts were identified, including 10 (23%) Asian and 33 (76%) Caucasian or other non-Asian pts. Sixty-five per cent of these pts (28/43) presented lesions in the upper aerodigestive tract, 8 in skin and 7 in other sites. Twenty-six (60%) were at an early stage, and of those, 20 received CHOP or modified-SMILE (m-SMILE) chemotherapy followed by involved site radiotherapy (ISRT). Four early stage pts had chemotherapy alone. Seventeen pts (40%) were presented at an advanced stage, and of those, 10 received chemotherapy alone, and 4 were given combined modality. Seven pts also received autologous stem cell transplantation (ASCT) after chemotherapy or chemo-RT. Three pts received no treatment due to poor physical condition with rapidly progressing disease. PET/CT was incorporated in treatment evaluations and radiotherapy design in 27 (73%) pts.

Results: After 1–3 cycles of chemotherapy, 19/37 pts (51%) were in CR and 11/37 (30%) in PR. With additional chemotherapy (11 cases), 2 PR pts converted to CR, while 3 PR pts had disease progression. CR rate was significantly higher in the m-SMILE group than in the CHOP group (80% vs 30%, $p = 0.015$). Only 1 (3%) among 30 irradiated pts developed an in-field failure; another 3 pts (10%) had disease failures at adjacent sites. Overall, 22/43 (51%) pts had progression or relapse and 21/43 (49%) died. With a 29-month median follow-up for responders (range, 3–186), the estimated 5-year PFS and OS were 39.6% and 54.8%, respectively. Those with localized disease had significantly higher 5-year PFS (55.6% vs 17.6%, $p = 0.000$) and 5-year OS (77.8% vs 20.6%, $p = 0.000$) than those with advanced stage. Eighty per cent of disease progressions occurred within 1 year after diagnosis. Stage, serum LDH, IPI and ECOG PS were found to be significant prognostic factors for OS and PFS. Ethnicity (Asian vs Caucasian) had no prognostic difference ($p = 0.8$).

Conclusions: EN-NK/TCL-NT in non-Asians shared similar disease characteristics and treatment outcomes with pts of Asian origin. Short course m-SMILE chemotherapy induced a high response rate. ISRT of 45 Gy administered immediately following chemotherapy demonstrated excellent local control and was well tolerated. With short course m-SMILE chemotherapy followed promptly by ISRT, most early stage pts have achieved durable remissions. However, advanced stage disease still remains challenging even with current regimens, with frequent progression and high mortality.

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CLINICAL OUTCOME OF P-GEMOX FOR NEWLY DIAGNOSED STAGE III/IV OR RELAPSED/REFRACTORY ENKTL

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Background and purpose: Extranodal natural killer/T-cell lymphoma (ENKTL) is an aggressive form of non-Hodgkin's lymphoma; the prognosis for patients with newly diagnosed stage III/IV or relapsed/refractory ENKTL is extremely poor. Optimal combined chemotherapy is not being defined yet so far. The purpose of this study evaluated efficacy and safety of P-Gemox regimen in patients with newly diagnosed stage III/IV or relapsed/refractory ENKTL.

Methods: We retrospectively analysed the efficacy and safety of pegaspargase combined with gemcitabine and oxaliplatin (P-Gemox) in 60 patients with newly diagnosed stage III/IV and relapsed/refractory ENKTL between February 2008 and August 2014. The P-Gemox dosage was as follows: days 1 and 8, 30-min intravenous infusion of 1000 mg/m^2 gemcitabine; day 1, 2-h intravenous infusion of 100 mg/m^2 oxaliplatin; day 1, deep intramuscular injection of 2000 U/m^2 PEG-ASP at two different sites. The regimen was repeated every three weeks for a maximum of six cycles. Patients underwent autologous hematopoietic stem cell transplantation (ASCT) if they achieved CR.

Results: The objective response and complete remission (CR) of the whole cohort were 73.7% (42/57) and 36.8% (21/57), respectively. The median follow-up was 39.1 (range, 2.4–54.2 months). Median overall survival (OS) and progression-free survival (PFS) was 23.0 (95% confidence interval [CI], 16.441–29.559) and 12.8 months (95% CI, 8.109–17.491), respectively. The 4-year OS and PFS rates were 43.0 ± 7.3% and 36.5 ± 6.9%, respectively (Figure1A,B). There was no difference between newly diagnosed stage III/IV and relapsed/refractory in terms of OS and PFS (Figure2A, B). The OS and PFS of patients who achieved CR were superior to patients with PR and SD/PD, and there was significant difference between the three groups (Figure3A, B, $P < 0.001$). Eleven patients received ASCT after achievement of CR. Three-year OS rate was better than other patients without ASCT (68.2% vs 36.6%, $P = 0.08$, Fig. 4). Common toxicities (>50%) were neutropenia (85.0%), thrombocytopenia (72.0%), hypoproteinemia (86.7%) and anorexia (63.3%). The most common grade III/IV toxicities (>10%) were granulocytosis (31.6%), thrombocytopenia (26.67%) and hypoproteinemia (13.3%). In addition, hypofibrinogenemia was 46.7%. Intracranial bleeding occurred in one patient during the first cycle of P-Gemox. No treatment related to death was found. Some cycles were administered in outpatients' clinic.

Conclusion: The P-Gemox regimen is a safe and promising therapeutic regimen for newly diagnosed stage III/IV and relapsed/refractory ENKTL with acceptable toxicity. This simple and convenient, P-GEMOX regimen warrants further clinical investigation.

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INTERIM RESULTS OF A PHASE II STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FIL) ON GEMCITABINE PLUS ROMIDEPSIN (GEMRO) IN RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA PATIENTS

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Introduction. Relapsed and primary refractory peripheral T-cell lymphomas (PTCL) show a dismal outcome, with 5-year overall survival of only 30%. There is no standard salvage chemotherapy for these patients. Gemcitabine has demonstrated to be an effective monotherapy, yielding 60–70% overall response rates in patients with advanced and heavily pre-treated diseases. Romidepsin, a recently Food and Drug Administration-approved histone deacetylase inhibitor, has demonstrated an overall response rate (ORR) of 30% and a complete response (CR) rate of 16%. We have recently designed a multicentric trial to investigate the role of the combination of gemcitabine plus romidepsin (GEMRO regimen) in relapsed or refractory PTCL, looking for a potential synergistic effect of the two drugs.

Methods. Twenty relapsed/refractory PTCL patients were included in a multicentric, prospective phase II trial that contemplated an induction with romidepsin 12 mg/m^2 intravenously (i.v.) on days 1, 8, and 15 and gemcitabine, 800 mg/m^2 i.v. on days 1 and 15, for 6 cycles, each cycle to be repeated every 28 days. After the induction phase, patients in at least a partial remission (PR) proceeded onto romidepsin maintenance phase, starting one month later, in which romidepsin was administered on days 1 and 15 at the dose of 14 mg/m^2 i.v. until

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disease progression. The primary endpoint was to evaluate the efficacy, as assessed by the CR rate, of GEMRO salvage treatment; safety assessment was regarded as a secondary objective. The trial was registered under EudraCT (2012-001404-38).

Results. At present time, 5 (25%) patients are still on treatment and 15 (75%) are evaluable for response and toxicity. The median age of patients was 55 (range, 24–77) years. According to histology, 10 patients had PTCL not otherwise specified, 9 angioimmunoblastic T cell lymphoma and 1 anaplastic large cell lymphoma (anaplastic large cell lymphoma kinase negative). The median number of prior therapies was 2 (range 1–4); 7/20 (35%) patients had failed a prior stem cell transplant. Nineteen out of 20 patients presented with advanced stage. Among the 15 evaluable patients, the ORR was 27%, including 2 CRs and 2 PRs. One of the 2 CR patients discontinued the treatment after 4 cycles due to cardiac toxicity, however maintaining a continuous CR with a follow-up of 2 years. Grade ≥3 adverse events were represented by thrombocytopenia (45%), neutropenia (26%) and anaemia (9%).

Conclusions. To date, preliminary data failed to show a superiority of the GEMRO combination regimen over single-agent romidepsin as a salvage therapy for refractory or relapsed PTCL patients. More mature data and an adequate follow up will be required to better understand the role of this combination regimen.

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THE ROLE OF TRANSPLANT IN THE TREATMENT OF PERIPHERAL T-CELL LYMPHOMAS (PTCLS): AN ANALYSIS FROM THE T-CELL PROJECT DATABASE

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Introduction: The role of high dose therapy followed by stem cell transplant (HDT) as consolidation of first remission in patients (pts) with PTCLs is controversial. While phase 2 studies have reported results that compare favourably to historical controls, no randomized studies are available. We analysed the cohort of transplanted pts of T-cell project in order to investigate if some indications could emerge supporting early consolidation with transplant after the achievement of initial response.

Methods: Eligible pts with first diagnosis of aggressive, nodal and extranodal subtypes of PTCLs were prospectively registered at a dedicated website via secure HTTP protocols, and baseline information including laboratory and disease extent data, therapy details and follow-up data were collected. Central review of diagnostic biopsy was planned.

Results: From September 2006 to January 2015, 1308 pts were registered by 73 sites worldwide from 14 countries; out of 1248 pts validated so far, 959 had available information on therapy. A total of 194 pts (20%) underwent HDT. Transplanted pts can be categorized in three groups: 56 (6%) and 15 (1%) pts were consolidated with HDT after achieving a CR (HDT-CR1) or a PR (HDT-PR1) with induction therapy, and 123 (13%) received HDT as salvage (HDT-S). HDT was autologous or allogeneic in 63 (89%) and 8 (11%) pts when given as consolidation and in 94 (76%) and 29 (24%) pts when given as salvage treatment, respectively. Main characteristics of transplanted pts are shown in the table. After a median follow-up of 37 mos, 5-yr overall survival (OS) of HDT-CR1, HDT-PR1 and HDT-S was 78% (95% CI 64–87), 43% (95% CI 18–66) and 53% (95%CI 41–63), respectively; 5-yr progression-free survival (PFS) was 63% (95% CI 45–76), 33% (95% CI 12–56) and 38% (95%CI 29–47), respectively. We also investigated on the outcome after relapse of pts who were treated with HDT at time of relapse: 5-yr survival after relapse (SAR) was 54% (95% CI 44–64).

Conclusions: In the T-cell project database, the use of HDT as consolidation of first remission was infrequent. Taking into account the observational nature of the study and the relatively small sample available for analyses, the data support very good outcomes among the small group of pts who received HDT in CR1, which compared favourably to those who received HDT in PR1. Patients receiving HDT as salvage show a satisfactory SAR. These results are to be considered as preliminary, more mature data are needed for definite conclusions.

Abstract 247 Table

Baseline characteristics of transplanted patients [194]	*N*	%
Age, yr; mean (range)	48 (18–72)	
Male gender	129	66
Clinical data [194]:		
ECOG-PS >1	34	17
B-symptoms	109	56
Disease-related symptoms	136	70
Disease extent [155]:		
Stages III–IV	123	79
Number of extranodal sites >1	53	34
Bulky disease (>5 cm)	30	19
Transplant use by subtype [959]		
PTCL, NOS (*N* = 352)	62	18
AITL (*N* = 161)	37	23
ALCL, ALK– (*N* = 142)	30	15
ALCL, ALK+ (*N* = 77)	15	19
NKTCL (*N* = 110)	22	20
Extranodal PTCLs, other (*N* = 84)	21	5
Unclassifiable PTCLs (*N* = 33)	7	221

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PHASE II STUDY OF SMILE CHEMOTHERAPY FOR RELAPSED/ REFRACTORY PERIPHERAL T-CELL LYMPHOMA

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Introduction: We previously reported that SMILE (Steroid, Methotrexate, Ifosfamide, L-asparaginase and Etoposide) is effective for newly diagnosed stage IV, relapsed or refractory extranodal NK/T-cell lymphoma, nasal type (J Clin Oncol 2011; 29: 4410-6). Because of the many similarities in extranodal NK/T-cell lymphoma, nasal type and peripheral T-cell lymphoma (PTCL), efficacy of SMILE was examined for relapsed/refractory PTCL.

Methods: The phase II study of SMILE for PTCL was carried out according to the Simon's two-stage design. Patients with relapsed or refractory PTCL after first-line chemotherapy, aged 15–69 years, and with a performance status of 0–2 were eligible. Eight subtypes of PTCL were subjected, comprising PTCL, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), ALK-positive

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and -negative anaplastic large cell lymphoma (ALCL), hepatosplenic T-cell lymphoma, enteropathy-associate T-cell lymphoma (EATL), primary cutaneous gamma-delta T-cell lymphoma (PCGDTL) and primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma. The primary endpoint was overall response rate (ORR) after 2 cycles of SMILE chemotherapy.

Results: From November 2009 to February 2014, a total of 42 patients were enrolled. The median age was 56 years (range 28 to 69 years), and the men:women ratio was 33:9. The diagnosis was PTCL-NOS in 19, AITL in 14, ALK-positive ALCL in 1, ALK-negative ALCL in 4, EATL in 3 and PCGDTL in 1. Twenty-six were relapsed PTCL, and 16 were refractory to initial treatment with anthracycline-containing regimen. Complete and partial responses were achieved in 6 and 13 patients, respectively, with an ORR of 48% (90% confidence interval, 34–62%). The response was stable disease in 6, progressive disease in 15 but was not evaluable in 2. The ORR was 64% (90% confidence interval, 46–80%) for relapsed PTCLs, but was 27% (90% confidence interval, 10–51%) for relapsed patients, and the difference was statistically significant ($P = 0.048$). One patient died of sepsis, and another patient died of disease in the treatment period.

Conclusion: These results indicate that SMILE regimen is effective for relapsed or refractory PTCL. The efficacy was lower than that for extranodal NK/T-cell lymphoma, but the present T-SMILE study included more relapsed patients. Further follow-up defined in the protocol is required to determine the duration of response and long-term efficacy.

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BENDAMUSTINE TREATMENT IN REFRACTORY/RELAPSED T CELL LYMPHOMAS: A RETROSPECTIVE MULTICENTRE STUDY

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Introduction: Peripheral T-cell lymphoma (PTCL) is an aggressive disease with poor outcome. First-line therapies are usually unsatisfactory with frequent relapses. Median progression-free survival (PFS) and overall survival (OS) for relapse PTCL patients are very short with few available therapeutic options. Bendamustine has been shown to be effective in this setting.

Methods: In order to assess the efficacy of bendamustine outside clinical trials, we conducted a national retrospective study of patients with the diagnosis of PTCL and who were treated with bendamustine. Between 2011 and 2013, about 200 patients with the diagnosis of PTCL have been treated with bendamustine. We present the results of the first 64 patients with complete clinical and biological data.

Results: The population median age was 65 y (range 28–89) with male/female sex ratio of 2 (49/25). Histology were angio-immunoblastic (AILT = 33), PTCL-nos ($n = 18$), anaplastic-large (ALCL = 6), NK/TCL ($n = 2$), mycosis fungoid (MF = 2), subcutaneous panniculitis-like-TCL ($n = 2$), hepato-splenic-TCL ($n = 1$). The majority of patients (92%) had stage-disseminated disease and 67% of them had extranodal localizations. The median number of chemotherapy lines prior to bendamustine was 2 (range 0–5). Three patients have received allogeneic stem cells transplantation (SCT) and four autologous SCT prior to bendamustine. The median duration of response (DoR) after the last chemotherapy was 6.5 months (range 2–71), and 45% of patients had refractory disease at bendamustine treatment.

Fifty-three per cent of patients received fewer than 3 cycles, mostly because of disease progression. Overall, they received a median of 2 cycles (range 1–6) at a median dose of 90 mg/m^2 (range 50–150). The best overall response rate (ORR) was 36% (23/64) with partial response of 9% (PR = 6), complete response (CR = 17) of 27% and metabolic CR of 14% (n = 9). The median DoR was 3.2 months (1–29). In patients with AITL, ORR was 52% (17/33) with 12% of PR and 40% of CR. In patients with PTCL-nos, ORR was 17% (3/18) with 6 and 11% of PR and CR, respectively. The two patients with MF were no responders to bendamustine. Four patients (6%) received allogeneic SCT in CR. Median PFS was 4 months (IC_{95} 2.96–5.04) and median OS was 5 months (IC_{95} 2.64–7.36).

With a median follow-up 5 months (1–55), 70% of patients (45/64) died. The causes of death were disease progression (94%) or toxicities (6%). Grade 3/4 thrombocytopenia, neutropenia and infections occurred in 19%, 9% and 19% of cases, respectively.

Conclusion: Bendamustine as single agent must be considered as a therapeutic option for relapsed or refractory PTCL. The safety profile was good. Combination of bendamustine with other drugs needs to be evaluated.

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THE mTORC1 INHIBITOR EVEROLIMUS PRODUCES TUMOUR RESPONSES IN PATIENTS WITH RELAPSED T-CELL NON-HODGKIN LYMPHOMA

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Introduction: Everolimus is an oral agent that targets the mTORC1 pathway. Multiple studies in B-cell malignancies and relapsed Hodgkin lymphoma have shown clinical activity and studies of combinations are underway. Patients with T-cell non-Hodgkin lymphoma (TCL) often have B-symptoms indicating that cytokines are likely elevated. Many of these are pro-inflammatory and signal through the PI3K/mTOR pathway. For these reasons, we studied the effects of everolimus on TCL *in vitro* and then *in vivo* in a pilot phase II trial in patients with relapsed TCL.

Methods: In this study, we investigated mTOR activation in TCL cell lines pre- and post-everolimus and assessed anti-tumour activity in patients with relapsed/refractory TCL in a phase II trial. Patients received everolimus 10 mg PO daily and were evaluated monthly. Blood cytokines were analysed pre- and post-everolimus in 8 of the patients.

Results: The mTOR pathway was activated in all 6 TCL cell lines tested. Everolimus strongly inhibited malignant T-cell proliferation in all cell lines but had minimal cytotoxic effects. *In vitro* data demonstrate that everolimus completely inhibited phosphorylation of ribosomal S6, a raptor/mTORC1 target, without a compensatory activation of the rictor/mTORC2 target AKT (S475). For the trial, 16 patients with relapsed TCL were enrolled: 7 (44%) had cutaneous (all mycosis fungoides); 4 (25%) peripheral T-cell NOS; 2 (13%) anaplastic large cell; and 1 each of extranodal NK/T-cell, angioimmunoblastic and precursor T lymphoblastic leukaemia/lymphoma types. The overall response rate was 44% (7/16; 95% CI: 20–70%); median PFS 4.1 months (95% CI: 1.5–6.5); and the median OS 10.2 months (95% CI: 2.6–44.3). The median duration of response for the 7 responders was 8.5 months (95% CI: 1.0–not reached). Treatment with single-agent everolimus for two cycles (56 days) led to a reduction in plasma levels of several cytokines but was most evident for IL-12, IL-1RA, sIL-2Rα and IL-8. Specifically, IL-12 and IL-8 plasma levels were inhibited in 7/8; whereas IL-1RA and sIL-2Rα plasma levels were inhibited in 6/8 patients.

Conclusions: Everolimus has anti-tumour activity and provides proof of concept that targeting the mTORC1 pathway in TCL is clinically relevant. Future studies of combinations in TCL are recommended.

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IMAGING

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NOVEL CARDIAC MRI AND CIRCULATING BIOMARKERS MODEL OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY SUGGESTS THAT 'HEALTHIER HEARTS' MAY BE AT GREATEST RISK

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Introduction: Lymphoma survivors are at high risk of treatment related to cardiovascular morbidity and mortality. Anthracycline chemotherapy is one of the major causative factors but accurate methods of identifying patients at risk that are lacking. Cardiac magnetic resonance imaging (CMR) can detect subtle changes in myocardial tissue and has the potential to identify subclinical damage at an earlier reversible stage. This study explored whether a combination of novel imaging and existing circulating biomarkers may be more powerful than clinical assessment to risk stratify lymphoma patients for monitoring and intervention.

Methods: Thirty patients receiving 1st-line anthracycline-based chemotherapy for lymphoma (doxorubicin) or breast cancer (epirubicin) were recruited December 2012 to May 2013. Patients underwent contrast enhanced CMR (1.5T Philips®) with 0.15 mmol/kg gadolinium before, during, after and 1 year following treatment to assess cardiac function, strain and tissue characterisation using T1 and T2 mapping. Pre and post contrasts T1 values and haematocrit were used to estimate myocardial extracellular volume (ECV), a surrogate for fibrosis, and T2 mapping was used to estimate myocardial oedema. Blood was taken for circulating biomarkers at corresponding time points.

Results: Seventeen patients (57%) experienced significant decline in LVEF over 18 months. Paradoxically, an inverse relationship was observed between baseline fibrosis, measured by ECV, and subsequent decline in LVEF suggesting that patients with 'healthier hearts' at baseline may be at greatest risk. ECV increased over time in patients with significant LVEF decline but T2 mapping remained unchanged. Greater dyssynchrony (strain rate) at baseline was associated with significant LVEF decline. Longitudinal strain worsened during and after treatment, but these changes occurred simultaneously with and did not precede LVEF decline. Principle component analysis (PCA) combining baseline ECV, strain rate, anthracycline dose, peak trop I and peak MMP9 decline showed distinct clustering of patients.

Conclusions: The ECV findings in this study generated a new hypothesis that fibrosis due to damage from processes such as ageing and ischaemia may slow entry of anthracyclines into myocardial cells, thus limiting exposure. Contrary to current dogma 'healthier hearts' may therefore be at greatest risk and the commonly employed clinical assessments (pre-existing cardiovascular risk factors and baseline LVEF) inadequate to identify high-risk patients. ECV increased over time in patients with LVEF decline but no significant oedema was seen suggesting that collagen deposition occurs with little overt inflammation. Combining key imaging and circulating biomarkers using a PCA model may be useful in risk stratifying patients for monitoring and intervention, but longer follow-up is required before any strong conclusions can be drawn.

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BASELINE METABOLIC TUMOR BURDEN IN DLBCL AFFECTS RESPONSE TO IMMUNO-CHEMOTHERAPY AND PATIENTS OUTCOME THROUGH INFLUENCE OF RITUXIMAB PHARMACOKINETICS. A LYSA GROUP STUDY

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Introduction: Immuno-chemotherapy associating rituximab (MabThera® and Rituxan®) and anthracycline-based chemotherapy (i.e. CHOP or ACVBP) has dramatically improved survival of patients with diffuse large B-cell lymphoma (DLBCL). However, some patients failed to respond or relapse early after treatment with poor prognosis. Among factors affecting response to treatment, a murine model has suggested that tumour burden could affect rituximab exposure and efficacy. To evaluate the role of tumour burden on response to immuno-chemotherapy, we have analysed the metabolic baseline tumour volume (MTV_0) using TEP and rituximab pharmacokinetics (PK) in a cohort of patients receiving immuno-chemotherapy.

Patients and Methods: One hundred eight patients with DLBCL from two prospective multicentre studies were evaluated for tumour volume and rituximab PK. Patients with localized stage ($n = 19$) were included in GOELAMS 0203 trial (NCI number: NCT00841945), whereas advanced stage ($n = 89$) were included in GELA 073B trial (NCT00498043). All patients received rituximab (375 mg/m^2) associated with anthracycline-based chemotherapy administered every 14 days (CHOP14 or R-ACVBP). Rituximab concentrations were measured before and after each rituximab infusion, on day 5 of each cycle, and 2 to 3 weeks after the fourth cycle. Baseline tumour volume was evaluated by PET (metabolic tumour volume: MTV_0). Rituximab pharmacokinetics was assessed using compartmental modelling, and the influence of MTV_0 was tested as a covariate on PK parameters. Logistic regression was applied to evaluate the influence of MTV_0, on rituximab exposure (AUC), metabolic response (after C4, according to international criteria) and patients' outcome (PFS and OS). AUC values were dichotomized using ROC curve, and the PFS and OS of patients above or below the cut-off were compared using a log-rank test.

Results: At baseline assessment, median MTV_0 was 313.5 cm^3 (range 0.8–4339 cm^3). After four cycles, ORR was 91.7% including 39.3% of CR. Lower rituximab AUC_1 was observed for high tumour volume at baseline assessed by TEP (MTV_0 ; $R^2 = 0.51$, $p < 0.0001$). The cut-off of AUC_1 allowing with the best sensitivity and specificity to discriminate responder patients and patients with different outcomes was 9600 mg.h/L. With a median follow-up of 48 months, patients with an $AUC_1 \geq 9600$ mg.h/l ($n = 48$, 44%) had a significantly better 4-y PFS (87% vs 62%; HR = 0.276, $P = 0.0026$) and 4-y OS (98% vs 69%; HR, $P = 0.0004$) than those with an AUC_1 under the cut-off.

Conclusion: Baseline metabolic tumour volume significantly influences response and outcome of DLBCL patients treated by immuno-chemotherapy and is therefore an important prognostic factor. Our results demonstrating that MTV_0 influences rituximab PK advocate the development of MTV_0 adapted rituximab dosing.

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COMPARISON OF DYNAMIC VISUAL SCORE AND DEAUVILLE SCORE ACCURACY OF INTERIM PET/CT IN 339 PATIENTS WITH HODGKIN LYMPHOMA TREATED ON THE ISRAELI H2 PROTOCOL

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Introduction: The aim of Hodgkin lymphoma (HL) therapy is to maximize response and minimize long-term treatment related toxicity.

Methods: This prospective multicentre study started in 2006. Pts with classic HL aged 18–60 ys, stages I–IV were eligible. Early HL (stage IA/IIA) was categorized to favourable (EF) and unfavorable (EU). After 2 ABVD cycles, EF pts with negative interim PET/CT (PET-2) underwent involved site radiation therapy (ISRT) and EU pts received 2 more ABVD cycles (a total of 4) followed by ISRT. If PET-2 was negative, ISRT could be substituted with additional 2× ABVD. Pts with positive PET-2 were given 2 more ABVD cycles (total 4 or 6) + ISRT. Pts with advanced HL (B symptoms or stage III/IV) were assigned to therapy based on IPS. Pts with IPS 0–2 initially received 2× ABVD. Those with IPS of ≥3 initially received 2× escalated BEACOPP (EB). If PET-2 was negative, additional 4× ABVD were given. If PET-2 was positive, therapy was escalated to EB with ISRT given to bulky mediastinal masses. A dynamic visual score (DS) comparing PET-2 to baseline PET was used for decision-making regarding PET positivity (Dann et al, Haematologica, 2010). For pts with a single site of uptake on PET-2, residual uptake was considered positive if its intensity was ≥normal mediastinal or liver blood pool (the hottest of these two). For pts with multiple initial HL sites, disappearance of uptake in all sites or residual single-site uptake with markedly lower intensity compared to baseline was considered a negative result. Pts with DVL£3 were considered to have a negative PET-2 scan.

Results: Three hundred thirty-nine of 355 pts enrolled in the study were evaluated using both scoring systems: 86% of pts were negative and 7% were positive (93% concordance by both systems; kappa 0.627). Among relapsed patients, 64% had negative PET-2 by both DS and DVL. The values for sensitivity were 0.23 vs 0.28; specificity 0.91 vs 0.93; positive predictive value (PPV) 0.29 vs 0.38; negative predictive value (NPV) 0.88 vs 0.89; and accuracy 0.81 vs 0.83, for DS and DVL, respectively.

Conclusions: Tailored-therapy based on PET-2 is feasible in HL. Similar results were obtained with both scores, while the DVL score seems to provide higher PPV. In the current study, most relapsing pts had negative PET-2 results by both methods. Additional prognostic markers would be beneficial to define the subset of HL patients with negative PET-2 who will eventually relapse.

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ROLE OF BONE MARROW BIOPSY (BMB) IN CLASSIC HODGKIN LYMPHOMA (CHL) STAGING IN FDG-PET/CT ERA

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Background: In recent years, several studies were conducted to investigate the role of routine BMB in newly diagnosed cHL staged with PET/CT: recently, a meta-analysis reported data of 955 patients in 9 different studies to determine whether BMB is still necessary in patients staged at diagnosis with PET/CT. Here we report data of patients (pts) with cHL assessed at diagnosis with both BMB and PET/CT, to evaluate their concordance in the detection of bone marrow lymphomatous involvement.

Methods: We retrospectively analysed data of consecutive pts since 2007 to 2013 referring to 16 haematology departments of the Fondazione Italiana Linfomi (FIL). All pts underwent at baseline to both unilateral and bilateral BMB and PET/CT; stage assessment was performed with PET/CT according to the Ann Arbor classification, and it was compared to that resulting from PET/CT combined to BMB. The predictive significance of PET/CT was determined in terms of positive (PPV) and negative predictive values (NPV), sensitivity and specificity.

Results: In this survey, we included 1244 pts, 159 were excluded due to the lack of baseline BMB or PET/CT. Median age 32 years (range 14–80 years), 567 men (52%). Nodular sclerosis (70.9%) and mixed cellularity (19.3%) were the most common histotypes; bulky disease and B symptoms were present in 27% and 42% of pts, respectively. One hundred sixty-nine pts (16%) presented 1 or more focal skeletal lesions at PET/CT and 55 (5%) had a positive BMB; other pts characteristics are summarized in Table 1. In 34/55 pts, focal skeletal lesions evidenced by PET/CT revealed a positivity of BMB, while in 948/1030 pts, the absence of skeletal lesions or a diffuse skeletal FDG uptake combined with a negative BMB. Based on these data, PPV and NPV resulted to be 20% and 98%, respectively; sensitivity and specificity were 62% and 87%, respectively. In 54/55, patients with positive BMB had PET/CT in stage III or IV, while 1/1043 (0.09%) would have been treated differently if he or she had not performed the BMB.

Abstract 254 - Table 1 Correlation between PET/CT-assessed staging and BMB results

	Patients with negative BMB (*n* = 1030)		Patients with positive BMB (*n* = 55)	
	No	**%**	**No**	**%**
PET/CT Ann Arbor stage				
I	55	5.3	0	0
II	531	51.5	1	1.8
III	250	24.3	8	14.6
IV	194	18.9	46	83.6
Focal skeletal PET/CT lesions				
Unifocal	48	4.7	8	14.5
Bifocal	30	2.9	2	3.6
Multifocal	57	5.5	24	43.6
No focal lesion	895	86.9	21	38.3
Homogeneous diffuse skeletal FDG uptake	53	5.1	9	16.4
Risk group according to GHSG				
Early stage	184	17.9	0	0
Intermediate stage	290	28.1	1	1.8
Advanced stage	530	51.5	54	98.2
Unspecified limited stage	26	2.5	0	0

Conclusions: NPV of PET/CT for bone marrow involvement was very high (98%). Moreover, the influence of BMB on the planning of treatment was minimal. BMB may be omitted in cHL patients staged with PET/CT.

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HODGKIN LYMPHOMA: PET-CT OR BONE MARROW BIOPSY?

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Introduction: Whether to perform routine bone marrow biopsies (BMB) in the PET-CT era for Hodgkin lymphoma has recently come into question. There is mounting evidence that PET-CT is highly sensitive in detecting bone marrow involvement, and a bone marrow biopsy would be unlikely to change management, especially in advanced stage disease. We performed this study to determine degree of concordance between PET-CT and BMB in identifying bone marrow involvement and whether a positive BMB is of prognostic significance when PET scan is negative for bone involvement.

Methods: We reviewed records of all HL patients treated at MDACC between April 1999 and March 2014. Inclusion criteria were stage IV classical HL, age of at least 15 years and presence of pre-treatment PET/CT scan performed within 30 days of therapy initiation. Baseline PET-CT imaging and BMB pathology were reviewed on the 187 eligible patients. Positive bone involvement on PET scan (PET+) was defined as increased uptake in the marrow with or without cortical erosion. Cohen's kappa coefficient was used to measure agreement between PET and BMB in delineating BM involvement. Relapse-free survival (RFS) and overall survival (OS) were assessed via Kaplan–Meier analysis. Comparisons were made by the log-rank test.

Results: Median age was 35 years (range 15–86). The majority of patients had nodular sclerosing HL (70%), with 2–3 extra-nodal sites of involvement (57%). Of the 187 patients, 24.9% had a positive BMB while 46% were PET+. Systemic therapy consisted predominately of ABVD or an ABVD-like regimen (95%). The 5-year RFS and OS of the whole group was 75% and 88%, respectively. Relapses occurred in 42 patients, eventually 21 died with disease.

Kappa coefficient was computed to measure agreement between BMB and PET scan and was found to be 0.446, demonstrating intermediate concordance between the two tests. Of the PET+ patients, 31.35% had a negative BMB, validating prior reports demonstrating higher sensitivity for PET scan in detecting bone marrow involvement. However, 10.3% of patients with negative PET scan had a positive BMB. Among patients with negative PET scan for bone involvement, the 5-year relapse-free survival rate was significantly lower in the subgroup of patients with positive BMB (83.5% vs 64%, $P = 0.03$).

Conclusion: Our results validate that PET scan is more sensitive than bone marrow biopsy in detecting bone marrow involvement in stage IV HL. However, there is a small subgroup with a negative PET for bone involvement but a positive BMB, suggesting that PET-CT is not 100% sensitive. Furthermore, among patients with negative PET scans, those with a positive BMB had a significantly increased risk of relapse. Consequently, while BMB is unlikely to change management in stage IV disease, its continued use in this setting may be of utility in predicting outcome. Future investigations will attempt to determine which patient population would most benefit from BMB.

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THE ROLE OF PET-CT IN ASSESSMENT OF BONE MARROW INVOLVEMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Objectives: Follicular lymphoma (FL) is the second most common type of lymphoma diagnosed in the Western World. It is an indolent B-cell lymphoproliferative disorder, characterized by diffuse lymphadenopathy and splenomegaly, with common involvement of the bone marrow (BM). BM involvement is an adverse prognostic factor in FL, therefore is routinely assessed during disease staging, by an arbitrary biopsy of the BM at the iliac crest. This study was aimed to assess the role of PET-CT in identifying BM involvement.

Methods: Seventy-one patients with FL were enrolled. Visual assessment of BM uptake was categorized as normal, increased in a diffuse pattern or focal increased uptake. Quantitative FDG uptake in the BM was measured using mean standardized uptake value (BM-SUV_{mean}). The diagnosis of BM involved by lymphoma was based either by BM histological findings or by disappearance of an increased uptake at the end-treatment PET-CT, in patients that responded to treatment. Uninvolved BM was defined as no increased uptake and no evidence of BM involvement on biopsy and/or clinical and imaging follow-up.

Results: Twenty-one patients (29.6%) had BM involvement, 17 (24%) had a biopsy proven involvement and 4 (5.6%) had a negative iliac crest biopsy but increased medullary uptake in other skeletal sites that normalized after successful anti-lymphoma therapy. Visual assessment of BM involvement in patients with BM involvement identified diffuse increased marrow uptake in 9 (42.9%) or and focal in 11 (52.4%) and was falsely normal in a single case. However, diffuse uptake was associated with 18 false positive cases. Overall, visual assessment for BM involvement had a positive predictive value (PPV) of 51% and a negative predictive value (NPV) of 97%. On a quantitative assessment, BM-SUV_{mean} was significantly higher in patients with confirmed BM involvement (4.1 ± 1.9 vs 1.4 ± 0.5, $p < 0.0001$). On ROC curve analysis, a BM-SUV_{mean} value greater than 2.51 had a positive predictive value (PPV) of 100% for BM involvement with a sensitivity of 86%, while BM-SUV_{mean} value lower than 1.7 had a negative predictive value of 97.5% and a specificity of 74% for excluding BM involvement. Patients with an involved BM per PET-CT had a mean survival of 85.5 months vs 104.0 months in patients with no BM involvement ($p = 0.001$).

In conclusion: Visual assessment of FDG PET-CT is associated with a high false positive rate but appropriate for ruling out BM involvement. Quantitation of uptake by measurement of BM-SUV_{mean} improved the diagnostic accuracy in assessment of BM involvement in patients with FL. This approach can identify additional 25% patients with BM involvement that would have been missed by an arbitrary bone marrow biopsy of the iliac crest.

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PROGNOSTIC VALUE OF INTERIM FDG-PET/CT IN 100 DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP ACCORDING TO INTERPRETATION CRITERIA AND PRETHERAPEUTIC PROGNOSTIC FACTORS

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Introduction: Positive interim PET-CT (iPET+) is an adverse prognostic factor in DLBCL. Positivity criteria are still debated. The aim of this study was to assess the prognostic value of interim PET-CT (iPET) according to qualitative criteria (Deauville 5-point scale: $iPET_{5PS}$), quantitative criteria (ΔSUVmax : $iPET_{\Delta SUV}$) and to an algorithm combining those two ($iPET_{algorithm}$) in DLBCL. We also evaluated impact of other prognostic factors (IPI, genomic expression profile and Bcl2 status).

Methods: Retrospective analysis of first-line DLBCL was evaluated by PET-CT at baseline and after 2 to 4 cycles of R-CHOP (2: 17%, 3: 68% and 4: 15%) and treated in our institute from 2006 to 2013, with no therapeutic change based on the results of interim PET. The response of the iPET was assessed by qualitative parameters ($iPET_{5PS}$ positive when score $\geq$4), ΔSUVmax ($iPET_{\Delta SUV}$ positive when $\leq$71%) and a combination of those 2. The algorithm criteria were identical to Deauville ones, except for score 4: $iPET_{Algorithm}$ was considered negative when ΔSUVmax $>$71% and positive when ΔSUVmax $\leq$71%. Pretherapeutic factors including GCB gene expression profile (Hans classifier), Bcl2 status and IPI score were also studied. Results were correlated with EFS and OS using curves computed with Kaplan–Meier and compared with log-rank test. Predictive factors were finally selected with multivariate cox regression (IPI, GCB, Bcl2 status and iPET results).

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Results: One hundred patients were included with a median follow-up of 40 months 95%CI [36.1–44.5]. Overall 3-year EFS and OS were 63.7% and 75.8%. Every PET criteria was significantly discriminating for outcome in terms of EFS and OS ($p < 0.0001$), slightly less in OS discrimination using quantitative criteria ($p = 0.0082$). Three-year and 5-year EFS were, respectively, 83.1% and 73.9% in $PET_{Algorithm}$ negative versus 14.3% and 0% in $PET_{Algorithm}$ positive, 3-year and 5-year OS were, respectively, 81.6% and 81.6% in $PET_{Algorithm}$ negative versus 50% and 43.8% in $PET_{Algorithm}$ positive.

Evaluation of pretherapeutic factors showed significantly different EFS and OS in GCB versus non-GCB (respectively $p = 0.087$ and $p = 0.031$), as well as in low IPI (1, 2, 3) versus high IPI (4, 5), respectively, $p = 0.014$ and $p = 0.031$. BCl2 status did not show any significant statistical difference in terms of EFS ($p = 0.13$).

In multivariate Cox regression, positive $iPET_{algorithm}$ (hazard ratio = 11.7) and GCB status (HR = 0.431) were independent risk factors of EFS. Combining algorithm and GCB status splits sample in 3 groups with significant different 5-year EFS and OS, respectively, 93% and 93% in the (GCB and negative $iPET_{algorithm}$) group; 60% and 81% in the (non-GCB and negative $iPET_{algorithm}$) group, and 0% and 44% in the (positive $iPET_{algorithm}$ and non-GCB) group, $p < 0.0001$.

Conclusions: The algorithm we proposed successfully stratified patients according to EFS and OS. Integrating genomic expression profiles study improves the outcome prediction.

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BLINDED EVALUATION OF THE PROGNOSTIC VALUE OF FDG-PET/CT AFTER 2 CYCLES OF RCHOP IN DLBCL – UK-NCRI STUDY

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Purpose: The prognostic value of early PET scanning in diffuse large B-cell lymphoma (DLBCL) has not been established. The UK-NCRI initiated a prospective blinded study (UKCRN-ID 1760) to assess the prognostic value of FDG-PET/CT after 2 cycles of RCHOP in a subset of patients treated in the prospective NCRI study comparing RCHOP-21 and RCHOP-14.

Methods: One hundred eighty-nine eligible patients had baseline (PET-0) and post-cycle 2 PET (PET-2) scans according to study protocol in centres, which satisfied QA requirements. The PET-2 scan was not reported but archived for central reporting at least 3 months later. The primary endpoint was 2-year progression-free survival (PFS). Response was assessed using visual [NCRI criteria and Deauville score (DS)] and quantitative methods [reduction in maximum standardised uptake value (SUVmax)]. Baseline metabolic tumour volume (MTV) was also measured. Cox regression and Kaplan–Meier curves were used to examine the relationship between PFS and the study variables; receiver operator characteristic (ROC) analysis was used to determine the optimal cut-off for continuous variables.

Results: After a median follow-up of 53.8 months, the 2-year PFS was 79.8% (95% CI: 74.1–85.5) and 2-year overall survival (OS) was 85.6% (95% CI: 80.5–90.7). Univariate Cox regression analysis showed the following parameters to be associated with worse PFS: NCRI score 2c–2d (HR = 8.2, 95% CI: 2.6–26.7, $p < 0.001$), DS-5 (HR = 3.0, 95% CI: 1.3–6.7, $p = 0.007$), International Prognostic Index (IPI) score 4–5 (HR = 2.7, 95% CI: 1.4–5.2, $p = 0.003$), baseline MTV ≥1856 cc as defined by ROC (HR = 2.5, 95% CI: 1.3–4.8, $p = 0.009$) and SUVmax reduction <66% (HR = 2.2, 95% CI: 1.1–4.5, $p = 0.035$). The number of patients in the worse prognostic group for each variable was relatively small (4, 14, 26, 25 and 21 respectively). Multivariable analysis showed the following variables to be independent predictors of worse PFS: NCRI score 2c–2d (HR = 7.2, 95%CI: 1.7–31.0, $p = 0.008$), DS-5 (HR = 3.3, 95%CI: 1.2–9.2, $p = 0.022$) and IPI score 4–5 (HR = 2.7, 95%CI: 1.3–5.3, p = 0.005).

Abstract 258 Table. Two-year progression-free survival (PFS) rates by prognostic group

Prognostic group	2-year PFS % (95% CI)
NCRI score	
1–2b (*n* = 185)	81.0 (75.3–86.7)
2c–2d (*n* = 4)	25.0 (0–67.5)
Deauville score	
1–4 (*n* = 175)	81.6 (75.9–87.3)
5 (*n* = 14)	57.1 (31.2–83.0)
SUVmax reduction	
≥66% (*n* = 168)	81.5 (75.6–87.4)
<66% (*n* = 21)	66.7 (46.5–86.9)
Baseline MTV	
<1856 cc (*n* = 164)	83.5 (77.8–89.2)
≥1856 cc (*n* = 25)	56.0 (36.6–75.4)
IPI	
1–3 (*n* = 161)	82.6 (76.7–88.5)
4–5 (*n* = 26)	61.0 (42.0–80.0)

Conclusion: FDG-PET/CT after 2 cycles of RCHOP identifies a small group of patients who have worse PFS. Baseline characteristics have independent prognostic value from early PET-2 response. Improving prognostic value of PET-2 may be possible by combining baseline characteristics with PET-2 response.

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NO SURVIVAL BENEFIT ASSOCIATED WITH ROUTINE IMAGING FOR CLASSICAL HODGKIN LYMPHOMA IN COMPLETE REMISSION: A DANISH-SWEDISH POPULATION-BASED STUDY

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Background: Patients with classical Hodgkin lymphoma (HL) in first remission are followed closely for signs of relapse, but the use of routine surveillance imaging for this purpose is controversial. The follow-up (FU) strategies in Denmark and Sweden are similar except for the routine imaging practice for patients in complete remission (CR). The aim of this study was to examine the impact of routine imaging on the post-remission survival in HL.

Methods: Patients registered in the Danish (LYFO) and Swedish population-based lymphoma registries were included by the following criteria: (i) classical HL in 2007–2012, (ii) aged 18–65 years at diagnosis, and (iii) CR after treatment with ABVD or BEACOPP ± radiotherapy. The FU for Danish and Swedish patients included symptom assessment, clinical examinations and blood tests with three months intervals in the first two years after treatment and with longer intervals later in FU. In Sweden, imaging was only performed in response to clinically

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suspected relapse. In Denmark, routine imaging was included in the standard FU programs, and the most common imaging practice was half-yearly computed tomography (CT) for two years. Overall survival (OS) was defined as the time from end of treatment until death/censoring. Cox regression models were used to assess prognostic factors for post-remission OS. Cumulative incidences for progression/relapse were calculated for Danish patients, as updated relapse data were available for this group.

Results: A total of 317 Danish and 454 Swedish patients were included. The men : women ratio and the frequencies of advanced stage disease (stages III–IV) and B-symptoms were comparable for Danish and Swedish patients. However, the Swedish patients had slightly lower median age (34 vs 38, $P = 0.02$). Age ≥45 years (HR 5.07, 95%CI 2.37–10.85, $P < 0.01$) was the only adverse prognostic factor for post-remission OS in multivariate analysis, but ECOG performance score ≥2 was associated with a strong trend for inferior OS (HR 2.65, 95%CI 0.99–7.06, $P = 0.05$). An imaging-based FU strategy (Danish patients) had no positive or negative effects on post-remission survival for the entire cohort ($P = 0.2$) or for patients grouped according to the Ann Arbor stage ($P = 0.5$ for stages I–II and $P = 0.4$ for stages III–IV). The 2-year cumulative progression rates (death/relapse) were 4% (95%CI 1–7) for patients with stages I–II disease and 12% (95%CI 6–18) for patients with stages III–IV disease.

Conclusions: Disease progression is rare among young HL patients in first CR, and few patients will benefit from a relapse-oriented FU program. More importantly, there were no differences in the post-therapy survival between Danish and Swedish patients despite the widespread use of routine imaging in Denmark, and an imaging-based FU for HL in 1st CR is of no benefit.

PRE-CLINICAL STUDIES

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A CROSS-SPECIES PAN-OMICS APPROACH IN A TRANSGENIC MOUSE LYMPHOMA MODEL TO DECIPHER TREATMENT FAILURE IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Introduction: Treatment failure is the key determinant of poor outcome in lymphoma therapy. Unveiling the underlying molecular mechanisms is critical to overcome drug insensitivity, may identify novel targets and direct the development of conceptual treatment alternatives. Since patient samples are rarely available as matched pairs at diagnosis and at a resistant state and cannot be further drug-challenged or subjected to functional and material-demanding validation experiments, we considered transgenic mouse models of cancer as valuable tools for the molecular dissection of treatment responsiveness. Notably, we previously demonstrated the predictive cross-species power of our murine lymphoma model for patients diagnosed with diffuse large B-cell lymphoma (DLBCL) (Reimann-M, Cancer Cell, 2010; Jing-H, Genes Dev., 2011). Here, we employ genomics, transcriptomics, proteomics and metabolomics in a 'pan-omics' approach to decipher mechanisms of treatment resistance in the well-established Eμ-*myc*-driven lymphoma mouse model.

Methods: Seventy-nine immunocompetent recipient mice were transplanted with 34 primary Eμ-*myc* transgenic mouse B-cell lymphomas and exposed to cyclophosphamide (CTX) upon tumour manifestation. Whole-exome sequencing, copy number alteration analysis, array-based transcriptomics and kinomics, mass spectrometry-based proteomics and metabolomics and functional assays (e.g. apoptosis and cellular senescence) were applied, and the data subjected to bioinformatics processing to unveil mechanisms of treatment resistance.

Results: After treatment of lymphoma-bearing mice, lasting remissions (reflecting cure) were observed in about half of the cohort (comparable to DLBCL patients after induction therapy). Repetitive treatments of relapsing mice resulted in progressively shortened remission times and finally full-blown resistance, thereby recapitulating clinical courses of patients with drug-insensitive aggressive lymphomas. Multipe omics technologies were applied to the large sample panel to compare curable vs relapse-prone and resistant lymphomas, all with or without an additional short-term exposure to CTX to acutely challenge drug-specific response programmes. Candidate findings and our integrative trans-omics bioinformatics strategy, targeting one of the most critical needs in omics-based cancer research and beyond, will be discussed at the conference.

Conclusions: Eμ-*myc* lymphoma-bearing mice treated in a clinical trial-like fashion were established as a faithful and versatile model of clinical chemoresistance. Going beyond a transcriptome-restricted investigation, our pan-omics strategy aims to dissect underlying mechanisms that will be further exploited as targets on their own for novel lesion-based (co-) therapies or advanced, condition-based targeting strategies in future cancer precision medicine.

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BIOLOGICAL RATIONAL FOR SEQUENTIAL TARGETING OF BRUTON TYROSINE KINASE AND BCL2 TO OVERCOME CD40-INDUCED BH3-MIMETICS RESISTANCE IN MANTLE CELL LYMPHOMA

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Aims: The aggressive biological behaviour of mantle cell lymphoma (MCL) and its short response to current treatment highlight a great need for better rational therapy. Recently, it has been demonstrated that tumour microenvironment strongly influence drug resistance in MCL cells. In the present study, we investigated the apoptotic efficiency of the first-in-class orally bioavailable Bcl-2-selective BH3 mimetic ABT-199 by integrating the key role of the microenvironment.

Methods: To determine sensitivity of MCL cells to ABT-199, cell lines ($n = 8$) and primary MCL cells obtained from peripheral blood of patients at diagnosis or relapse ($n = 11$) were treated with ABT-199. To mimic the lymph node microenvironment where CD40-CD40L interaction takes place, MCL cells were cultured on CD40L-expressing cells.

Ibrutinib has been shown to induce a redistribution of lymph node resident MCL (lymphocytosis). Thus, we investigated the lymphocyte population in the peripheral blood of two patients that received ibrutinib. Blood was collected and analysed for the presence of $CD19^+$ $CD5^+$ MCL cells before treatment and at days 2 and 7 following treatment. Peripheral blood population obtained on day 7 was treated with ABT-199 to analyse the cytotoxic efficiency.

Results: All primary MCL samples from peripheral blood ($n = 11$) were highly sensitive to ABT-199. In contrast, among MCL cell lines tested ($n = 8$), only three were sensitive and *BCL2/(BCLXL + MCL1)* mRNA ratio is a strong predictor of sensitivity.

By mimicking the microenvironment through CD40 stimulation, we show that ABT-199 sensitivity is impaired through activation of NF-kB pathway and Bcl-x_L up-regulation. We further demonstrate that resistance is rapidly lost when MCL cells detach from CD40L-expressing fibroblasts. Ibrutinib induces lymphocytosis *in vivo* holding off malignant cells from their protective microenvironment, and we show

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here for two patients undergoing ibrutinib therapy that mobilized MCL cells are highly sensitive to ABT-199. This led us to propose a rational combination strategy to overcome microenvironment-dependent ABT-199 resistance through prior induction of cellular egress in peripheral blood using the selective BTK inhibitor ibrutinib.

Conclusion: Our results provide evidence that *in situ* ABT-199 resistance can be overcome when MCL cells escape from the lymph nodes. Altogether, our data support the clinical application of ABT-199 therapy both as a single agent and in sequential combination with BTK inhibitors.

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INHIBITION OF PIM KINASES DECREASES PROTEIN TRANSLATION AND ATTENUATES MYC AND NFKB ACTIVITY IN DIFFUSE LARGE B-CELL LYMPHOMA CELL LINES

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Introduction: Diffuse large B-cell lymphoma (DLBCL) accounts for 30–40% of all non-Hodgkin lymphomas. Current treatment options are ineffective in 1/3 of DLBCL patients who die of the disease, underscoring the need for development of more effective, targeted therapies. Simultaneous genetic ablation of three PIM kinases (PIM-1/2/3) or their pharmacological inhibition induces apoptosis in DLBCL cell lines, providing proof of concept that these proteins are promising targets in this disease. To better understand mechanisms of toxicity of PIM inhibition in DLBCL, we investigated biochemical and biological consequences of PIM inhibition using novel pan-PIM inhibitor (SEL24-B489) in a panel of DLBCL cell lines representing GCB- and ABC-DLBCL subtypes.

Methods: Protein expression and phosphorylation status of PIM substrates was assessed by immunobloting. Proliferation and apoptosis were assessed by MTS assay and PI/Annexin V staining, respectively. RNA content analysis was performed via FACS-acridine orange staining. NFkB target gene expression was assessed by real-time PCR (RT-PCR).

Results: The newly developed pan-PIM inhibitor (SEL24-B489) was toxic to DLBCL cell lines in low-micromolar or sub-micromolar concentrations (IC50 ranging from 0.29 to 1.17 μM). To determine mechanisms underlying toxicity of PIM inhibition in DLBCL, we first assessed the activity of 4EBP1 and ribosomal protein S6, proteins involved in protein translation. PIM inhibitor rapidly and uniformly decreased 4EBP1 and S6 phosphorylations in both ABC- and GCB-DLBCL cell lines. Since PIM kinases have been shown to increase stability and/or activity of Myc and NFκB-p65 (RelA), we assessed the abundance/activity of these transcription factors upon PIM inhibitor treatment. PIM inhibition led to downregulation of Myc protein in GCB-DLBCL cell lines but not in ABC cells. Since Myc has been shown to act as a general amplifier of actively expressed genes in lymphocytes, we assessed global RNA levels upon PIM inhibitor treatment. Consistent with decreased MYC activity, the RNA abundance decreased by 22–37% in GCB cells, whereas in ABC-DLBCL cells, it remained unchanged. Since PIM kinases have been shown to increase NFkB activity via multiple mechanisms, we assessed expression of NFκB target genes in DLBCL cell lines. In cells treated with SEL-B489, we observed marked downregulation of known NFκB target genes *IkBα*, *Bfl*-1 and mir-155 in cells representing ABC but not GCB subtype.

Conclusions: A novel pan-PIM kinase inhibitor (SEL24-B489) exhibits universal and subtype-specific activities in DLBCL cells. Decreased protein translation represents a general and universal mechanism of PIM inhibition-associated toxicity in DLBCL cells. Downregulation of Myc and decreased RNA transcription occurs predominantly in GCB-type cells, whereas attenuation of NFκB-p65 activity is observed only in ABC-type cells.

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PIM KINASES PROMOTE CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS SURVIVAL THROUGH A PLEIOTROPIC MECHANISM INVOLVING MODULATION OF CXCR4-TRIGGERED mTOR PATHWAY

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Introduction. Lymph node microenvironment supports CLL cells through multiple contact-mediated and paracrine signals. Highly conserved PIM kinases support tumour cell growth through modification of proteins regulating transcription, translation, apoptosis, cell cycle and adhesion/motility. Therefore, PIM inhibitors might exhibit pleiotropic effects via modulation of these processes and disruption of microenvironmental interactions. Herein, we assessed the PIM kinase expression in CLL patients and investigated the consequences of PIM kinase inhibition in primary CLL cells.

Methods. The expression of PIM kinases was analysed in CD19+ cells from 71 newly diagnosed CLL patients using RQ-PCR. Apoptosis was measured by Annexin V/PI staining. Protein expression and phosphorylation status were assessed by immunoblotting. The surface expression of CXCR4 was estimated with flow cytometry. CXCR4-mediated migration in CXCL12 chemokine gradient was assessed using transwell chamber assay.

Results. PIM2 transcript abundance was higher than PIM1 and PIM3 ($p < 0.0001$). PIM2 expression was higher in patients with advanced disease (Rai 3–4, $p = 0.004$). Higher PIM2 expression was also observed in patients who relapsed after first-line treatment ($p = 0.005$). Incubation of primary CLL cells with a novel PIM kinase inhibitor SEL24-B489 (1–10 μM) decreased levels of p-4EBP1(S65), p-FOXO1 (T24)/FOXO3a(T32) and induced dose-dependent apoptosis. We next tested, whether PIM inhibitor might interfere with CXCR4-mediated migration. SEL24-B489 reduced phospho-CXCR4 (Ser339) level and decreased CXCR4 surface expression. Consistent with this, SEL24-B489 significantly (up to 80%) altered the migration of primary CLL cells towards CXCL12 gradient. To investigate the role of PIM kinases in modulation of CXCR4-dependent signals in CLL cells, we assessed the activity of mTOR pathway after incubation with CXCL12 and found increased levels of p-mTOR (S2448) and mTOR substrates, p-AKT (S473) and p-4EBP1 (T37/T46). Activation of mTOR was blocked by SEL24-B489 in a mechanism involving altered phosphorylation of PRAS40 (T246) and p-TSC2 (S1798). Consistent with the role of mTOR signalling in CLL cell migration, mTOR inhibitor OSI-027 and PI3Kδ inhibitor idelalisib also decreased CLL cell migration in the CXCL12 gradient.

Conclusions. We demonstrate herein that PIM kinases control CLL cell migration not only by regulating CXCR4 phosphorylation and expression but also by altering CXCR4-triggered mTOR activity. Novel pan-PIM kinase inhibitor SEL24-B489 decreases cellular migration by targeting both mechanisms, inhibits cap-dependent translation and induces apoptosis. Since overexpression of PIM kinases might be associated with adverse clinical characteristics at diagnosis, PIM inhibition might be a rational therapeutic strategy in CLL.

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PRECLINICAL EVALUATION OF THE NOVEL PLEIOTROPIC BCR KINASE INHIBITOR IQS019 IN IN VITRO AND IN VIVO MODELS OF B LYMPHOID NEOPLASMS

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Introduction: Inhibition of B cell receptor (BCR) signalling has recently emerged as one of the most promising therapies in lymphoid neoplasms. The cascade of effectors downstream of the BCR includes many kinases that are amenable to therapeutic intervention, including the SRC-family kinases, Syk and Btk. Despite the promising results obtained with the first BCR kinase inhibitors in some B lymphoid neoplasms, such as the Syk-targeting drug fostamatinib or the Btk inhibitor ibrutinib, the design of new compounds is warranted to improve treatment efficacy and to bypass the resistance appearing in patients primarily responsive to current BCR-targeting therapies. In this context, our aim was to evaluate the antitumour activity of IQS019, a new 4-aminopyrido[2,3-d]pyrimidine with broad BCR kinase inhibiting properties, in preclinical models of B lymphoid neoplasms.

Methods: We determined by MTT assay and flow cytometry the time- and dose-dependent cytotoxic effect of IQS019 in a panel of 21 human cell lines and 17 primary samples corresponding to representative B-cell neoplasms, including chronic lymphocytic leukaemia (CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). The phosphorylation status of the BCR-related kinases Syk, Lyn and Btk with or without IgM-mediated BCR stimulation, as well as cell chemotaxis, was assessed after IQS019 treatment by western blot and flow cytometry. Safety and efficacy of the compound was assessed in healthy mice and xenotransplant mouse models of FL and MCL, respectively.

Results: Doses of IQS019 in the micromolar range engaged a rapid and dose-dependent dephosphorylation of both constitutively and IgM-activated Syk, Lyn and Btk in CLL, MCL, FL and DLBCL cell lines and CLL primary cultures, leading to impaired cell proliferation and CXCL12-dependent cell migration, followed by induction of caspase-dependent apoptosis. Accordingly, mice bearing MCL and FL tumours and receiving a daily treatment of IQS019 showed a reduced tumour outgrowth characterized by a decreased mitotic index and a lower infiltration of malignant cells in the spleen, in tight correlation with the downregulation of phospho-Syk, phospho-Lyn and phospho-Btk.

Conclusions: These results define the BCR inhibitor IQS019 as a potential candidate in antitumour therapy against a variety of B-cell lymphoid neoplasms.

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SYK INHIBITION TRIGGERS DLBCL CELL DEATH VIA A MECHANISM LINKING FOXO1 ACTIVATION, DREAM CLEAVAGE AND EXPRESSION OF A PROAPOPTOTIC BCL2 FAMILY MEMBER, HRK

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Introduction: In normal B lymphocytes, B-cell receptor (BCR)-induced activation of PI3K-AKT kinases and subsequent inactivation of FOXO1 is critical pro-survival component of tonic BCR signalling. Disruption of the BCR signalling by SYK inhibitor leads also to the apoptosis of BCR-dependent DLBCL, at least in part via a mechanism involving decreased activity of PI3K/AKT axis. Herein, we investigated the role of FOXO1 in toxicity of BCR pathway/SYK inhibition in human BCR-dependent lymphomas.

Methods: The expression of p-SYK and FOXO1 in DLBCL samples was assessed by immunohistochemistry (IHC) in 60 DLBCL patients. FOXO1 knock-down in DLBCL cell was achieved with shRNA. FOXO1 target gene expression was assessed by qPCR. DLBCL cell viability and apoptosis after incubation with SYK and/or AKT inhibitors R406 and MK2206 were assessed by MTS assay and Annexin V/PI staining. Interactions were computed by CompuSyn software. DREAM cleavage and HRK expression were assessed by western blotting and qPCR, respectively, in the absence or presence of caspase inhibitors. Transfections were achieved with retroviral vectors.

Results: FOXO1 expression was present in 80% of DLBCL samples and correlated with SYK activity ($p = 0.009$). High levels of FOXO1 protein expression were associated with longer OS (log rank, $p = 0.04$). Since FOXO1 is a major effector of tonic BCR signal, we assessed the activity of FOXO1 in DLBCL cells after SYK inhibition. R406 decreased phosho-FOXO1 levels and triggered FOXO1 target genes BIM, TRAIL, GADD45A and p27. Cells with depleted FOXO1 exhibited 70% lower sensitivity to SYK inhibitor than control cells ($p < 0.0001$). Since in previous studies, expression of the proapoptotic member of BCL2 family, HRK, was required for SYK-inhibitor induced cell death in DLBCL cells, we determined the role of FOXO1 activation in HRK expression. HRK expression was dramatically increased in SYK-inhibitor treated control cells but not in FOXO1 – deficient cells. Consistent with this, SYK inhibitor triggered cleavage of HRK transcriptional repressor DREAM only in control cells but not in FOXO1-depleted cells. HRK induction was blocked by caspase inhibitors. Since AKT is major kinase regulating both FOXO1 activity and HRK induction, we assessed the combined effects of the AKT inhibitor MK-2206 with R406 and found markedly synergistic toxicity (combination index [CI] 0.5–0.8). Combination of inhibitors in FOXO1-depleted cells did not trigger cell death, highlighting the critical effector role of FOXO1.

Conclusions: Taken together, our results demonstrate that FOXO1 is an important effector of SYK and AKT inhibition in DLBCLs, and its expression is required for SYK-inhibitor-induced toxicity. The underlying mechanism linking FOXO1 activation and cell death involves caspase-dependent cleavage of transcriptional repressor DREAM and subsequent induction of a proapoptotic BCL2 family member, HRK.

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TRANSCRIPTOMIC PROFILING OF RESPONSE TO PI3K INHIBITION BY BKM120 IN NON-HODGKIN LYMPHOMA (NHL) AND HODGKIN LYMPHOMA (HL)

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Introduction: BKM120 is a novel oral pan-class I PI3K inhibitor with antitumour activity and efficacy reported in solid tumours. Phase I/II BKM120 clinical trials are ongoing for treatment of relapsed/refractory NHL; however, there are a relative paucity of data towards molecular activity and biological mechanisms of resistance in NHL or HL.

Methods: T-cell NHL (TCL) (Jurkat, Hut78 and HH), HL (L428 and L540) and diffuse large B-cell lymphoma (DLBCL) cell lines (SUDHL4, SUDHL6, SUDHL10, OCILY3 and OCILY19) were treated with BKM120 (0.16–10 μM) in 96 well plates, and cell viability was assessed by MTT assay. For gene expression profiling (GEP), RNA isolated from SUDHL6, OCILY3, Jurkat, Hut78 and L540 cells treated with BKM120 at IC_{50} were analysed using Illumina human HT12 gene chip. Gene set enrichment (GSEA) and biological network analysis were performed using ingenuity pathway analysis and cytoscape.

Results: BKM120 resulted in dose-dependent decrease in cell viability in all NHL and HL cells, with IC_{50} between 0.316 μM and 2.80 μM at 72 hours. GEP analyses following BKM120 exposure identified significant genes with expression changes >1.2-fold change, OCILY3 (1886 genes), SUDHL6 (1884 genes), Hut78 (1474 genes), L540 (859 genes) and Jurkat (212 genes). Venn diagrams comparing differential gene expression showed significant overlap of 991 genes within OCILY3 and SUDHL6, while the differentially expressed genes (859 genes) identified in L540 (HL) showed better overlap with DLBCL (366 genes with OCILY3 and 315 genes with SUDHL6); the overlap with TCL was relatively poor. Among TCL lines, only 52 overlapping gene sets with BKM120 were identified among 212 and 1474 differentially expressed genes identified in Jurkat and Hut78, respectively. Of note, loss of PTEN associated with excessive PI3K activity in Jurkat may impact biological

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responses to PI3K inhibition in these cells. Despite these differences, GSEA showed conserved decrease in the expression of genes associated with cell cycle, DNA replication and metabolic processes in all cell lines indicating in part that these are the core biological responses to PI3K inhibition. Furthermore, these effects were confirmed by western blot analyses of associated cell cycle proteins. In addition, we found that BKM120 resulted in apoptosis in all NHL and HL cell lines with prominent cleaved PARP and caspase 3, 8 and 9 by western blot.

Conclusions: BKM120 induced potent dose-dependent cell death in multiple NHL and HL cell lines. Transcriptome analyses of BKM120 treated cells showed prominent inhibition of cell cycle, DNA replication, metabolic function and induction of apoptosis in varied NHL and HL cell lines. Further investigation of this novel therapeutic agent is warranted to determine predictive biomarkers of response and to identify rational synergistic drug combinations in NHL and HL.

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EXPRESSION OF PI3K ISOFORMS AND PTEN IN FOLLICULAR LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction: Follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) are two of the most common non-Hodgkin lymphomas (NHL) worldwide. Although introduction of anti-CD20 antibodies has improved the outcome of patients with NHL, the PI3K pathway and its role in the pathogenesis of FL have also been clinically validated with various inhibitors in clinical trials. However, the relative importance of specific PI3K isoforms and the role for PTEN loss in NHL are not clear. We therefore analysed PI3K isoforms and PTEN from primary tumours to gain insight into their expression and functional role in FL and DLBC.

Methods: A set immunohistochemistry (IHC) assays for analysis of p110α, p110β, p110δ and p110γ isoforms of PI3K and PTEN were developed. Evaluation of PI3K isoforms/PTEN status and their correlation with patient demographics and disease characteristics were conducted with 45 FL and 45 DLBLC primary tumours as well as a panel of 15 DLBCL cell lines. The role of each PI3K isoform in regulating B-cell lymphoma proliferation and survival was addressed using PI3K inhibitors with differential isoform profiles in various cellular assays.

Results: High expression of PI3Kδ (score: >3+) was found in both FL (87%) and DLBCL (96%) patients, while the expression of PI3Kα (>3+) was found in 18% of FL and significantly increased in DLBCL (62% >3+). PTEN-loss was observed in 24% of FL and 27% of DLBCL. In FL, high expression of PI3Kα showed a trend of correlation with late stage (III/IV) of disease ($PI3K\alpha^{high}$: 7/8 [88%] vs $PI3K\alpha^{low}$: 22/37 [59.4%]) and high FLIPI risk group ($PI3K\alpha^{high}$: 4/8 [50%] vs $PI3K\alpha^{low}$: 10/37 [27%]). Furthermore, FL patients who expressed low or no PI3Kδ also showed low or no PI3Kα (6/6) but exhibited PTEN-loss. In DLBCL, 9/45 patients with translocations or gains of *BCL2*, all showed high expression of PI3Kα ($p = 0.009$). GCB-DLBCL showed high incidence of PTEN-loss (7/10, 70%) compared to ABC-DLBCL (5/35, 14%, $p = 0.001$). C-myc translocation often coexisted with PTEN-loss (5/6, 83%, $p = 0.003$). Similar molecular features were observed in 15 DLBCL cell lines. Using selective inhibitors targeting PI3Kα (BYL-719), PI3Kβ (TGX-221), PI3Kδ (idelalisib; CAL-101), PI3Kα/δ/β/γ (copanlisib) and Bcl2 (ABT737), we confirmed a role for PI3Kα, PI3Kβ, PTEN and Bcl2 in promoting tumour cell proliferation and survival. Furthermore, simultaneous inhibition of PI3Kα/δ in $PI3K\alpha/\delta^{high}$ or PI3Kα/β in PTEN-loss DLBCL cells was more effective than PI3K isoform-selective inhibitors.

Conclusion: High levels of PI3Kδ were observed in a majority of primary FL and DLBCL. A subset of FL showed alternative activation of the PI3K pathway through overexpression of PI3Kα or loss of PTEN and the majority of DLBCL expressed high levels PI3Kα. These results justify clinical trials with dual PI3Kα/δ inhibitors such as copanlisib in patients with indolent and aggressive NHL.

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COPANLISIB ATTENUATES BOTH BCR-DEPENDENT AND BCR-INDEPENDENT ACTIVATION OF NFKB IN DLBCL CELLS

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common and incurable subtype of aggressive non-Hodgkin lymphoma (NHL). Although the BTK inhibitor ibrutinib (PCI-32765) has shown a promising overall response rate in $CD79B^{mut}$ ABC-DLBCL (71%), patients with $CD79B^{wt}$ and $MYD88^{mut}$ or patients with $CARD11^{mut}$ did not respond. Therefore, novel therapeutics targeting BCR/BTK-independent activation of NFkB are needed for the treatment of DLBCL. Here we tested whether copanlisib, a highly selective pan-class 1 PI3K inhibitor with predominant activity against PI3Kα and PI3Kδ, attenuated BCR-dependent and BCR-independent activation of NFkB in DLBCL cells.

Methods: BCR-dependent and BCR-independent NFkB activation were investigated using TMD8 ($CD79B^{mut}$ and $MYD88^{mut}$), HBL-1 ($CD79B^{mut}$ and $MYD88^{mut}$), U2932 ($CD79B^{wt}$ and $TAK1^{mut}$) and WSU-DLCL2 ($CD79B^{wt}$ and $NFkB^{high}$) DLBCL cell lines with stably transfected NFkB luciferase reporter construct. Functional evaluation of PI3K and BTK inhibitors was conducted in *in vitro* tumour cell proliferation and apoptosis assays as well as *in vivo* xenograft tumour models in mice.

Results: PCI-32765 (ibrutinib) and copanlisib demonstrated potent and comparable IC_{50}s in NFkB reporter assay in $CD79B^{mut}$/$MYD88^{mut}$ TMD8 (2 nM vs 5 nM) and HBL-1 cells (3 nM vs 4 nM), respectively. However, 90% inhibitory concentration (IC_{90}) was not reached with 2000 nM of PCI-32765, whereas copanlisib reached IC_{90} at 36 nM and 107 nM, suggesting that BCR-independent, $MYD88^{mut}$-induced NFkB activation might not be suppressed by PCI-32765. This hypothesis was supported by the published synergistic inhibition of NFkB of PCI-32765 with IRAK inhibitor (Yang et al., Blood, 2013). Furthermore, copanlisib also potently inhibited NFkB activity and cell proliferation in BCR-independent WSU-DLCL2 and U2932 cells with IC_{50}s <100 nM, while PCI-32765 was inactive. The inhibitory effect of copanlisib on NFkB required dual inhibition of PI3Kα and PI3Kδ as the PI3Kα-selective inhibitor BYL-719 and the PI3Kδ-selective inhibitor idelalisib (CAL-101) could not effectively inhibit NFkB as single agents, while combination of BYL-719 and CAL-101 showed strong synergistic effect (combination indexes of 0.18, 0.24 and 0.23 in TMD8, HBL-1 and WSU-DLCL2 cells, respectively). Furthermore, combination of copanlisib and PCI-32765 showed synergistic effects on induction of tumour cell apoptosis *in vitro*, in multiple tumour cell lines, and led to 100% RR (50% complete response and 50% partial response) *in vivo* in TMD8 DLBCL xenograft model in mice.

Conclusion: In addition to the important role of PI3K in regulating BCR signalling in NHL, a novel mechanism by which copanlisib potently inhibits BCR-independent activation of NFkB in DLBCL via PI3K α/δ inhibition was identified. These results provide molecular mechanism-based rationale for developing copanlisib for the treatment of DLBCL.

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PRE-CLINICAL LYMPHOMA MODELS SHOW HIGH SENSITIVITY TO THE SMALL MOLECULE YK-4-279

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Up to 20% of diffuse large B-cell lymphomas (DLBCL) present recurrent 11q23 gains leading to upregulation of ETS1 and FLI1, two transcription factors of the

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ETS family and FLI1 silencing leads to cell death in DLBCL cell lines (*Blood* 2013). Friend leukaemia integration1 (FLI1) was initially identified as a proto-oncogene in Friend virus-induced erythroleukaemia and is expressed in endothelial and haematopoietic cells. The EWS1-FLI1 translocation is a hallmark of Ewing sarcoma (ES) and the fusion product functions as an aberrant transcription factor with the ETS-DNA binding domain of FLI1 recognizing GGAA microsatellites upstream of its target genes. In pre-clinical models of ES, YK-4-279 inhibits binding of the transcriptional co-activator RNase helicase A (RHA) to EWS1-FLI1 leading to growth arrest and apoptosis (*Nat Med* 2009). Here, we assessed whether YK-4-279 could exert an anti-proliferative effect in lymphomas expressing FLI1 or other ETS family members.

Methods: Forty-eight cell lines [27 derived from DLBCL, 10 from mantle cell lymphoma (MCL), 3 from splenic marginal zone lymphoma (SMZL) and 8 from anaplastic large cell lymphoma (ALCL)] were treated with increasing doses of YK-4-279), and IC50s were calculated with the MTT assays after 72 hrs exposure. Baseline gene expression profiling was performed to determine which gene profiles might render the cells either more or less sensitive to YK-4-279. Levels of apoptosis cell cycle arrest were also quantified upon treatment with the compound.

Results: YK-4-279 showed potent dose-dependent anti-proliferative activity in most cell lines with the majority of IC50 values below 1000 nM. The median IC50 value was 393 nM (95% C.I., 272–3622 nM). There were no apparent differences among the different lymphoma subtypes: DLBCL (median IC50 = 386 nM; 95% C.I., 223–497), MCL (596 nM; 95% C.I., 230–1305), SMZL (217 nM; 95% C.I., 183–524) and ALCL (403 nM; 95% C.I., 170–3387). Results were confirmed by performing further MTT experiments using the inactive (R)-YK-4-279 and the active (S)-YK-4-279, showing activity only when cells were exposed to the latter enantiomer.

The comparison of baseline gene expression profiles between 14 less sensitive (IC50 > 500 nM) and 9 highly sensitive (IC < 200 nM) B-cell lines highlighted that the expression signature of the latter group was enriched of E2F1 and PAX5 targets (including FLI1), genes involved in germinal centre, cell cycle, sequence-specific DNA binding and RNA processing. Conversely, the low sensitivity was associated with NFKB-related genes. Genes such as BCL6, BACH2, LMO2, CXCR4 and FAK were among the 50 most up-regulated transcripts in the sensitive cells.

When we treated five YK-4-279 sensitive DLBCL cells lines (3 ABC and 2 GCB) with (S)-YK-4-279 for 72 hrs, cells underwent apoptosis rather than cell cycle arrest.

Conclusion. YK-4-279 shows strong anti-proliferative activity in lymphomas and it is worth of further investigations as an anti-lymphoma compound.

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IRAK1/4 AND BET BROMODOMAIN INHIBITIONS CONVERGE IN NF-KB BLOCKADE AND OFFER SYNERGISTIC ANTITUMOURAL ACTIVITY IN ABC-DLBCL WITH MYD88 MUTATION

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Introduction: A high proportion of diffuse large B-cell lymphomas (DLBCL) can be cured with combination of rituximab, cyclophosphamide, adriamycin, vincristine and prednisone. However, almost a third of the patients, mainly the activated subtype (ABC), does not achieve complete remission (CR) or relapse shortly after CR. It is believed that these tumours rely almost exclusively on constitutive NF-κB signalling for their survival, a phenomenon that has recently been related to mutations in *MYD88* gene and consequently enhanced interleukin-1 receptor-associated kinase 1 (IRAK1) activity. In this setting, our aim was to evaluate therapeutic strategies to counteract IRAK- and NF-κB-dependent signalling in ABC-DLBCL.

Methods: A set of 3 ABC-DLBCL cell lines harbouring the L265P *MYD88* mutation (OCILY3, OCILY10 and HBL1) and 3 germinal centre (GC)-DLBCL control cell lines (SUDHL4, SUDHL8 and OCILY8) was exposed to a selective IRAK1/4 inhibitor (IRAKi) and/or to the BET bromodomain inhibitor CPI203 (Constellation Pharmaceuticals), and response to drugs or drug combination was analysed by MTT assay, flow cytometry, western blot, gene expression profiling (GEP) and real-time PCR.

Results: Although the IRAKi efficiently blocked the phosphorylation of IRAK1 at Thr209 residue, we observed only a partial and transitory response to the compound in ABC-DLBCL cell lines and xenotransplant murine model, when used at physiological doses (50 μM and 5 mg/kg/BID, respectively). The mean cytotoxicity of the compound *in vitro* was 25.5% and 19% at 24 and 72 hours, respectively. GEP analysis showed that IRAKi treatment slightly affects NF-κB related gene expression (mean normalized enrichment score (NES) ≤ 1), being genes like *IL6* and *IRF4* unaffected by the treatment. In contrast, when combined with CPI203, IRAKi induced a significant downregulation of NF-κB-related genes (mean NES > 1.8), including *IL6*. Accordingly, the drug combination led to the intracellular accumulation of IκB and to a synergistic antitumoural effect *in vitro* (mean combination index: 0.52). Moreover, while IRAKi and CPI203 single agents harboured limited pro-apoptotic activities, the drug combination induced a 36% increase in apoptosis rate ($p < 0.04$), related to the downregulation of the antiapoptotic NF-κB factor, MCL-1.

Conclusions: These results suggest that IRAKi single agent is modestly effective in *in vitro* and *in vivo* models of ABC-DLBCL, in which it achieves a partial inhibition of NF-κB signalling. We confirm that BET inhibition is an efficient strategy to counteract NF-κB activity in these models, as it offers synergistic antitumoural and pro-apoptotic activities with IRAK inhibition *in vitro*. Results of the ongoing evaluation of IRAKi-CPI203 combinatorial activity in *in vivo* settings will be presented.

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THE BET BROMODOMAIN INHIBITOR OTX015 (MK-8628) AFFECTS THE EXPRESSION OF MICRORNAS INVOLVED IN THE PATHOGENESIS OF LYMPHOMA: *IN VITRO* AND *IN VIVO* EVIDENCE

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Background: Aberrant changes in histone modifications, DNA methylation and expression levels of non-coding RNA, including microRNA (miRNA), contribute to lymphoma pathogenesis and represent potential therapeutic targets. A new class of epigenetic drugs, the BET bromodomain inhibitors such as OTX015 (MK-8628), has demonstrated promising preclinical activity in hematologic and solid tumour models more recently in an ongoing phase I study (NCT01713582). To better understand the mechanism of action of OTX015, we studied its effects on miRNAs in lymphomas.

Methods: RNA was isolated from two diffuse large B-cell lymphoma (DLBCL), three splenic marginal zone lymphoma (SMZL) and three mantle cell lymphoma (MCL) cell lines and from SU-DHL-2 murine xenografts using Qiagen miRNeasy Serum/Plasma kit following OTX015 exposure. Samples were analysed with Agilent Human microRNA microarray v.3 and the Illumina HumanHT-12 v4 Expression BeadChip. Limma was used to detect differentially expressed transcripts. Selected miRNA changes were validated by real-time PCR using TaqMan miRNA assays. All fold changes are represented in log2 scale and a *P*-value of <0.05 is considered statistically significant.

Results: miRNA profiling of the germinal centre B-cell (GCB) DLBCL DOHH2 and activated B-cell-like (ABC)-DLBCL SU-DHL-2 exposed to DMSO or 500 nM OTX015 (4, 8 h) identified four miRNAs with decreased expression ranging from −0.37 to −2.01 log2 FC (miR-21-3p, miR-92a-1-5p, miR-196a-3p and hsa-miR-29b-1-5p) and seven with increased expression ranging from 0.36 to 0.64 log2 FC (miR-96-5p, miR-630, miR-935, miR-1181, hsa-miR-765, miR-345-5p and miR-1246). We focused on miR-92a-1-5p (log2 FC, −2.01; $P = 0.004$) and miR-21-3p (log2 FC, −0.37; $P = 0.0045$), and the tumour suppressor miR-96-5p

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(log2 FC, 0.39; $P = 0.041$). Changes in these oncomirs matched variations of validated target genes (e.g. miR-92a-1-5p: CDKN1A, log2 FC, 0.81, CDKN2A, log2 FC, 0.81 and miR-96-5p: MYC, log2 FC, -0.57, MYD88, log2 FC, -0.35). In SU-DHL-2 xenografts treated with OTX015 or control for 3 days, miRNA expression was similarly up- or downregulated but only changes in miR-92a-1-5p reached statistical significance ($P = 0.03$). miR-92a-1-5p was also decreased by OTX015 (500 nM, 8 h) in 1/3 SMZL and 3/3 MCL cell lines, including Rec1 and JeKo1 with high expression of this oncomir due to DNA amplification. miR-21-3p was decreased in 2/3 MCL but none of the SMZL lines. miR-96-5p was increased in 2/3 SMZL but none of the MCL lines.

Conclusions: Expression changes of miRNAs may contribute to OTX015 antitumour activity and, in particular, the oncomir miR-92a-1-5p, which was strongly and systematically downregulated by OTX015. Expression of specific oncomirs may represent pharmacodynamic biomarkers for BET bromodomain inhibitors. Further evaluation is needed to identify miRNAs targeted in different lymphoma types and in the clinical setting.

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OTX015 (MK-8628) TARGETS GENES WITH HIGH LEVELS OF PROMOTER H3K4ME3 INVOLVED IN KEY SIGNALLING PATHWAYS IN SPLENIC MARGINAL ZONE LYMPHOMA AND MANTLE CELL LYMPHOMA

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Background: BET bromodomain inhibitors have shown preclinical activity in several lymphoma models, including splenic marginal zone lymphoma (SMZL) and mantle cell lymphoma (MCL). OTX015 (MK-8628) is a new selective oral inhibitor of BET proteins currently in clinical development in haematologic malignancies and solid tumours. Downregulation of NFKB and JAK/STAT signalling and E2F1 transcriptional activity have been proposed as mechanisms of action in diffuse large B-cell lymphoma (DLBCL). We studied gene pathways potentially affected by OTX015 in lymphoma cell lines.

Methods: Gene expression profiling (GEP) in four MCL and three SMZL cell lines was performed with Illumina HumanHT12 Expression BeadChips. ChIP-Seq was performed in SMZL cell lines using antibodies against histone H3K4me3 and, as control, against IgG. Libraries were prepared with the ChIP-Seq sample preparation kit (Illumina) from immunoprecipitated DNA and also from input DNA samples. Sequencing was performed on an Illumina HiScanSQ sequencer with 50-bp single reads. Sequence tags were aligned to the human genome (build hg19) using Bowtie software with default settings. Redundant reads were removed, and reads mapping uniquely to the reference genome were used for further analysis. A maximum of one mismatch per read was allowed. 'Peak calling' was performed with the Homer genomic suite. Promoter peaks were defined as peaks whose apex was located within a ±2 kb window from a representative transcription start site. ChIP-Seq data were normalized by total tags count.

Results: OTX015 (500 nM; 4 h, 8 h and 24 h) did not downregulate the driver genes CCND1 or NOTCH2 in MCL ($n = 4$) or SMZL ($n = 3$) cell lines, respectively. GEP in OTX015-sensitive cell lines (3 MCL and 3 SMZL) exposed to DMSO or 500 nM OTX015 for 2, 4, 8, 12 and 24 h showed that OTX015 downregulated transcripts involved in NFKB/BCR/TLR signalling and RNA metabolism, as well as MYC and NOTCH target genes. Upregulated transcripts included genes involved in chromosome formation and maintenance, the cell cycle and response to UV. The OTX015 GEP signature overlaps the HDAC and BET bromodomain inhibitors signature in other tumour models, with respect to genes upregulated by HDAC/BET bromodomain inhibitors. The five most strongly downregulated genes were NAPSB, SLC2A5, TNFRSF17, TLR10 and FCRL2 in MCL and TNFRSF17, MYC, TLR10, LRRC33 and PLD6 in SMZL. Clusters 1 and 2 histones were the most upregulated genes in both lymphoma types. Genes downregulated by OTX015 had significantly more H3K4me3 bound to their promoter regions than expected for random gene sets. Conversely, H3K4me3 binding on promoters of genes not affected by OTX015 was coherent with that seen for random gene sets.

Conclusions: In MCL and SMZL, the main mechanism of OTX015 activity appears to be via the NFKB and TLR pathways. Genes with a high degree of H3K4me3 binding to their promoters were more sensitive to OTX015 in both MCL and SMZL.

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PIXANTRONE HAS PRE-CLINICAL SYNERGISTIC ACTIVITY WHEN COMBINED WITH TARGETED AGENTS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction. Diffuse large B-cell lymphoma (DLBCL) is the commonest lymphoma and still at least one quarter of patients present with a refractory disease or relapse after first-line chemotherapy. Pixantrone (Pix) is an aza-anthracenedione with reduced cardiotoxicity, which has received a conditional marketing approval in the E.U. as a monotherapy for the treatment of adults with multiply relapsed or refractory aggressive B-cell non-Hodgkin lymphomas. Here, we evaluated the preclinical activity Pix in combination with a series of additional anti-lymphoma drugs.

Methods. DLBCL cell lines derived from activated B-cell like (ABC) DLBCL (U2932, TMD8 and HBL1) and from germinal centre B-cell (GCB) DLBCL (DOHH2, SU-DHL-4 and OCI-Ly19) were studied. Pix was used in combination with bendamustine, ibrutinib, idelalisib, lenalidomide, rituximab, vorinostat and bortezomib. Due to the mechanism of action, lenalidomide, bortezomib and ibrutinib were evaluated only in ABC-DLBCL cell lines. Cell lines were exposed (72 h) to increasing Pix doses alone or in combination with increasing doses of other agents, followed by MTT assay. The Chou-Talalay combination index (CIndex) was estimated using the Synergy R package: CI between 0.9 1.1 defined an additive effect; $CI < 0.9$, synergism; $CI > 1.1$, no benefit.

Results. Pix was active in all the cell lines as single agent [median IC50 (50% – inhibitory concentration) at 72 h, 175 nM (95%, C.I., 35–245)]. Across all the cell lines, a synergism was observed with the combination of Pix with ibrutinib (median CIndex = 0.6, 95%, C.I., 0.51–0.74), idelalisib (median CIndex = 0.7, 95%, C.I., 0.63–0.83) and lenalidomide (median CIndex = 0.78, 95%, C.I., 0.71–0.84). In particular, Pix/ibrutinib were synergistic in 3/3 ABC-DLBCL cell lines (Table 1). Pix/idelalisib were synergistic in 3/4 cell lines and additive in the remaining one. Synergism was observed in 2/5 cell lines when Pix was combined with rituximab. Pix/vorinostat were synergistic in 1/4 and additive in 1/4. An additive effect was observed in 3/4 cell lines exposed to Pix/bendamustine. None of the two ABC-DLBCL cell lines benefited from exposure to Pix/bortezomib.

Conclusions. Positive results were obtained when combining Pix with different drugs. The combinations with the BTK inhibitor ibrutinib or with the PI3K-delta inhibitor idelalisib gave the best results and could be considered for further studies at pre-clinical and clinical levels.

Abstract 273 Table. Combination index values obtained for pixantrone combined with a second drug in DLBCL cell lines

DLBCL subtype	Cell line	Second drug	Median CIndex	95% CI
GCB-DLBCL	DOHH2	Bendamustine	0.98	0.90–1.08
GCB-DLBCL	SUDHL4	Bendamustine	1.02	0.93–1.21
ABC-DLBCL	TMD8	Bendamustine	1.3	0.87–1.45
ABC-DLBCL	U2932	Bendamustine	1.03	0.95–1.24
ABC-DLBCL	TMD8	Bortezomib	1.21	0.85–1.6
ABC-DLBCL	U2932	Bortezomib	1.31	0.97–1.82
ABC-DLBCL	HBL1	Ibrutinib	0.50	0.45–0.58
ABC-DLBCL	TMD8	Ibrutinib	0.65	0.49–0.79

(*Continues*)

Abstract 273 - Table 1 (Continued)

DLBCL subtype	Cell line	Second drug	Median CIndex	95% CI
ABC-DLBCL	U2932	Ibrutinib	0.82	0.7–1.35
GCB-DLBCL	DOHH2	Idelalisib	0.67	0.59–0.82
GCB-DLBCL	SUDHL4	Idelalisib	0.82	0.71–0.92
ABC-DLBCL	TMD8	Idelalisib	0.11	0.09–0.18
ABC-DLBCL	U2932	Idelalisib	1.01	0.96–1.13
ABC-DLBCL	TMD8	Lenalidomide	0.61	0.39–0.72
ABC-DLBCL	U2932	Lenalidomide	0.93	0.83–1.01
GCB-DLBCL	DOHH2	Rituximab	0.79	0.74–0.9
GCB-DLBCL	OCILY19	Rituximab	>3	Min. >3
GCB-DLBCL	SUDHL6	Rituximab	>3	Min. >3
ABC-DLBCL	TMD8	Rituximab	0.74	0.63–0.79
ABC-DLBCL	U2932	Rituximab	>3	Min. >3
GCB-DLBCL	DOHH2	Vorinostat	0.92	0.77–0.99
GCB-DLBCL	SUDHL4	Vorinostat	1.24	0.91–1.63
ABC-DLBCL	TMD8	Vorinostat	0.74	0.63–0.92
ABC-DLBCL	U2932	Vorinostat	1.14	1.09–1.18

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NOVEL CD37-TARGETING ANTIBODY-DRUG CONJUGATE (ADC), IMGN529, HAS SYNERGISTIC ACTIVITY IN COMBINATION WITH RITUXIMAB IN NON-HODGKIN LYMPHOMA (NHL) MODELS

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Introduction: IMGN529 is a CD37-targeting ADC consisting of a CD37-binding antibody, K7153A conjugated to the maytansinoid anti-mitotic, DM1. In preclinical studies, IMGN529 exhibits targeted, potent activity against NHL cells via antibody-mediated direct cell-killing and effector function, and via tubulin disruption from the DM1 payload. IMGN529 has demonstrated preliminary single-agent clinical activity in an ongoing phase I study in adult patients with relapsed or refractory NHL (NCT01534715). Rituximab, a monoclonal antibody against CD20, is widely used for NHL therapy and remains the standard frontline regimen for diffuse large B-cell lymphoma (DLBCL), used in combination with cyclophosphamide/doxorubicin/vincristine/prednisone (R-CHOP). The combination potential of IMGN529 with rituximab was evaluated in clinically relevant preclinical models of NHL.

Methods: IMGN529 was evaluated in combination with rituximab and with other CD20-targeting antibodies across a panel of twenty NHL cell lines *in vitro* using a combination dose-response matrix. A statistical method was used to identify synergies significantly superseding baseline additivity values. The IMGN529/rituximab combination was further evaluated in select cell lines using a higher resolution dose matrix of IMGN529 and its components: its CD37-binding antibody and its payload, as DM1-Me. The benefit of the IMGN529/rituximab combination was also evaluated *in vivo* using human xenograft models of DLBCL in mice.

Results: Robust synergy was observed between IMGN529 and the CD20-targeting antibodies tested—rituximab, obinutuzumab and ofatumumab—in cell lines representative of different NHL sub-types including the CLL cell line JVM13, activated B-cell (ABC) DLBCL cell lines U2932 and HBL-1 and germinal centre B-cell (GCB) DLBCL cell lines DOHH2, OCI-Ly7 and SUDHL-4. Significant synergy was also observed in the OCI-Ly18 cell line, representative of 'double-hit' lymphoma, characterized by overexpression of MYC and BCL2. While both the antibody and the DM1 components of IMGN529 showed synergy with rituximab in some cell lines, the synergy scores obtained with the complete ADC were typically 3–10-fold higher. Consistent with the *in vitro* data, the combination of IMGN529 and rituximab was more active against NHL xenograft models *in vivo* than either agent administered as a monotherapy. In addition, the combination was well tolerated.

Conclusions: In *in vitro* models of different NHL subtypes, IMGN529 demonstrated synergistic activity in combination with CD20-targeting antibodies including rituximab. In NHL *in vivo* models, the combination of IMGN529 and rituximab was more active than either agent alone and well tolerated. These results support clinical assessment of IMGN529 used in combination with rituximab.

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A VARIANT OF OBINUTUZUMAB WITH ABOLISHED ADCC, ADCP AND CDC IS AS EFFICIENT AS RITUXIMAB IN B CELL DEPLETION AND ANTITUMOUR ACTIVITY

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Introduction: Obinutuzumab (GA101 and GAZYVA®/GAZYVARO®) is a glycoengineered type II CD20 antibody mediating superior direct cell death induction, ADCC and ADCP compared to rituximab while CDC is strongly reduced. Obinutuzumab is approved for the 1st-line treatment of CLL in combination with chlorambucil in the USA and Europe. The contribution of these mechanisms of action (MOA) to the efficacy of obinutuzumab in patients is unclear. In order to further dissect the MOA of GA101 and rituximab and the variants with abolished FcgR and C1q binding were assessed *in vitro* and *in vivo*.

Methods: Glycoengineered GA101, non-glycoengineered wild type (wt) GA101, GA101 and rituximab with P329G LALA mutations in the Fc were generated and characterized for (i) binding to FcgRs and CD20 positive cells by FACS, (ii) cell death induction by Annexin V/PI staining, (iii) ADCC, ADCP and CDC assays, and (iv) depletion of B cells in whole blood from healthy volunteers. Anti-tumour efficacy was assessed using established 300 mm^2 s.c. SU-DHL4 xenograft tumours in Scid beige mice (30 mg/kg, q7 d × 4, ip).

Results: Variants of GA101 and rituximab retain CD20 binding and cell death-inducing properties of the respective parental antibodies. The introduction of P329G LALA in the Fc part abolishes binding to Fcg receptors and C1q, so that ADCC, ADCP or CDC is abolished, while direct cell death induction is not affected. In whole blood assays, GA101 showed the best B cell depletion in terms of potency and absolute B cell depletion followed by wt GA101, rituximab and GA101 P329G LALA, while rituximab P329G LALA did not mediate B cell depletion. Thus, in whole blood B cell depletion, ADCC/ADCP plays an important role, but direct cell death induction is equally important for the MOA of GA101, whereas it does not contribute to B cell depletion of rituximab. In the SU-DHL4 xenograft model, GA101 (tumour growth inhibition (TGI) >100%, non-parametric tumour control ratio (npTCR) 0 with 95% confidence interval (CI) 0–0 and wt GA101 (TGI > 100%, npTCR 0, 95% CI 0–0.006) showed equivalent efficacy with complete tumour remission in 9/10 mice for GA101 and 8/10 mice for wt GA101. GA101 P329G LALA showed anti-tumour efficacy (TGI 62%, npTCR 0.43, 95% CI 0.30–0.60) comparable to rituximab (TGI 71%, npTCR 0.37, 95% CI 0.29–0.50), while rituximab P329G LALA showed only residual anti-tumour activity (TGI 26%, npTCR 0.73, 95% CI 0.44–0.92).

Conclusions: These data show that in whole blood B cell depletion and xenograft tumour models, direct cell death and ADCC/ADCP through the glycoengineered Fc part are required for anti-tumour efficacy of obinutuzumab. Most notably, the variant of obinutuzumab with completely abolished ADCC, ADCP and CDC activities, but retained direct cell death induction, is as efficacious as rituximab *in vivo* demonstrating the important role of direct effects for the efficacy of obinutuzumab.

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THE YIN-YANG OF KIR3DL2 IN CTCL MALIGNANT T CELLS

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Introduction: We previously reported the specific expression of the NK cell receptor KIR3DL2 by the skin resident and circulating tumoural T cell clone of CTCL patients. This selective expression raised the issue of its function in the malignant cells and its potential use as a therapeutic target.

Methods: KIR3DL2 receptor activity was tested on the CD3-mediated proximal signals, proliferation and activation-induced cell death (AICD) of $CD4^+$ T cells from late stage Sézary syndrome patients. Furthermore, a humanized monoclonal antibody, IPH4102, has been developed to target KIR3DL2 on CTCL tumour cells. The anti-tumour activity of IPH4102 was addressed in allogeneic *in vitro* tests on cell lines, *in vivo* mouse models of KIR3DL2+ tumours and *ex vivo* autologous antigen-dependent cell cytotoxicity (ADCC) assays.

Results: Biochemical and functional data showed that KIR3DL2 is functional in Sézary cells where it acts as an inhibitory co-receptor that promotes malignant cells resistance to AICD. In addition, the targeting of KIR3DL2 with IPH4102 results in the generation of a potent antitumour activity mediated through antibody-dependent cell cytotoxicity and phagocytosis. Thus, IPH4102 improved survival and reduced tumour growth in mice inoculated with $KIR3DL2^+$ tumours. IPH4102 *ex vivo* efficacy was also evaluated using primary Sézary patient cells, in sorted NK-based autologous assays or after direct spiking into Sézary patient peripheral blood mononuclear cells (PBMC). In both systems, it efficiently and selectively induced primary Sézary cell death, including at the unfavourable effector to target ratio found in unsorted PBMC.

Conclusions: Altogether, our data provide evidence for a role of KIR3DL2 in the maintenance of a high circulating malignant-cell burden in Sézary patients and strongly support the development of the anti-KIR3DL2 monoclonal antibody IPH4102 for the treatment of advanced CTCL patients.

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TARGETING THE MUC1-C ONCOPROTEIN IS ASSOCIATED WITH DOWNREGULATION OF TIGAR AND ROS-MEDIATED CUTANEOUS T-CELL LYMPHOMA CELL DEATH

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Introduction: Cutaneous T-cell lymphoma (CTCL) is an aggressive haematologic malignancy, which demonstrates resistance to reactive oxygen species (ROS) mediated apoptosis characteristic of normal T cells upon activation. MUC1-C oncoprotein governs critical pathways of tumourigenesis including those regulating apoptosis and is aberrantly expressed in certain hematologic malignancies. The expression and functional significance of MUC1 in CTCL have not been previously investigated.

Methods and Results: Our studies demonstrate that MUC1-C is overexpressed in CTCL cell lines (HuT-78, H9, Myla and SeAx) and primary CTCL cells but is absent in normal resting T cells from healthy donors and B-cell lymphoma cells when incubated with anti-MUC1-N antibody and analysed by flow cytometry (FCM) and confirmed by immunoblotting (IB) with another antibody directed against MUC1-C, the oncogenic subunit. The expression profile was found to be similar at the mRNA level by RT-PCR analysis. We have developed a cell-penetrating peptide that disrupts homodimerization of the MUC1-C subunit necessary for nuclear translocation and downstream signalling, and thus functions as a MUC1-C inhibitor. Growth inhibition was determined using CellTiter-Glo that measures ATP produced by live and proliferating cells and demonstrated that the concentration required to inhibit growth by 50% (IC50) following 72-hour exposure that ranged from 3 uM to 3.5 uM in most cell lines (HuT-78, H9 and Myla) and primary patient sample. Analysis of cell death with Annexin V (AV) and propidium iodide (PI) staining by FCM confirmed these results. To determine mechanism of action of GO-203 induced apoptosis *in vitro*, level of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS) was measured using FC-based conversion of carboxy-2′7′ dichlorodihydrofluorescein diacetate (H_2DCFDA) to 2′7′-dichlorodihydrofluorescein (DCF). Cell lysates were analysed for expression of the protein TIGAR, a fructose-2-6-bisphosphatase p53-inducible regulator of glycolysis and apoptosis by IB. Exposure of Hut-78, Myla and H9 cells to GO-203 for 72-hours was associated with an increase in ROS and downregulation of TIGAR expression. Further, downregulation of TIGAR upon MUC1-C inhibition was associated with decrease in NADPH and glutathione (GSH) levels measured by colorimetric assays and increased late apoptosis/necrosis validated by AV/PI staining. To confirm ROS-mediated apoptosis by GO-203, *in vitro* treatment with the antioxidant *N*-acetylcysteine (NAC) was performed, which blocked both the decrease in TIGAR levels and apoptosis. Targeting MUC1-C in CTCL tumour xenograft models further demonstrated statistically significant decrease in disease burden.

Conclusion: Our findings indicate that MUC1-C maintains redox balance in CTCL cells and is thereby a novel target for the treatment of patients with CTCL.

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MYC AND CHK1 DEPENDENT CELL DEATH IN T-CELL LYMPHOMA AND HODGKIN LYMPHOMA CELLS AND HUMAN XENOGRAFT MODELS WITH ANTI-PROTEASOMAL THERAPY

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Introduction: Proteasome inhibition has become an important strategy for anticancer treatment. We investigated the potency, biological mechanisms of action and examined rational synergistic drug combinations with the proteasome inhibitor, ixazomib, in TCL and HL tumour models.

Methods: TCL cell lines (Jurkat, Hut78 and HH) and HL cell lines (L428, L540 and L1236) were treated with ixazomib for 24–72 hours; cell viability was analysed by MTT and apoptosis by flow cytometry (Annexin/PI). Survival of SCID mice and tumour growth were determined using xenografts derived from Jurkat and L540 cell lines. Transcriptome analyses were completed with gene expression profiling (GEP) using Human HT 12 Genechip Illumina and Affy Human Gene Chip 2.0; gene set enrichment analysis (GSEA) was performed using leading edge analysis, and pathway relationships were constructed utilizing ingenuity pathway analysis (IPA).

Results: Treatment with ixazomib resulted in dose-dependent cytotoxicity and apoptosis in all cell lines with all IC50's <75 nM. SCID tumour xenografts showed significant inhibition of tumour growth ($P < 0.001$) with significantly improved survival ($P < 0.001$) in both Jurkat and L540 models with ixazomib-treated mice vs controls. Global transcriptome analyses showed significant gene changes following ixazomib exposure with a 1.2-fold change for Jurkat (508 genes), L540 (4765 genes) and L428 (423 genes). Venn diagrams comparing differential gene expression showed significant and conserved overlap in biological functions involved in regulation of cell cycle, chromatin modification and DNA repair in Jurkat and L540 cells; however, there was minimal overlap with the ixazomib-resistant cell line, L428. IPA predicted activation and inhibition status of tumour suppressors and oncogenes strongly favouring ixazomib inhibition of tumour progression (in all cell lines except L428). Most notably, ixazomib has prominently down-regulated Myc and its target genes. Additionally, chromatin immunoprecipitation showed that Chk1 and histone H3 acetylation strongly affected Myc levels and cell death response to ixazomib. Finally, ixazomib in combination with AZD7762, Belinostat or JQ1 resulted in synergistic cell death in L428.

Conclusions: Ixazomib induced potent *in vitro* cell death and inhibited *in vivo* tumour growth in TCL and HL models at nanomolar concentrations. Global changes in the transcriptome were consistent with inhibition of tumour progression. Furthermore, ixazomibinduced cell death in a Myc-dependent mechanism that involved Chk1 and histone acetylation. Continued examination of ixazomib in lymphoma is warranted and rational combinations with Chk1, Myc and HDAC inhibitors should be explored.

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ALISERTIB EXHIBITS BROAD ACTIVITY AND SELECTIVELY SYNERGIZES WITH ROMIDEPSIN IN PRECLINICAL MODELS OF T-CELL LYMPHOMA

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Introduction: Aurora A kinase (AAK), a serine-threonine kinase, regulates mitotic entry, spindle formation and cytokinesis. Alisertib (A) is a selective AAK inhibitor with clinical activity in haematologic malignancies. We demonstrate the potent cytotoxicity and apoptotic effects of alisertib in a panel of T-cell lymphoma (TCL) and B-cell lymphoma (BCL) cell lines. We also describe marked synergy with the histone deacetylase inhibitor romidepsin (R) but not with pralatrexate or the proteasome inhibitor, ixazomib.

Methods: A low throughput screening approach was employed to identify drugs that were potentially synergistic in combination with alisertib. Live cell imaging was used to explore mechanistic basis for drug: drug interaction between A and R. An *in vivo* xenograft TCL model was used to confirm *in vitro* results.

Results: *In vitro*, alisertib exhibited concentration-dependent cytotoxicity in B- and TCL cell lines. A + R was synergistic **only in TCL and not in BCL**. Using a live cell imaging time lapse experiment (0–72 hr), we found that combination treatment in the H9 cell line led to cytokinesis failure leading to apoptosis. Apoptosis was confirmed in H9 and HH cell lines after 72 hrs of combination treatment through increased Puma, Caspase 3 and PARP cleavage and decreased BCL-xL and BCL2 expression. Annexin V/propridium iodide via FACS analysis confirmed induction of apoptosis. Cell cycle analysis was performed following 24 hrs of treatment of alisertib and romidepsin as a single agent and in combination. Alisertib produced a G2/M arrest while A + R induced polyploidy (up to 8N). This finding may also complement the increase in apoptosis in the combination treatment versus the single agents. An *in vivo* xenograft demonstrated that the A+R cohort showed a statistically superior decrease in area under the curve (AUC) as compared to control and single agents ($P < 0.05$). A Kaplan–Meir survival analysis showed that the combination cohort surpassed survival of all other cohorts and was statistically significant when compared to control and single agent arms ($P < 0.05$). Using pharmacokinetic analyses, we found that in animals treated with the combination, the alisertib concentration in tumour tissue was increased from 100 nM to 400 nM (1 hr post treatment) when compared to single-agent alisertib tumour concentration. Interestingly, synergy was not seen with the combination of alisertib and pralatrexate or ixazomib. These observations support the lineage specific activity of the A + R combination in models of TCL and suggest it is highly dependent on the drug : drug combinations.

Conclusions: These data support the observation that alisertib produces marked synergy with R, but not pralatrexate or ixaxomib, in *in vitro and in vivo* models of TCL, supporting the concept that these combinations might be lineage restricted. Ongoing early phase clinical studies are exploring the merits of the combination in the clinic.

EARLY CLINICAL TRIALS

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INTERIM ANALYSIS OF A PHASE 1B STUDY EVALUATING THE SAFETY OF GS-9820, A SECOND-GENERATION PI3Kδ-INHIBITOR, IN RELAPSED/REFRACTORY LYMPHOID MALIGNANCIES

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Background: In B-cells, phosphatidylinositol 3-kinase delta (PI3Kδ) mediates cell survival and proliferation, and its activity is critical for retaining B cells in lymphoid tissues. Idelalisib inhibits PI3Kδ in B-cell malignancies and is indicated for the treatment of 1st line 17p deleted and relapsed chronic lymphocytic leukaemia (CLL) in combination with rituximab. Idelalisib also demonstrated high response rates in relapsed follicular lymphoma (FL) and small lymphocytic leukaemia (SLL). GS-9820 is a second-generation, PI3Kδ-inhibitor and was evaluated in B-cell malignancies including CLL and non-Hodgkin's lymphoma (NHL). The primary objective was to determine the maximum tolerated dose (MTD) and to assess safety including the incidence and severity of elevated transaminase levels, diarrhoea and pneumonitis, which are observed with idelalisib.

Methods: Subjects with relapsed B-cell malignancies and measurable lymphadenopathy (LAD) with >1 prior therapy received GS-9820 at doses of 50, 100, 200, or 400 mg, orally, twice daily (BID). The dose-escalation stage ($n = 12$) had a 3 + 3 design and measured safety, efficacy and pharmacologic properties. Additional subjects ($n = 27$) enrolled in the dose-expansion cohort at 400 mg BID. Antitumour activity was evaluated every 2 months including CT scans, with adjustments for redistribution lymphocytosis, consistent with PI3Kδ inhibition. Nodal PR (nPR) is defined as ≥50% reduction in LAD. Subjects received GS-9820 until disease progression or unacceptable toxicity.

Results: As of July 2014, 15/39 subjects remain on treatment. Reasons for discontinuation include disease progression (11), death (4) (all unrelated to GS-9820), adverse events (AE) (14) and other (9). Response rates assessed by independent review committee were overall 33.3% (95%CI (19.1–50.2)), for CLL subset ($n = 22$) 33.3%, for NHL subset ($n = 17$; 8 MCL and 4 DLBCL) 28.6%. nPR in CLL subset was 84.6%. The median duration of response was not reached, and maximum duration of response was up to 11.9 months.

Safety: No dose-limiting toxicities were reported. AEs (subject incidence >20%) were cough, diarrhoea, dyspnea, fatigue, peripheral edema and rash. Severe adverse events (grade 3 or 4) reported by >1 subject were pneumonia[a] (5, 12.8%), pyrexia (4, 10.3%), sepsis (2, 5.1%) and diarrhoea (2, 5.1%). AEs considered related to study drug by the investigator (subject incidence >10%) were pyrexia, dysgeusia, diarrhoea and increase in AST or ALT. Three subjects had elevations in transaminase levels at grade 3 or 4 in the 400 mg BID dose cohort. No subjects reported pneumonitis and 8 subjects reported pneumonia.

Conclusion: Interim analysis of this phase 1b study of GS-9820 demonstrates clinical efficacy and safety comparable to idelalisib. Based on the similarity to idelalisib, no further clinical development is planned.

[a]Pneumonia included AEs of pneumonia, viral pneumonia and organizing pneumonia.

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DUVELISIB, AN ORAL DUAL INHIBITOR OF PI3K-δ,γ, MODULATES SERUM CHEMOKINES AND CYTOKINES IN PATIENTS WITH RELAPSED/REFRACTORY B AND T CELL LYMPHOMAS

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Introduction: Phosphoinositide-3-kinase (PI3K)-δ and PI3K-γ isoforms are preferentially expressed in normal and malignant B-cells and T-cells. The tumour microenvironment (TME) is known to play an important role in the pathogenesis of both B-cell and T-cell lymphomas. For example, in B-cell lymphomas, CCL17 and CCL22 are produced by malignant cells and are chemotactic for CCR4 expressing T-regulatory cells, which promote immune evasion. In cutaneous T-cell lymphomas, CCL17 produced in the skin recruits CCR4-expressing malignant T-cells. Inhibition of both PI3K-δ and PI3K-γ can have complementary effects by impeding the function of both malignant cells and nonmalignant tumour-promoting cells in the TME. Duvelisib, an oral dual inhibitor of PI3K-δ,γ, has shown clinical activity in a phase 1 study in patients (pts) with advanced hematologic malignancies (Study IPI-145-02), including pts with relapsed/refractory (R/R) iNHL (Flinn, ASH 2014), T-cell lymphoma (TCL) (Horwitz, ASH 2014) and aggressive NHL (aNHL) (Porcu, ASH 2014).

Methods: Serum from 25 pts with R/R iNHL, 28 pts with R/R TCL and 25 pts with R/R aNHL was analysed for 72 analytes (cytokines, chemokines and matrix metalloproteinases) using Luminex xMAP technology at baseline and after 1 week of treatment (Cycle 1 Day 8). In addition, serum from 33 healthy donors was analysed for the same analytes. Change from baseline was analysed for statistical significance using a paired *t*-test with Bonferroni correction for multiple hypotheses. Comparison between each disease subtype and healthy donors utilized a 2-sample *t*-test with Bonferroni correction.

Abstract 281 Table Results: analytes with a statistically significant decrease from baseline to cycle 1 day 8

Disease subtype	Analyte
iNHL	CCL1, CCL4, CCL17, CCL22, CXCL10, CXCL13, IL-1RA, IL-10, IL-16, MMP-9, MMP-12, TNF-α
aNHL	CCL1, CCL3, CCL22, CXCL10, IL-1RA, IL-10, MMP-9, TNF-α
TCL	CCL1, CCL17, CCL22, CXCL10, CXCL13, IL-10, IL-12p40, MMP-9, MMP-12, TNF-α

Note: Statistical significance defined as ≤70% of baseline at C1D8 ($p = 0.0007$). Across all 3 disease subtypes, CCL1, CCL22, CXCL10, IL-10, MMP-9 and TNF-α were statistically significantly reduced following treatment with duvelisib.
All analytes with a statistically significant decrease were elevated at baseline relative to healthy donors, with the exception of CXCL10 and IL-1RA in iNHL.

Conclusions: Most of the analytes that were significantly reduced following duvelisib treatment are known to be involved in the communication between lymphoma cells and the TME. Modulation of the TME via dual inhibition of PI3K-δ,γ, as evidenced by decrease in numerous cytokines and chemokines, may be an important mechanism of action supporting the clinical activity of duvelisib observed in pts with iNHL, TCL and aNHL.

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EVEROLIMUS PLUS R-CHOP AS INITIAL THERAPY FOR DLBCL: A PHASE I/FEASIBILITY STUDY (NCCTG N1085 [ALLIANCE])

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Background: Everolimus was demonstrated to have single-agent activity in relapsed DLBCL (*Leukemia*. 2011; 25(2):341-7). However, the safety and efficacy of everolimus in combination with R-CHOP is unknown.

Methods: A phase I study was designed to determine the maximum tolerated dose (MTD) of everolimus on days 1–10 or 1–14 in combination with R-CHOP given every 21 days, with a feasibility cohort to examine response in patients with newly diagnosed CD20+ DLBCL. MTD was defined as the highest safely tolerated dose where at most 1 out of 6 patients experienced DLT. Starting everolimus dose was 10 mg days 1–10, and the planned dose escalation was 10 mg days 1–14. DLT was defined as any grade 3 or higher non-haematologic toxicity or a haematologic toxicity within the first cycle resulting in a delay of the next cycle of chemotherapy. The response was evaluated using PET/CT by standard criteria. A fourteen-patient feasibility extension was planned.

Results: In the phase I portion, 3 patients received 10 mg everolimus daily for days 1–10 and 6 patients received 10 mg everolimus daily for days 1–14. No DLT was seen, and no MTD was achieved; therefore, the dose for everolimus was determined to be 10 mg daily × 14 for the extension phase. Fifteen additional patients were enrolled in the feasibility portion. For the 24 patients: median age 58.5 years, 58% men and 50% with stage IV disease. The most common grades 3 and 4 adverse events in patients were febrile neutropenia (21%), anaemia (17%), thrombocytopenia (21%) and neutropenia (75%). Grade 3 hypertriglyceridemia occurred in 12.5% and grade 3 pneumonitis occurred in one patient. Overall response rate at completion of 6 cycles was 100% in the phase I cohort (8 CR and 1 PR). The patient achieving a PR became PET negative by 12 months without further therapy, thus achieving a CR. Feasibility cohort data will be available for the presentation.

Conclusions: Everolimus when combined with R-CHOP combination immunochemotherapy is well tolerated at 10 mg daily on days 1–10 and 1–14 of a 21-day cycle. The initial response rates in the phase I portion appear promising. A larger trial will be necessary to confirm the benefits of this novel combination.

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SAFETY AND CLINICAL ACTIVITY OF TEMSIROLIMUS IN COMBINATION WITH RITUXIMAB AND DHAP FOR THE TREATMENT OF PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

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Purpose: The aim of this study was to evaluate the safety, tolerability and efficacy of the combination of the mTOR inhibitor Temsirolimus with the regimen of DHAP and rituximab (STORM) in patients with relapsed or refractory diffuse large cell B-Cell lymphoma (DLBCL).

Patients and Methods: This is a prospective multicentre, phase II, open-label study. Patients with relapsed or refractory DLBCL exposed to at least one prior but a maximum of two prior therapies were eligible. The STORM regimen consisted of Rituximab 375 mg/m^2 (day 2) and DHAP (dexamethasone 40 mg day 3–6, cisplatine 100 mg/m^2 day 3, cytarabine 2 × 2 g/m^2 day 4) with temsirolimus added on days 1 and 8 of a 21-day cycle. In part I, a dose escalation for the mTOR inhibitor temsirolimus from 25 up to 50 mg was conducted.

Results: Here we report on the preliminary results of part I of this clinical trial. Fifteen patients were included – 8 patients in the 25 mg cohort and 7 patients in the 50 mg cohort. Median age was 70 (49–76) years and median number of prior regimen was 1. Two DLTs (esophagus infection and venous thrombosis) were observed. The most frequent non-haematologic side effects were nausea (9 pts), epistaxis (7 pts), fatigue (6 pts), increased ALT (6 pts) and increased creatinine (6 pts). Frequent grade 3/4 events (n>2) in both cohorts (25 mg|50 mg) included leukopenia (6|5 pts – with a mean duration of 4.4 days | 6.7 days), thrombocytopenia (5|6pts – with a mean duration of 4.6 days | 11.9 days), lymphopenia (2|4 pts), anemia (2|3 pts), neutropenia (3|0 pts), renal failure (2|1 pts) and infections (1|3 pts) (bladder infection, esophagus infection, central venous access infection, soft tissue infection and mucositis). For the part II proportion of the trial, a temsirolimus dose of 25 mg given on days 1 and 8 was

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defined as recommended dose. Evaluation for best response showed a response in 10/11 patients, with 2 CRs and one CRu. Four patients could not be evaluated for response at the time of this report. At a median follow-up of 6 months, 77% of evaluable patients are without signs of progression.

Conclusion: Temsirolimus can be safely added to DHAP and rituximab with promising activity. Recruitment into part II is ongoing.

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UBLITUXIMAB + TGR-1202 DEMONSTRATES ACTIVITY AND FAVOURABLE SAFETY PROFILE IN RELAPSED/REFRACTORY B-CELL NHL AND HIGH-RISK CLL

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Introduction: Ublituximab (UTX) is a novel anti-CD20 mAb that has been glycoengineered for enhanced ADCC. TGR-1202 is a novel once daily oral PI3Kδ inhibitor with demonstrated clinical activity in B-cell lymphoma and notably absent hepatotoxicity associated with similar agents. The combination of UTX + TGR-1202 showed strong synergistic activity *in vitro* (Lugano 2013). This phase 1 trial evaluates the safety and efficacy of the combination of UTX + TGR-1202 in patients with heavily pre-treated rel/ref NHL and CLL.

Methods: A 3 + 3 design was utilized with rel/ref NHL and CLL patients accruing independently. There were no limits on number of type of prior therapies. Patients refractory to prior PI3K or BTK inhibitors were eligible. UTX was administered D 1, 8, 15 of Cyc 1 and 2, followed by D 1 of Cyc 4, 6, 9 and 12. TGR-1202 was administered orally once daily. Primary endpoints: safety and dose-limiting toxicities (DLT). Secondary endpoints: efficacy (ORR and CR rate).

Results: Thirty-seven patients were enrolled and evaluated for safety: 13 CLL/SLL, 12 FL, 9 DLBCL, 2 MZL and 1 Richter's transformation. Med age 64 yo (range 29–86); 24 M/13 F; median number prior treatment regimens = 3 (range 1–9). Day 1 infusion reactions (3% G 3/4), neutropenia (32% G 3/4), diarrhoea (3% G 3/4) and nausea (0% G 3/4) were the most commonly reported adverse events regardless of causality. To date, TGR-1202 related hepatotoxicity has not been observed. One DLT occurred: a patient with Gr 3 neutropenia at study entry, which worsened (cohort 1). A dose-response relationship was observed with TGR-1202; greater clinical activity was observed at higher doses. Twenty-nine over thirty-seven were evaluable for efficacy (7 too early and 1 was ineligible) with best response to treatment as follows:

Abstract 284 Table

	TGR-1202 higher[a] dose						TGR-1202 lower[b] dose					
Type	Pts (*n*)	CR	PR	ORR *n* (%)	PD	Type	Pts (*n*)	CR	PR	ORR *n* (%)	PD
CLL/SLL	3	–	3	3 (100)	–	**CLL/SLL**	7	–	4	4 (57)	–
DLBCL	4	2	1	3 (75)	1	**DLBCL**	3	–	–	–	2
FL/MZL	7	1	4	5 (71)	–	**FL/MZL**	4	–	1	1 (25)	–
Richter's	1	–	1	1 (100)	–	**Richter's**	–	–	–	–	–
Overall	15	3	9	12 (80)	1	**Overall**	14	-	5	5 (36)	2

[a]Higher dose = 1200 original formulation and 600 or >micronized.
[b]Lower dose = 800 original formulation and 400 micronized.

To date, of the 29 patients evaluable for response, 87% (13/15) in the higher dose cohorts remain progression-free compared to 43% (6/14) in the lower dose cohorts. All but 1 of the CLL patients (lower dose cohort patient) remain progression free with a median follow-up time of 9 months (range 1–12+ months).

Conclusions: The chemotherapy-free combination of UTX + TGR-1202 is highly active and well tolerated in patients with both indolent and aggressive rel/ref NHL and CLL. Dose escalation continues with enrollment ongoing at the highest dose cohort and in recently opened expansion cohorts.

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BLINATUMOMAB TREATMENT IN RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA IN AN OPEN-LABEL, PHASE 2 STUDY: SAFETY, EFFICACY AND LATE RESPONSES

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Introduction: In a phase 1 study, blinatumomab, a bispecific T-cell engager (BiTE®) antibody construct, resulted in an overall response rate (ORR) of 55% in a subset of patients with diffuse large B-cell lymphoma (DLBCL). This phase 2 study evaluated stepwise or flat dosing of blinatumomab in patients with relapsed/refractory (r/r) DLBCL.

Methods: Eligible patients were adults ≥18 years old with r/r DLBCL. Blinatumomab was infused continuously over 8 weeks. In stage 1, stepwise dosing (cohort I: 9 μg/day in week 1, 28 μg/day in week 2, then 112 μg/day) was compared with flat dosing (cohort II: 112 μg/day throughout). Based on benefit/risk in stage 1, stepwise dosing was chosen for cohort III in stage 2 (see Results). Responders could receive a 4-week consolidation cycle after a 4-week treatment-free period. All patients received prophylactic dexamethasone. The primary endpoint was ORR by independent radiologic assessment per Cheson revised response criteria 2 weeks after each cycle and then every 3 months. Patients who received 112 μg/day for ≥1 week were considered evaluable for response.

Results: In all, 25 patients were treated: 9, 2, and 14 in cohorts I, II, and III, respectively. Median age was 66 years and 56% were men. Blinatumomab followed a median (range) of 3 (1–7) prior treatments. All patients experienced ≥1 adverse event (AE), including grade 3 AEs in 96% and grade 4 AEs in 20%. Regardless of causality and grade, the most common AEs were tremor (52%), pyrexia (44%), diarrhoea (24%), fatigue (24%), edema (24%) and pneumonia (24%). Grade 3 neurologic AEs were reported in 28%, including 33% and 100% in cohorts I and II, respectively. Thus, stepwise dosing was selected for cohort III. Grade 3 neurologic AEs in >1 patient overall were disorientation, encephalopathy, aphasia and epilepsy ($n = 2$ each). There were no grade 4 or 5 neurologic AEs. Deaths were due to disease progression ($n = 11$), cardiogenic shock ($n = 1$), organ failure following transplantation ($n = 1$) and unreported cause ($n = 1$). Two of the deaths were reported as grade 5 (fatal) AEs (pneumonia and disease progression); neither fatal AE was considered related to blinatumomab. The ORR for patients who were evaluable per protocol was 43% (9 of 21), including 4 CR and 5 partial remissions (PR). Median duration of response was 11.6 months. One investigator reported that 3 patients with PRs during blinatumomab therapy developed CRs during follow-up without additional anti-lymphoma therapy.

Conclusions: In this phase 2 study, stepwise dosing of blinatumomab had an acceptable safety profile and resulted in objective responses in heavily pretreated patients with r/r DLBCL, including late CR after blinatumomab therapy in some patients.

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PHASE I DOSE-ESCALATION STUDY OF BI 836826 IN PATIENTS (PTS) WITH RELAPSED OR REFRACTORY NON-HODGKIN LYMPHOMA (NHL) OF B CELL ORIGIN

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Introduction: BI 836826 is a chimeric IgG1 type II antibody targeting CD37, which is predominantly expressed on normal and malignant B cells. BI 836826 is engineered to improve Fc-receptor binding and has high intrinsic pro-apoptotic activity on malignant B cells. This study evaluates the safety, maximum tolerated dose (MTD), pharmacokinetics (PKs) and activity of BI 836826 in pts with relapsed/refractory NHL of B cell origin (NCT01403948; 1270.2).

Methods: The trial has a dose-escalation phase (modified 3 + 3 design) and an expansion phase at the MTD. Eligible pts were treated with up to 3 courses comprising an intravenous infusion (starting dose: 1 mg) once weekly for 4 weeks followed by an observation period of 27 (courses 1 and 2) or 55 days (course 3). Dose-limiting toxicities (DLTs) were defined as grade (G) ≥3 drug-related non-haematologic adverse event (AE) except infusion-related reactions (IRRs) evaluated for MTD determination up to 7 days after the 2nd administration. Pts treated beyond course 2 had to demonstrate clinical benefit.

Results: As of 14 October 2014, 37 pts (mean age: 67.5 yrs; ECOG score 0/1/2: 24%/57%/19%; subtypes: follicular lymphoma [FL; 51%], diffuse large B-cell lymphoma [38%], mantle cell lymphoma [MCL; 8%]) were treated in the dose-escalation phase. Pts had a median of 5 prior lines of therapy, and 62% were refractory to last therapy. At 200 mg ($n = 7$), 1 pt had DLTs (oral herpes, stomatitis and febrile neutropenia, all G3) and 4 additional pts had neutropenia >7 days, hence 200 mg was considered to exceed the MTD. At 150 mg ($n = 6$), 3 pts had DLTs (G3 hypocalcemia and G3 hypokalemia [$n = 1$]; G4 hypophosphatemia [$n = 2$]); 4 pts also had G4 leukopenia and/or neutropenia >7 days. The MTD was established at 100 mg as no DLTs were observed in the 6 pt cohort. Twenty-four (65%), 9 (24%) and 4 (11%) pts initiated 1, 2 and 3 courses, respectively; 3 pts completed 3 courses. As of 14 October 2014, 36 (97%) pts had discontinued, most due to progressive disease (PD, 64%). All pts had ≥1 AE, most commonly leukopenia (60%), neutropenia (57%), thrombocytopenia (49%) and IRRs (41%). G3/4 AEs were reported in 78% of pts; most frequent were leukopenia (57%), neutropenia (57%) and lymphopenia (27%). Most IRRs were G1/2; 1 pt had a G4 IRR (infusion schedule was changed hereafter to a slowly increasing rate controlled infusion) and 2 pts had G3 IRRs (all reversible). After the infusion schedule was changed, IRRs have been manageable. There were 7 deaths, all due to PD and not considered treatment related. One pt (leukemic MCL) had a partial response and 2 pts (FL) had 33% and 44% tumour reductions according to Cheson et al 1999. Plasma exposure increased with increasing doses.

Conclusions: The MTD for BI 836826 was defined as 100 mg, which is being used in the expansion phase of the study, and AEs were considered to be manageable. Most frequent AEs were hematologic and IRRs. IRRs were manageable after amending the infusion schedule and mainly G1/2.

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RESULTS OF A PHASE I STUDY OF ^{177}LU-DOTA-HH1 ANTIBODY RADIONUCLIDE CONJUGATE FOR PATIENTS WITH RELAPSED CD37+ NON-HODGKINS LYMPHOMAS

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Introduction: The CD37 surface antigen is expressed by normal B-cells and most malignant B-cell lymphomas and represents an interesting therapeutic target. 177Lu-DOTA-HH1 (Betalutin™) is a novel anti-CD37 radioimmunoconjugate in early clinical development. It consists of the β-emitting isotope lutetium-177 (T1/2 = 6.7 days) chelated to p-SCN-Bn-DOTA, which is conjugated to the murine mAb HH1. Betalutin™ is delivered as a ready to use formulation. The current phase I study aims to determine the maximum tolerated dose, overall safety and tumour response.

Methods: Pts with relapsed CD37+ non-Hodgkins lymphoma; follicular (FL) grades I–IIIA, marginal zone, mantle cell (MCL), lymphoplasmacytic or small lymphocytic lymphoma with platelet counts ≥150 × 109/L and <25% bone marrow involvement were eligible. On days (d) 1 and 8, pts received pre-treatment with single infusions of rituximab (375 mg/m^2) in order to deplete normal B cells. On d 29 a predosing with HH1 (50 mg and cold CD37 antibody) was followed by Betalutin™ i.v.. In a 3 + 3 study design, 10 MBq/kg was the starting dose. Pts were assessed for distribution of radioactivity by whole body scans and SPECT/CT. Tumour response was assessed by PET/CT and CT according to the Cheson criteria. Adverse events (AEs) were monitored and scored according to the CTCAE grading (G).

Results: Thirteen pts (12 FL and 1 MCL) have been enrolled and 12 treated to date. The range of prior therapies was 1–8, 4/12 pts were refractory to rituximab. Serious AEs were reported in 5 pts: thrombocytopenia requiring platelet transfusions (2 pts) and epistaxis in 1 of them; pneumonia and pulmonary embolism (PE) in 1 pt with history of PE; transient atrial fibrillation unlikely related to the study drug (2 pts). Most common toxicities were hematologic; the median time to nadir for platelets and neutrophils was 39 and 48 days, respectively. All dose-limiting toxicities (DLT) were reversible and manageable: at 20 MBq/kg G 3/4 neutropenia and/or thrombocytopenia were observed in all pts. DLTs at 15 MBq/kg (5 pts) were 1 G 3 thrombocytopenia lasting >14 days and 1 G 4 neutropenia/ thrombocytopenia lasting >7 days. Tumour responses observed across all dose levels were 4 complete, 3 partial remissions (PR), 2 stable disease (1 of which had disease regression at 3 months not yet qualifying for PR) and 3 progression of disease. Further data on efficacy, safety, intertumour variability in uptake of Betalutin™ and correlation of CD37 expression with tumour response will be presented.

Conclusions: In this study Betalutin™ as a single dose has demonstrated predictable and manageable safety, haematological toxicity as expected and tumour responses across all dose levels. DLTs were observed at 20 MBq/ kg, and 15 MBq/kg is the recommended dose for the phase 2 part of this trial, open for enrolment. Betalutin™ is a promising new treatment for NHL.

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