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POSTER PRESENTATIONS

MOLECULAR BIOLOGY

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BURKITT'S LYMPHOMAS THAT ARISE SPONTANEOUSLY IN VK*MYC MICE SHARE SIMILAR MUTATIONS WITH THE HUMAN DISEASE AND ARE ACCELERATED BY DIVERSE SURVIVAL SIGNALS

M. Chesi[1], V. M. Garbitt[1], S. E. Palmer[1], L. Bergsagel[1].
[1]*Comprehensive Cancer Center, Mayo Clinic, Scottsdale, USA.*

Introduction: We previously reported that transgenic mice in which AID-dependent MYC expression is activated sporadically in germinal centre (GC) cells by somatic hypermutation only infrequently develop Burkitt's lymphoma (BL) (20%),but uniformly develop multiple myeloma. We concluded that sporadic MYC expression alone is not sufficient to drive lymphomagenesis in mature B cells. Aiming to identify MYC-cooperating genetic lesions in the development of spontaneous BL in Vk*MYC mice, we analyzed genomic DNA from a cohort of BLs by sequencing and aCGH. In a parallel approach, we investigated the contribution of known oncogenes/tumour suppressor genes to the development of MYC-driven BL by crossing Vk*MYC mice to available mice transgenic or knockout for the gene of interest.
Methods: BLs from Vk*MYC mice were characterized by FACS analysis as B220 +CD19+CD38-FAS+PNA+ IgM+or−, Kappa dim, and by expression of high levels of MYC and BCL6 by western blot. The GC origin of the BL tumours was confirmed by direct sequencing of the rearranged *V–J* segment from 50 independent tumours, revealing an average of 7% somatic mutations. aCGH was performed on the Agilent 244K array.
Results: Sequencing in four tumours identified missense mutations of genes frequently mutated in human BL: Tp53 gene in four; Gna13, Arid1a, and Lrp1b in two; and Ddx3x, Ncor2, Notch1, Mki67, Foxo1, and Whamm in one tumour each. Consistent with AID-dependent somatic mutation, there were multiple silent and missense mutations of Pim1 in all four tumours, a gene more frequently mutated in human diffuse large B-cell lymphoma then BL. As expected, Vk*MYC mice crossed to floxed TP53 mice developed very aggressive BL. Mutations in the Ccnd3 or NFKB genes were not identified, but crosses of the Vk*MYC with Emu-CCND1 or TRAF3 floxed mice increased lymphomagenesis, as did crosses with vav-BCL2 (but not Emu-BCL2).
Conclusions: Sporadic high expression of MYC driven by Ig enhancers is not tolerated by GC cells. Spontaneous mutations of TP53 allow the development of BL in Vk*MYC mice; however, multiple independent survival signals including activation of the NFkB pathway or overexpression of anti-apoptotic proteins can also allow GC cells to sustain high levels of MYC, stimulating lymphomagenesis.

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PRO-ONCOGENIC FUNCTIONS OF HSP105 IN HUMAN B-CELL NON-HODGKIN LYMPHOMAS

M. Di Nicola,[1] R. Zappasodi,[1] A. Cavan,[1] M. Tortoreto,[2] C. Tringali,[3] A. Cabras,[4] G. Ruggiero,[1] L. Castagnoli,[2] B. Venerando,[3] N. Zaffaroni,[2] S. M. Pupa,[2] A. M. Gianni.[1]
[1]*Medical Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy;* [2]*Experimental Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy;* [3]*Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy;* [4]*Pathology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy.*

Introduction: We have reported that heat shock protein (HSP)105 is an immunogenic antigen of human B-cell non-Hodgkin lymphoma (B-NHL) expressed in function of their aggressiveness and proliferation index. We have now set out to clarify how HSP105 contribute to the malignant phenotype in these diseases.
Methods: A model showing HSP105 stably knocked down Namalwa aggressive lymphoma was generated by using a lentiviral vector carrying an HSP105-targeting pre-microRNA in frame with the green fluorescent protein (GFP) gene. GFP+infected cells were FACS sorted and cloned. GFP clones were analyzed by western blot, and their growth was monitored *in vitro* and in severe combined immunodeficiency mice.
Results: GFP cells displayed a constitutive and specific downregulation of HSP105, with no alteration of HSP90 and HSP70, the two major HSP family members. Despite no sign of cytotoxicity, the two best-silenced clones (B1 and C5) showed a slower *in vitro* proliferation rate and a significant doubling time increase compared with the MOCK-infected cells ($p = 0.03$, 0.001). Accordingly, their *in vivo* tumour-forming capability was significantly hampered ($p = 0.0009$, 0.019) up to the loss of tumourigenicity in 100% and 83% of the mice inoculated with 10^4 B1 or C5 cells, respectively. Western blot analysis of six silenced clones showed that BCL-6 and c-Myc were both down-modulated in function of HSP105 knockdown levels. In two aggressive B-NHL models, HSP105 co-immunoprecipitated with BCL-6 and c-Myc, suggesting that it may favour the stabilization of these oncoproteins. Along this line, immunohistochemistry analysis of 40 primary human aggressive B-NHLs revealed that HSP105 expression is significantly higher in c-Myc Burkitt's ($p = 0.0264$) or BCL-6 diffused large B-cell lymphomas ($p = 0.0068$) than in other aggressive histotypes that do not overexpress these oncoproteins. In the same cases, HSP105 expression levels positively correlate with those of BCL-6 and c-Myc (p 0.0001, 0.0075).
Conclusions: Our findings point to HSP105 as a *per se* non-oncogenic molecule that can contribute to lymphomagenesis by stabilizing key lymphoma oncoproteins. They equally provide the rationale for developing HSP105 inhibitors as a new therapeutic strategy for aggressive B-NHLs.

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DIMERIZATION AND MONOUBIQUITINATION OF THE PARACASPASE MALT1 ARE REQUIRED FOR THE SURVIVAL OF CELLS DERIVED FROM ABC DLBCL

M. Thome,[1] C. Pelzer,[1] K. Cabalzar,[1] A. Wolf,[2] G. Lenz,[2] S. Hailfinger.[1]
[1]*Department of Biochemistry, University of Lausanne, Epalinges, Switzerland;* [2]*Department of Hematology, Oncology and Tumor Immunology, Charite University Medical Center, Berlin, Germany.*

The mucosa-associated lymphoid tissue protein-1 (MALT1, also known as paracaspase) is a protease whose activity is essential for the activation of lymphocytes via the NF-kB transcriptional pathway. MALT1 activity is also essential for the growth of cells derived from human diffuse large B-cell lymphomas of the activated B-cell subtype (ABC DLBCL). Recently, we have shown that the MALT1 protease activity is regulated by monoubiquitination, which favours or stabilizes MALT1 dimerization. Mutation of the monoubiquitination site K644 impaired antigen-induced MALT1 activation in T cells and interfered with the growth of cell lines derived from ABC DLBCL, in which MALT1 was constitutively monoubiquitinated. Thus, monoubiquitination of Malt1 is essential for its catalytic activation and is therefore an interesting target for treatment of ABC DLBCL and immune modulation. Here, we identify a charged residue localized at the dimerization interface of MALT1 as essential for the formation of catalytically active MALT1 dimers. Mutation of this residue impaired the capacity of MALT1 to undergo monoubiquitination and to promote the growth of ABC DLBCL cell lines. We conclude that dimerization via the protease interface and subsequent monoubiquitination are required for the full enzymatic and biological activity of MALT1.

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BCL2 MUTATIONS IN B-CELL LYMPHOMAS AND IN THE EVOLUTION OF FOLLICULAR LYMPHOMA

R. Burkhard,[1] G. Bhagat,[2] S. Cogliatti,[3] D. Rossi,[4] M. Fangazio,[5] G. Gaidano,[4] L. Pasqualucci,[5] U. Novak.[6]
[1]*Experimental Oncology/Hematology, Department of Clinical Research, University of Bern, Bern, Switzerland;* [2]*Department of Pathology and Cell Biology and the Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY, USA;* [3]*Institute of Pathology, Kantonsspital St. Gallen, St. Gallen, Switzerland;* [4]*Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy;* [5]*Institute for Cancer Genetics and the Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, USA;* [6]*Department of Medical Oncology, Inselspital Bern University Hospital, Bern, Switzerland.*

Introduction: Somatic hypermutation (SHM) is active in germinal centres (GC) and randomly introduces mutations in the immunoglobulin heavy chain genes (IgH) of B cells. Unlike normal B cells, 50% of diffuse large B-cell

lymphomas (DLBCL) have somatic mutations in several proto-oncogenes through an aberrant activity of the SHM process (ASHM). BCL2 was identified as a target of ASHM in a subset of DLBCLs, independent from the translocation t(14;18) (Saito, Novak; 2009). Deregulated BCL2 expression due to the t (14;18) joining BCL2 to the Ig enhancer is the genetic hallmark of follicular lymphoma (FL) and present in one third of GC-derived DLBCL. FL is characterized by relapses and transformation to DLBCL. We assessed the prevalence of BCL2 mutations in B-cell non-Hodgkin's lymphomas (B-NHLs) and explored BCL2 mutations in the clinical course of FL.

Methods: One hundred four primary cases of B-NHLs [t(14;18) positive and negative FL, HIV-DLBCL, chronic lymphocytic leukaemia in Richter's transformation, post-transplant lymphoproliferative disorder, and marginal zone lymphoma] and 10 matched pairs of FL later relapsing to FL or DLBCL were Sanger-sequenced for BCL2 mutations (promoter, exons 1 and 2, and intron). The clonal relationship in sequential biopsies was assessed by IgH-fragment or BCL2-rearrangement analysis.

Results: With 0.63%, the BCL2 mutation frequency was highest in the 2/16 mutated t(14;18) negative FLs and 0.43% in the 15/21 mutated t(14;18) positive FLs. The other B-NHL entities had few or no BCL2 mutations. BCL2 sequence analysis in paired samples revealed two patterns of genomic evolution of FL: (i) direct evolution from the antecedent FL clone with few novel clonal mutations in the DLBCL compared with the FL and (ii) evolution from a common progenitor cell characterized by both shared and unique mutations in the initial FL and the relapsed or transformed FL counterpart. No common BCL2 mutations were found in the single clonally unrelated pair, suggesting that the second FL developed as a novel tumour rather than as a relapse of the first FL.

Conclusions: BCL2 mutations are not restricted to DLBCL and found in a subset of t(14;18) negative FL with features of ASHM. BCL2 sequence analysis in serial biopsies of relapsing or transforming FL revealed distinct patterns of genomic evolution, mirroring the complexity of FL biology and evolution.

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MYD88 L265P IN MARGINAL ZONE LYMPHOMAS

A. Martinez-Lopez,[1] S. Curiel del Olmo,[1] N. Martinez,[1] S. Montes-Moreno,[2] M. Mollejo,[3] F. Mazorra,[2] L. Cereceda,[1] J. Revert,[1] C. Almaraz,[1] M. Piris.[1]
[1]Genomica del Cancer, Instituto de Investigación y Formación Marqués de Valdecilla (IFIMAV), Santander, Spain; [2]Pathology, Hospital Universitario Marqués de Valdecilla (IFIMAV), Santander, Spain; [3]Pathology, Hospital Universitario Virgen de la Salud, Toledo, Spain.

Introduction: It has been described a recurrent somatic variant at the position 38 182 641 in chromosome 3p22.2 that results in an amino acid change from leucine to proline (L265P) in the Myd88 gene in a heterogeneous group of B-cell disorders. Recently, it has been reported that Myd88 may be useful in distinguishing lymphoplasmacytic lymphoma from marginal zone lymphoma (MZL) an often difficult task to overlapping morphologic, immunophenotypic, cytogenetic and clinical features. For this purpose, we develop a competitive allele-specific Taqman RT-PCR assay, a reliable method for the detection of the mutation. The aim of this study is to characterize the frequency of MYD88 (L265P) mutation in a series of MZL cases.

Patients: We studied 68 cases that comprises 36 splenic marginal zone lymphoma (SMZL), 16 nodal marginal zone lymphoma (NMZL) and 16 extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) with different localizations: 5 gastrointestinal and11 with other localizations (thyroid gland, parotid, endometrium, soft tissue, lung and kidney).

Methods: Cases were centrally reviewed and classified according to the criteria defined by WHO 2008 classification. Genomic DNA was extracted from FFPE diagnostic sample. Myd88 gene exon 5 Sanger sequencing was performed to identify positive controls (c.794T>C, COSM85940). Quantitative detection of Myd88 L265P mutation was developed using a Taqman allele-specific RT-PCR assay (Applied Biosystems, Foster City, CA). Mutated or wild-type sequences from samples were specifically amplified in a competitive allele-specific PCR using an ABI 7900 HT Fast Real-Time PCR system. The analytical sensitivity for the detection of the mutation by this method has a lower limit of 0.1%

Results:

Diagnosis	Total cases	No. positive cases for mutation	% positive cases for mutation
SMZL	36	3	8
NMZL	16	1	6.25
MALT	16	0	0

Conclusions: These findings suggest that Myd88 L265P mutation can be present in marginal zone lymphomas, being in a higher prevalence in SMZL.

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EBV-SPECIFIC MICRO-RNA VIA EXOSOME: A KEY INTER-CELLULAR MACHINERY BETWEEN EBV+ TUMOUR AND TUMOUR-SURROUNDING IMMUNE CELLS?

A. Kotani,[1] K. Yokoyama,[2] J. Lu,[3] T. Yahata,[4] K. Ando,[3] K. Imadome,[5] N. Nakamura.[6]
[1]Institute of Innovative Science and Technology, Tokai University, Isehara, Japan; [2]Immunology, Tokai University School of Medicine, Isehara, Japan; [3]Hematology, Tokai University School of Medicine, Isehara, Japan; [4]Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan; [5]Infectious Diseases, National Research Institute for Child Health and Development, Tokyo, Japan; [6]Pathology, Tokai University School of Medicine, Isehara, Japan.

Introduction: EBV is associated with heterogeneous lymphomas, which are subdivided into three types of latency. Hodgkin's lymphoma (HL) is characterized by a minority of neoplastic Hodgkin and Reed–Sternberg (HRS), which are embedded in non-neoplastic bystanders, mostly B and T cells, but also macrophages. Without these bystander cells, the HRS cells are incapable of being engrafted in immunodeficient mice. In this context, the bystanders are tumour-supportive 'inflammatory niche'. Because of the complexity of its interplay, the detailed mechanism remains elusive. Small RNAs including miRNAs are well-known intra-cellular regulatory elements of gene expression. Recently, they was reported to be conjugated in exosomes, transferred to cells, and involved in tumour metastasis. Moreover, EBV-infected cells produce exosomes that contain viral-encoded, EBV-specific miRNAs (EBV-miRNAs). Accordingly, we hypothesized that exosomal EBV-miRNAs might redirect tumour surrounding immune cells from tumour reactive into tumour-supportive 'inflammatory niche', which ultimately leads to tumour progression.

Methods: We evaluated the expression of EBV-miRNAs in EBV HL clinical specimens by *in situ* hybridization, its functional characterization *in vitro*, and its effects on persistent infection and tumour development *in vivo*.

Results: The exosomes produced by EBV cells (EBV-Ex) were harvested from the media of either the type III or type I EBV-infected cells, in which EBV-miRNAs were relatively rich or vacant.The EBV-Ex uptake was detected only in monocyte/macrophage (Mo/Mf), and EBV-miRNA effects were potent on Mo/Mf in inducing CD69, IL-10, and TNF, suggesting the possibility that EBV-miRNAs might polarize Mo/Mf into tumour-associated Mf. *In vitro*, EBV-miRNAs suppress cell proliferation, whereas in *in vivo* mouse model, they are required to develop lymphoproliferative disease (LPD), suggesting that EBV-miRNAs affect the microenvironment to cause LPD. Most importantly, exosomal EBV-miRNAs were transferred in Mf in human EBV HL samples.

Conclusion: EBV might utilize the exosomal machinery to secrete key viral-encoded miRNAs, through which a small number of neoplastic EBV cells could modulate the tumour microenvironment.

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RICHTER SYNDROME PROMOTER METHYLATION PATTERN DIFFERS FROM *DE NOVO* DIFFUSE LARGE B-CELL LYMPHOMA

A. Rinaldi,[1] A. A. Mensah,[1] I. Kwee,[1] F. Forconi,[2] E. M. Orlandi,[3] M. Lucioni,[4] V. Gattei,[5] R. Marasca,[6] F. Berger,[7] S. Cogliatti,[8] F. Cavalli,[9] E. Zucca,[10] G. Gaidano,[11] D. Rossi,[11] F. Bertoni[1].
[1]Lymphoma and Genomics Research Program, IOR Institute of Oncology Research, Bellinzona, Switzerland; [2]Cancer Sciences Unit, CRUK Clinical Centre, University of Southampton, Southampton, UK; [3]Department of Hematology Oncology, Hematology Unit, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy; [4]Pathology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy; [5]Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, Aviano, Italy; [6]Division of Hematology, Department of Oncology and Hematology, University of Modena and Reggio Emilia, Modena, Italy; [7]Department of Pathology, Centre Hospitalier Lyon-Sud, Lyon, France; [8]Institute of Pathology, Cantonal Hospital St Gallen, St Gallen, Switzerland; [9]IOSI, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [10]IOSI, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [11]Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy.

Introduction: Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in Western countries. Up to 15% of CLL transform to Richter syndrome (RS). To broaden knowledge of the relationship between RS and *de novo* large B-cell lymphoma (DLBCL), and the role of DNA methylation in RS transformation, we performed genome-wide DNA promoter methylation profiling of RS, CLL and DLBCL.

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Methods: Methylation profiling was performed using the Infinium HumanMethylation27 arrays in 19 RS (10 paired with CLL), 7 untransformed CLL (CLL-U), 10 DLBCL and 6 normal peripheral blood CD19+B-cells.
Results: Hierarchical clustering showed that RS clustered distinctly from *de novo* DLBCL and largely intermixed with CLL. The 2687 probes more methylated in DLBCL were mapped in the promoters of H3K27me3-marked genes ($p < 0.001$) and polycomb protein targets ($p < 0.001$). The 443 probes more methylated in RS mapped in the promoters of genes involved in TP53 signalling ($p = 0.006$) and cell cycle regulation ($p = 0.05$). RS differed from CLL-U in the methylation levels of 258 probes. Probes that were hypermethylated in RS compared with CLL-U mapped to the promoters of TP53 and RB1 gene targets ($p < 0.001$) and polycomb gene targets ($p < 0.001$). Probes mapping to the OSM and S1PR4 genes appeared as the most differentially methylated between RS and the preceding CLL phase. MSP confirmed OSM as more frequently methylated in RS compared with the CLL phase. qPCR showed lower OSM expression in RS compared with the paired CLL phase. OSM expression was restored in DLBCL cell lines methylated for OSM following treatment with decitabine in a dose-dependent manner.
Conclusions: The methylation profiles of RS and *de novo* DLBCL differ. RS and DLBCL show methylation of polycomb-marked genes, compatible with a cancer-related de-differentiation programme. OSM is specifically methylated in RS and DLBCL, and its suppression might contribute to the transformation of CLL to RS.AR and AAM equally contributed.

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THE GENOMIC LANDSCAPE OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

E. Braggio,[1] B. O'Neill,[2] S. VanWier,[1] J. Ojha,[1] R. Valdez,[1] R. Tibes,[1] K. Stewart,[1] R. Fonseca.[1]
[1]Hematology and Oncology, Mayo Clinic, Scottsdale, USA; [2]Neurology, Mayo Clinic, Rochester, USA.

Introduction: Primary central nervous system lymphoma (PCNSL) is a very aggressive and incurable lymphoma that is confined to the CNS because of a poorly understood neurotropism. Because of the still fragmented knowledge of the genomic basis of the disease, it is still a matter of debate whether PCNSL differs from systemic diffuse large B-cell lymphoma (DLBCL) with respect to their molecular features and pathogenesis and also if there is a genetic signature CNS-specific.
Methods: We performed an integrated genomic analysis in 21 immunocompetent, EBV-PCNSL and HIV-PCNSL. Samples were studied by aCGH ($N = 18$), paired-end whole exome sequencing ($N = 10$) and mate-pair whole genome sequencing ($N = 2$).
Results: We found a complex karyotype with a median of 21 copy-number abnormalities (range 10–47), 4 structural abnormalities, 6 frameshift indels and 59 nonsynonymous mutations. By integrating mutation and copy-number data, we found biallelic inactivation of CDKN2A in 89% of cases, being nearly a unifying event of the disease. Another interesting finding was the high prevalence of MYD88 mutations (L265P/M232T/V217F), mutated in 79% cases. Several other genes affected have been previously identified in nodal DLBCL, such as inactivated mutations of TNFAIP3 (16%), PRDM1 (16%), GNA13, TMEM30A, B2M and CD58 (11% each), activated mutations of CD79B (28%) and CARD11 (19%), and translocations of BCL6 (22%). On the other hand, several recurrent abnormalities have not been previously identified in DLBCL, suggesting that they are specific to PCNSL pathogenesis. Thus, 11% of PCNSLs show biallelic inactivation of TOX and PRKCD, and the other 17% of cases show monoallelic inactivation in these genes. Overall, we found components of the NF-kB pathways to be altered in >90% of PNCSL. Pathway analysis also showed an enrichment of networks associated with immune response, proliferation, regulation of the apoptosis, and lymphocyte differentiation and activation.
Conclusion: We found a genomic landscape in PCNSL similar to post-germinal centre DLBCL but reinforcing the existence of a subset of abnormalities specific to PCNSL, suggesting their potential relevance in the disease pathogenesis.

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QUANTITATIVE ULTRA DEEP SEQUENCING TO CHARACTERIZE CLONAL EVOLUTION OF FOLLICULAR LYMPHOMA

R. Burack, J. M. Spence, J. P. Spence.
Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, USA.

Introduction: Intra-clonal genetic diversity is the prerequisite for the clinical evolution of a tumor. Genetic diversity in follicular lymphoma (FL) has been documented qualitatively by studying the effects of Activation Induced Deaminase (AID) on IGH coding regions. To obtain a quantitative measure of genetic variation, we performed ultra-deep sequencing of IGH and selected non-coding regions known to be aberrantly targeted by AID allowing the extent and type of diversity to be compared between tumors.
Method: 10 FL specimens were studied. In addition to coding and non-coding portions of the IGH gene, ten AID-targeted non-coding regions scattered throughout the genome were sequenced to an average mapped coverage of > 20,000-fold. Array CGH was performed to further assess the extent of genome-wide damage. Data were analyzed by a novel algorithm that exploits partial assembly to reduce noise.
Results: 407 mutations reflecting AID-activity on the coding (1-77, median 34) and 1362 non-coding (7-290, median 101) regions of IGH were detected. Each mutation reflects a branch in a tumor's evolutionary tree, and 41% of branches included less than 1% of the tumor cells. 656 mutations were detected in non-coding regions of the 10 non-IGH genes. The extent of genome-wide damage attributable to AID was predicted by the number of mutations in the non-coding region of the translocated BCL2 gene (r2=0.85). However, no relationship was seen between the number of mutations detected in the IGH genes and the non-IGH genes. Furthermore, the extent of genomic complexity apparent by high-resolution arrray Comparative Genomic Hybridization was also independent of the AID-activity as assessed by the number of mutations. Finally, the branching pattern of the evolutionary trees qualitatively distinguishes grade 3 from grade 1/2 cases.
Conclusions: These data show: 1) AID-induced damage varies widely in FL; 2) genome-wide and IGH-directed effects of AID are quantitatively independent; 3) histologic grade correlates with distinctive branching patterns of the tumors' evolutionary trees. In contrast to prior methods, this quantitative approach to tumor evolution may allow correlation of intra-clonal genetic diversity with natural history, response to immune-chemotherapy and risk of transformation to diffuse large cell lymphoma.

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GENOME-WIDE MAPPING OF TRANSCRIPTION FACTOR BINDING SITES IN ACTIVE CHROMATIN IDENTITIES DEREGULATED IRF ACTIVITY AS MASTER REGULATOR OF HODGKIN LYMPHOMA-SPECIFIC GENE EXPRESSION

S. Kreher,[1] P. Chaudry,[2] A. Bouhlel,[2] S. Li,[3] B. Lamprecht,[1] I. Anagnostopoulos,[4] S. Wenzel,[1] G. Lenz,[1] M. Janz,[1] P. Cockerill,[2] B. Doerken,[1] C. Bonifer,[2] S. Mathas.[1]
[1]Haematology/Oncology, Charite - Universitaetsmedizin Berlin, Berlin, Germany; [2]Experimental Haematology, University of Birmingham, Birmingham, UK; [3]Hematology/Oncology, Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany; [4]Pathology, Charite - Universitaetsmedizin Berlin, Berlin, Germany.

Classical Hodgkin lymphoma (cHL) is a common human lymphoma characterized by the malignant Hodgkin–/Reed–Sternberg (HRS) cells, which usually represent only a small percentage of the cellular mass in the affected lymph nodes. A key molecular defect of HRS cells is the constitutive activation of various transcription factors (TFs) known to be only transiently activated in other cellular systems. Furthermore, cHL constitutes the most prominent example for lineage infidelity: although originating from B cells, the HRS cells have almost completely lost their B cell-specific gene expression programme and acquired expression of genes characteristic for other haematopoietic lineages. In order to extend our current model of deregulated TF activities in cHL and to examine how the lineage-specific TF network is disturbed in cHL, we performed a genome-wide analysis of accessible chromatin regions in cHL as well as non-Hodgkin lymphoma (NHL) cell lines. By combining these data with gene expression profiles, we identified *cis*-regulatory regions specifically active in HRS cells. An unbiased search for enriched TF binding motifs within such regions followed by functional studies identified an as yet unknown deregulation of interferon regulatory factors (IRFs) in HRS cells. We demonstrate that experimental deregulation of IRF activity in NHL cells induces gene expression changes reminiscent of cHL, including the upregulation of a whole set of cytokines, the TNFR family member CD30 and the TF AP-1 components JUN, JUNB and ATF3. Moreover, IRF modulation led to a shutdown of the B-cell programme and repression of the epigenetic regulator CBFA2T3, pointing to deregulated IRF activity as a key defect of HRS cell biology. In summary, we identified an as yet unknown key transcriptional pathway in HRS cells and described a powerful method for the identification of deregulated TF activities in human lymphoma.

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MULTIDIMENSIONAL SINGLE-CELL ANALYSIS OF B-CELL RECEPTOR SIGNALLING REVEALS PROXIMAL ACTIVATION DEFECT AS A HALLMARK OF CHRONIC LYMPHOCYTIC LEUKAEMIA B CELLS

M. Palomba,[1] K. Piersanti,[1] C. G. Ziegler,[1] H. Decker,[2] J. W. Cotari,[2] K. Bantilan,[1] I. Rijo,[1] J. R. Gardner,[1] M. Heaney,[1] D. Bemis,[2] R. Balderas,[3] S. N. Malek,[4] E. Seymour,[4] A. D. Zelenetz,[1] M. R. van den Brink,[1] G. Altan-Bonnet.[2]

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[1]Medicine, MSKCC, New York, USA; [2]Cancer Center Systems Biology, Memorial Sloan Kettering Cancer Center, New York, USA; [3]Biological Sciences, BD Biosciences, San Diego, USA; [4]Division of Hematology/Oncology, University of Michigan Health System, Ann Arbor, USA.

Purpose: Chronic lymphocytic leukaemia (CLL) is defined by a perturbed B-cell receptor (BCR)-mediated signalling machinery. We aimed to model differential signalling behaviour between B cells from CLL and healthy individuals to pinpoint modes of dysregulation.
Methods: We developed an experimental methodology combining immunophenotyping, multiplexed phosphospecific flow cytometry, and multifactorial statistical modelling. Utilizing patterns of signalling network covariance, we modelled BCR signalling in 67 CLL patients using partial least squares regression (PLSsR).
Results: Results from multidimensional modelling were validated using an independent test cohort of 38 patients.
Results: We identified a dynamic and variable imbalance between proximal (pSYK, pBTK) and distal (pPLCγ2, pBLNK, ppERK) phosphoresponses. PLSR identified the relationship between upstream tyrosine kinase SYK and its target, PLCγ2, as maximally predictive and sufficient to distinguish CLL from healthy samples, pointing to this juncture in the signalling pathway as a hallmark of CLL B cells. Specific BCR pathway signalling signatures that correlate with the disease and its degree of aggressiveness were identified. Heterogeneity in the PLSR response variable within the B-cell population is both a characteristic mark of healthy samples and predictive of disease aggressiveness.
Conclusion: Single-cell multidimensional analysis of BCR signalling permitted focused study of the variability and heterogeneity of signalling behaviour from patient to patient and from cell to cell. Disruption of the pSYK/pPLCγ2 relationship is uncovered as a robust hallmark of CLL B-cell signalling behaviour. Together, these observations implicate novel elements of the BCR signalling regime as potential therapeutic targets.

CLINICOLOPATHOLOGIC CORRELATIONS

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SELECTIVE QUANTIFICATION OF BMI-1 LEVELS IN THE NUCLEI OF FOLLICULAR LYMPHOMA CELLS SHOWS ITS ASSOCIATIONS WITH EZH2 GENE MUTATION AND REDUCED OVERALL SURVIVAL, INDEPENDENT OF FLIPI

N. I. AlJohani,[1] A. Alhejaily,[2] S.-J. Choi,[2] S. Virk,[2] T. Baetz,[3] D. P. LeBrun.[2]
[1]Hematology Department, Queen's University, Kingston, Canada; [2]Department of Pathology & Molecular Medicine, Queen's University, Kingston, Canada; [3]Medical Oncology, Queen's University, Kingston, Canada.

Introduction: Polycomb group (PcG) proteins remodel chromatin to enforce epigenetic transcriptional silencing of the CDKN2A tumour suppressor locus and genes that promote B-lymphoid specification, among other targets. High BMI-1 (PcG protein) expression has been associated with aggressive clinical behaviour in many human malignancies. Although gain-of-function mutations in the PcG gene EZH2 are common in follicular lymphoma (FL), the role of BMI-1 is unclear.
Methods: Pretreatment FL biopsy samples from 112 subjects were used for tissue microarray analysis; sections of these samples were co-immunostained for BMI-1 (detected with Cy5), CD20 (Alexa488), and DNA (DAPI) using quantitative multi-channel immunofluorescence. Automated quantitative analysis (AQUA) was then used to selectively quantify BMI-1 in the nuclei of CD20-expressing FL cells. EZH2 mutation status was determined using MassARRAY. Kaplan–Meier analysis was used for the relationship between BMI-1 abundance and patient survival and one-way ANOVA for associations with other clinical and pathological features.
Results: BMI-1 abundance determined by IF/AQUA analysis in 10 human cell lines correlated well with quantitative immunoblotting results ($r^2 = 0.7$), thus validating IF/AQUA as a quantitative assay. Dividing the cases into tertiles according to BMI-1 expression showed an association between BMI-1 expression and reduced overall survival (OS, $p = 0.02$). The association between BMI-1 expression and OS remained significant after accounting for the FLIPI score in a Cox regression model ($p = 0.001$). Greater BMI-1 abundance was associated with absence of EZH2 mutations ($p = 0.035$) and a histological grade of 1 or 2 ($p = 0.016$).
Conclusion: BMI-1-mediated gene silencing may contribute to relatively aggressive clinical behaviour in FL; BMI-1 can be considered a candidate biomarker that could help identify patient subgroups who might benefit from tailored clinical management; for example, post-translational modifications of chromatin by PcG proteins could eventually be susceptible to pharmacological intervention.

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MUM-1 IS A STRONG PROGNOSTIC IMMUNOHISTOCHEMICAL MARKER IN FOLLICULAR LYMPHOMA: COMPUTERIZED IMAGE ANALYSIS OF THE FL-2000 AND PRIMA RANDOMIZED GELA TRIALS

L. Xerri,[1] E. Bachy,[2] B. Fabiani,[3] D. Canioni,[4] C. Chassagne-Clement,[5] P. Dartigues,[6] F. Charlotte,[7] M. Rousselet,[8] B. Chetaille,[1] C. Foussard,[9] P. Brice,[10] P. Feugier,[11] F. Morschhauser,[12] A. Sonnet,[13] D. Olive,[14] N. Brousse,[4] G. Salles.[15]
[1]Bio-Pathology, Institut Paoli-Calmette, Marseille, France; [2]Hematology, CHU Pierre-Benite, Lyon, France; [3]Pathology, CHU Saint-Antoine, Paris, France; [4]Pathology, CHU Necker, Paris, France; [5]Pathology, Centre Leon Berard, Lyon, France; [6]Pathology, IGR, Villejuif, France; [7]Pathology, CHU Pitie-Salpetriere, Paris, France; [8]Pathology, CHU, Angers, France; [9]Hematology, CHU, Angers, France; [10]Hematology, CHU Saint-Louis, Paris, France; [11]Hematology, CHU, Nancy, France; [12]Hematology, CHU, Lille, France; [13]Hematology, Universite Catholic de Louvain, Louvain, Belgium; [14]Immunology, Institut Paoli-Calmettes, Marseille, France; [15]Hematology, CHU Lyon-Sud, Lyon, France.

The search for prognostic factors using immunohistochemistry (IHC) in follicular lymphoma (FL) has led to numerous discrepancies, because of the heterogeneity of treatments or the lack of standardized scoring method. To establish whether MUM1 and Ki67 can be used as predictive markers, we analyzed their IHC expression in two randomized trials using a computerized scoring. In the FL2000 trial, 358 patients were randomized to receive either CHVP-I or R-CHVP-I. In the PRIMA study, 1019 patients were randomized to receive 2 years of rituximab maintenance after induction. Biopsy samples from 156 FL2000 patients and 411 PRIMA patients were available for IHC. Slides were stained with anti-MUM1 and anti-Ki67 antibodies. Positive cells were detected using an automated scanning microscope and computerized image analysis system. The staining specificity was validated by visual supervision and double-staining experiments. Positive cells were quantified using two different automatized scoring methods, that is, the number of positive spots (NS) and the surface of the positive staining (SS). A first analysis was performed in the PRIMA trial, considering the median of the parameter for dichotomization and cutoff. In a second step, the significant median values were applied to the FL2000 cohort for validation. All tests were two-sided, and p-values less than 0.05 were considered as statistically significant. In the PRIMA cohort, high numbers of MUM1 cells (i.e. above the median score) were significantly associated with shorter event-free survival (EFS) whatever the counting method, that is, NS ($p = 0.004$, HR = 1.58) and SS ($p = 0.002$, HR = 1.63). These values remained significant after adjustment for the FLIPI and treatment arm in a multivariate Cox proportional hazards regression model for NS ($p = 0.004$, HR = 1.58) and SS ($p = 0.009$, HR = 1.52). In contrast, Ki-67 cell counts were not correlated with outcome. In the FL2000 cohort, the MUM1 predictive value was significant using univariate analysis of the NS ($p = 0.001$, HR = 2.1 for EFS) and SS ($p = 0.005$, HR = 1.9 for EFS). These values remained significant using a multivariate Cox regression model after adjustment for the FLIPI and treatment arm regarding both the NS ($p = 0.003$, HR = 2.1) and the SS ($p = 0.016$, HR = 1.8). These results show that MUM1 is a strong and robust predictive IHC marker in FL and that it is not circumvented by anti-CD20 therapy.

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STRONG CD180 EXPRESSION: A NEW MARKER OF MARGINAL B-CELL LYMPHOMAS

L. Baseggio,[1] L. Mauvieux,[2] L. Miguet,[2] A. Traverse-Glehen,[3] F. Berger,[3] E. Callet-Bauchu,[1] D. Morel,[1] J. Magaud,[1] G. Salles.[4]
[1]Hematology, GH Lyon Sud/Hospices Civils de Lyon, Pierre Benite, France; [2]Hematology, CH Strasbourg, Starsbourg, France; [3]Pathology, GH Lyon Sud/Hospices Civils de Lyon, Pierre Benite, France; [4]Hematology Clinic, GH Lyon Sud/Hospices Civils de Lyon, Pierre Benite, France.

Introduction: Among B-cell lymphoproliferative disease (LPD), the diagnosis of marginal zone lymphoma (MZL) may be still difficult in some cases, in the absence of positive diagnostic markers, especially immunological marker. By proteomic analysis, the CD180 (close to Toll-like receptors) has been identified as a potential immunological MZL marker (Miguet, leukemia 2013).
Methods: The relevance of CD180 expression in differential diagnosis was tested on peripheral blood samples obatined from patients with chronic lymphocytic leukaemia (CLL, $n = 80$*/29**), mantle cell lymphoma (MCL, $n = 10$*/14**), MZL ($n = 25$*/14**), lymphoplasmacytic lymphoma (LPL, $n = 15$**) and splenic red pulp lymphoma with villous lymphocytes (SDRPL, $n = 8$*) from two independent patient sets using multiparametric flow cytometry approach in two different laboratories (CHU Lyon Sud*/CHU Strasbourg**).
Results: The CD180 expression was significantly higher in MZL B-cells than CLL, MCL and LPL B-cells ($p < 0.05$, Mann Whitney U-test). These other B-cell entities showed indeed a very dim or negative CD180 expression. In blood specimens, the

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CD180 expression by flow could so positively discriminate MZL from CLL, LPL and MCL with more than 86% sensitivity and specificity. Within the MZL group, splenic MZL (SMZL) cases expressed stronger the CD180 than nodal MZL cases ($p < 0.05$) and SDRPL cases expressed stronger than SMZL cases ($p < 0.05$). The CD180 expression by MZL B-cells should allow better understanding of the mechanisms involved in the SMZL and SDRPL pathogenesis, because the CD180 cross-linking induced a stronger increase of CD86 expression in MZL B-cells than in control and CLL B-cells, suggesting an active NF-#x0392;B pathway.

Conclusion: This study showed the differential CD180 expression in circulating B-cell of LPD, with strong expression restricted to MZL B-cells, in particular SMZL and SDRPL B-cells and the contribution of C180 on MZL diagnosis.

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MULTIPARAMETER FLOW CYTOMETRY FOR IDENTIFICATION OF THE WALDENSTRÖM'S CLONE IN IGM-MGUS AND WALDENSTRÖM'S MACROGLOBULINEMIA: NEW CRITERIA FOR DIFFERENTIAL DIAGNOSIS AND RISK STRATIFICATION

B. Paiva,[1] M. Montes,[1] R. Garcia-Sanz,[1] E. Ocio,[1] J. Alonso,[1] N. de las Heras,[2] F. Escalante,[2] R. Cuello,[3] A. Garcia de Coca,[3] J. Galende,[4] J. Hernandez,[5] M. Sierra,[6] A. Martin,[1] E. Pardal,[7] A. Barez,[8] J. Alonso,[9] L. Suarez,[10] T. Gonzalez-Lopez,[11] A. Orfao,[1] M. Vidriales,[1] J. San Miguel.[1]

[1]Hematology, Hospital Universitario Salamanca, Salamanca, Spain; [2]Hematology, Complejo Hospitalario de Leon, Leon, Spain; [3]Hematology, Hospital Clinico Universitario, Valladolid, Spain; [4]Hematology, Hospital Comarcal del Bierzo, Ponferrada, Spain; [5]Hematology, Hospital General de Segovia, Segovia, Spain; [6]Hematology, Hospital Virgen de la Concha, Zamora, Spain; [7]Hematology, Hospital Virgen del Puerto, Plasencia, Spain; [8]Hematology, Hospital Nuestra Senora de Sonsoles, Avila, Spain; [9]Hematology, Hospital Rio Carrion, Palencia, Spain; [10]Hematology, Hospital Santos Reyes, Aranda de Duero, Spain; [11]Hematology, Complejo Asistencial Universitario de Burgos, Burgos, Spain.

Although multiparameter-flow-cytometry (MFC) has demonstrated clinical relevance in MGUS/myeloma, immunophenotypic studies on the full spectrum of Waldenström's macroglobulinemia (WM) remain scanty. Herein, a comprehensive MFC analysis on BM samples from 244 newly diagnosed patients with an IgM monoclonal protein was performed, including 67 IgM-MGUS, 77 smouldering and 100 symptomatic WM. Our results show a progressive increase on the number and light-chain restriction of B-cells from IgM-MGUS to smouldering and symptomatic WM ($p < 0.001$), with only 1% of IgM-MGUS patients showing >10% B-cells or 100% light-chain restricted B-cells ($p < 0.001$). Complete light-chain restriction of the B-cell compartment was an independent prognostic factor for time-to-progression in smouldering WM (median 26 months; HR: 19.8, $p = 0.001$) and overall survival in symptomatic WM (median 44 months; HR: 2.6, $p = 0.004$). The progressive accumulation of light-chain restricted B-cells accompanied the emergence of a characteristic Waldenström's phenotype ($CD22^{dim}$/CD25/CD27/IgM) that differed from other B-NHL by negative expression of CD5, CD10, CD11c or CD103. In contrast to myeloma, light-chain restricted plasma cells in IgM monoclonal gammopathies show otherwise normal antigenic expression. Our results highlight the potential value of MFC immunophenotyping for the characterization of the Waldenström's clone, as well as for the differential diagnosis, risk of progression and survival in WM. We are currently working with eight-colour MFC, and preliminary results will be presented.

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CD70+ T CELLS ARE INDICATIVE OF TGF-β-MEDIATED IMMUNE SUPPRESSION AND PREDICT A POOR OUTCOME IN FOLLICULAR LYMPHOMA PATIENTS

Z. Yang, D. Grote, B. Xiu, S. Ziesmer, T. Price-Troska, L. Hodge, T. Witzig, A. Novak, S. Ansell.

[1]Hematology, Mayo Clinic, Rochester, USA.

Introduction: TGF-β is known to be responsible for immune suppression and plays an important role in suppressing immune effector cells in B-cell lymphomas. We found that T cells treated with TGF-β significantly upregulated CD70. The aim of this study was to determine whether CD70 expression on T cells identified cells immunologically suppressed by TGF-β and whether increased numbers of these cells were associated with a poor prognosis in follicular lymphoma.

Methods: CD70 expression on T cells was determined by flow cytometry or fluorescent microscopy. Cytokine production by T cells was measured by intracellular staining. Progression-free survival of lymphoma patients was estimated using the Kaplan–Meier method. The univariate associations between individual CD70 expression and survival were determined with the log-rank test.

Results: We examined the effect of TGF-β on the phenotype and function of T cells in follicular B-cell lymphoma and found that CD70 was significantly upregulated by TGF-β. This led us to explore the underlying mechanism by which TGF-β upregulates CD70 expression on T cells and to define the function of CD70 cells in follicular lymphoma. We found that TGF-β differentially exerts its effects on naive and memory T cells by inducing CD70 expression on effector memory T cells and upregulating Foxp3 in naive T cells. CD70 induction is Smad3 dependent and involves Stat5 signalling. CD70 T cells are highly susceptible to TGF-β-mediated apoptosis and have diminished responses to immune stimulation with downregulation of costimulatory molecules CD27 and CD28. In follicular lymphoma specimens, CD70 was expressed on a subset of T cells, and these CD70 T cells failed to produce cytokines and displayed no signal transduction when stimulated. Clinically, increased numbers of CD70 cells in diagnostic biopsy specimens from follicular lymphoma patients correlated with an inferior progression-free survival ($p = 0.029$).

Conclusions: The data we present in this study not only reveal a novel mechanism by which the expression of CD70 is regulated but also identify CD70 as a prognostic factor in lymphoma and as a marker of TGF-β-mediated T-cell suppression. CD70 expression may therefore be useful as a biomarker in clinical trials using biologic agents in patients with B-cell lymphoma.

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MYC TRANSLOCATION PARTNER GENE IS A PREDICTIVE FACTOR OF SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMAS IRRESPECTIVE OF SINGLE OR DOUBLE HIT MYC GENE ALTERATIONS: A LYSA STUDY

C. Copie Bergman,[1] P. Cuillere Dartigues,[2] M. Baia,[1] J. Briere,[3] D. Canioni,[4] M. Parrens,[5] B. Fabiani,[6] R. Delarue,[7] T. Petrella,[8] G. Salles,[9] K. Belhadj,[10] C. Recher,[11] N. Ketterer,[12] C. Haioun,[13] F. Jardin,[14] K. Leroy,[1] J. Jais,[15] H. Tilly,[14] P. Gaulard,[1] T. Molina.[4]

[1]Departement de Pathologie, Hopital Henri Mondor, Creteil, France; [2]Departement de Pathologie, Institut Gustave Roussy, Villejuif, France; [3]Departement de Pathologie, Hopital Saint Louis, Paris, France; [4]Departement de Pathologie, Hopital Necker, Paris, France; [5]Departement de Pathologie, CHU Bordeaux, Bordeaux, France; [6]Departement de Pathologie, Hopital Saint Antoine, Paris, France; [7]Hematology Department, Hopital Necker, Paris, France; [8]Departement de Pathologie, CHU Dijon, Dijon, France; [9]Hematology department, CHLS, Lyon, France; [10]Hematology Department, Hopital Henri Mondor, Creteil, France; [11]Hematology Department, CHU Toulouse, Toulouse, France; [12]Hematology Department, University Hospital-CHUV, Lausanne, Switzerland; [13]Hematology Department, Hopital Henri Mondor, Creteil, France; [14]Hematology Department, Centre Henri Becquerel, Rouen, France; [15]Biostatistics, Hopital Necker, Paris, France.

Introduction: Diffuse large B-cell lymphomas (DLBCL) associated with *MYC* gene translocation are considered to have a poor outcome as compared with *MYC* translocation negative DLBCL. The aim of the study was to analyze the prognostic relevance of*MYC*, *BCL2* and *BCL6* rearrangements and the impact of *MYC*partner gene in a large series of DLBCL.

Methods: Patients with *de novo* CD20+ DLBCL enrolled in the randomized LNH-03 and LNH-01-5B GELA trials and treated with rituximab-based and anthracyclin-based chemotherapy (R-CHOP/R-miniCHO $p = 482$ or R-ACVB $p = 292$) were studied on tissue microarrays. Tumour samples were centrally reviewed and analyzed by fluorescence *in situ* hybridization using breakapart probes for *BCL2*, *BCL6*, *MYC*, *IGK*, *IGL* and fusion probe for *IGH/MYC*.

Results: Five hundred seventy-four DLBCL were evaluable for *MYC* rearrangement. Rearrangement of *MYC*, *BCL2* and *BCL6* genes were observed in 8.8% (51/574), 15.9% (82/515) and 23.8% (129/541) of DLBCL, respectively. *MYC* rearranged cases (*MYC-R*) included 19 *MYC*single hit (*MYC-SH*) and 32 *MYC* double hit (or triple hit) with concurrent *BCL2* and/or *BCL6* rearrangement (*MYC-DH*). Twenty eight *MYC-R* cases showed *IGH* or *IGK*or *IGL* partner gene (*MYC-IG*), whereas 23 *MYC-R* cases had a non immunoglobulin partner gene (*MYC-nonIG*). *MYC* rearrangement was associated with a shorter 5-year overall survival (OS) rate (56.5% vs 73.7%, $p = 0.007$), which remained significant in *MYC-SH* ($p = 0.04$) but not in *MYC-DH* DLBCL ($p = 0.06$). *MYC-IG* DLBCL, but not *MYC-non-IG* DLBCL, had a significant shorter progression-free survival ($p = 0.02$) and OS ($p = 0.001$) as compared with *MYC* translocation negative DLBCL. This adverse prognostic effect of *MYC-IG* on OS was maintained in *MYC-SH* ($p = 0.030$) and *MYC-DH* ($p = 0.036$) DLBCL. Multivariate analysis showed that in addition to international prognostic index, *MYC-IG* predicted a poor OS in *MYC-R* and *MYC-SH* DLBCL, but not in *MYC-DH*DLBCL.

Conclusion: Our study shows that *MYC* translocation is an adverse prognostic factor in DLBCL. It is strongly correlated to *IG* or non-*IG* translocation partner genes, suggesting that analysis of these partner genes should now be considered in DLBCL patients.

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OVEREXPRESSION OF MYC, BCL2, MYC/BCL2, IGM, AND NON-GERMINAL CENTRE B CELL-LIKE IMMUNOPHENOTYPE PREDICTS A WORSE PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN A SERIES OF 670 *DE NOVO* DIFFUSE LARGE B-CELL LYMPHOMAS: S LYSA STUDY

T. J. Molina,[1] J. Briere,[2] C. Copie-Bergman,[3] T. Petrella,[4] D. Canioni,[5] R. Delarue,[6] B. Fabiani,[7] F. Peyrade,[8] M. Parrens,[9] C. Recher,[10] J. Emile,[11] P. Cuillere-Dartigues,[12] K. Belhadj,[13] P. Ruminy,[14] C. Haioun,[13] K. Leroy,[3] G. Salles,[15] J. Jais,[16] H. Tilly,[14] F. Jardin,[14] P. Gaulard.[3]

[1]Pathology, Paris Descartes University, AP-HP, LYSA, Paris, France; [2]Pathology, AP-HP, Saint-Louis, Paris, France; [3]Pathology, AP-HP, Mondor, Creteil, France; [4]Pathology, CHU Dijon, Dijon, France; [5]Pathology, AP-HP, Necker, Paris, France; [6]Hematology, AP-HP, Necker, Paris, France; [7]Pathology, AP-HP, Saint Antoine, Paris, France; [8]Oncology, Centre Antoine Lacassagne, Nice, France; [9]Pathology, CHU Bordeaux, Bordeaux, France; [10]Hematology, CHU Toulouse, Toulouse, France; [11]Pathology, AP-HP, Ambroise Pare, Boulogne, France; [12]Pathology, Institut Gustave Roussy, Villejuif, France; [13]Hematology, AP-HP, Mondor, Creteil, France; [14]Hematology, Centre Henri Becquerel, Rouen, France; [15]Hematology, CHU Lyon, Lyon, France; [16]Statistics, AP-HP, Necker, Paris, France.

Introduction: Diffuse large B-cell lymphomas (DLBCL) represent a heterogeneous disease with variable clinical outcome. Our aim was to analyze by immunohistochemistry the prognostic relevance of the expression of MYC, BCL2, MYC/BCL2, IgM, and GCB/non-GCB subtype in a large series of DLBCL treated with rituximab (R) chemotherapy (anthracyclin based).

Methods: The 2003 programme included patients with *de novo* CD20+ DLBCL enrolled in six different LNH-03 GELA trials stratifying patients according to age and age-adjusted international prognostic index (IPI). Tumour samples were analyzed by immunohistochemistry using CD10, BCL6, MUM1, MYC (40% threshold), BCL2 (70% threshold), and IgM antibodies on tissue microarrays. Six hundred seventy patients were included in the study with 237 receiving intensive R-ACVBP regimen and 433 R-CHOP/R-mini-CHOP.

Results: Three hundred four (45.4%) DLBCL were classified as GCB and 366 (54.6%) as non-GCB according to Hans algorithm. IgM was recorded as positive in tumour cells in 52.4% of the cases. MYC was positive in 170/577 (29.5%) cases. BCL2 was positive in 356/644 (55.3%) cases and coexpression BCL2/MYC in 116/557 (21%) cases. Progression-free survival (PFS) was significantly worse among patients with high IPI score ($p < 0.0001$), non-GCB DLBCL subtype ($p < 0.0001$), and IgM positive ($p < 0.0001$), MYC positive ($p < 0.001$), BCL2 positive ($p < 0.001$), and MYC/BCL2 positive tumours ($p = 0.003$). Overall survival (OS) was also significantly worse among patients with high IPI score ($p < 0.0001$), non-GCB DLBCL subtype ($p < 0.0001$), and IgM positive ($p = 0.02$), MYC positive ($p < 0.01$), BCL2 positive ($p < 0.001$), and MYC/BCL2 positive ($p = 0.005$) tumours. Multivariate analyses using Cox Model showed that in addition to IPI, only BCL2 overexpression and the non-GCB subtype predicted significantly a worse PFS ($p = 0.0002$ and $p = 0.002$, respectively) as well as a worse OS ($p = 0.03$ and $p = 0.002$ respectively). This strong prognostic value of BCL2 and non-GCB subtyping was confirmed considering only patients treated with R-CHOP for PFS ($p = 0.002$ and $p = 0.002$, respectively) and for OS ($p = 0.02$ and $p = 0.002$, respectively).

Conclusion: Our study confirmed the relevance of immunohistochemistry to identify significant prognostic biomarkers for clinical use. Above all, we fully validated the strong and independent prognostic value of the Hans algorithm, daily used by the pathologists to subtype DLBCL as well as BCL2 overexpression.

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CLINICAL RELEVANCE OF MYC, BCL2 AND BCL6 REARRANGEMENTS IN DIFFUSE LARGE B-CELL LYMPHOMA

G. C. Caponetti,[1] B. J. Dave,[2] L. Smith,[3] M. Bast,[4] P. Meyer,[5] P. Bierman,[4] G. Bociek,[4] J. Vose,[4] J. Armitage,[4] K. Fu,[6] P. Aoun,[6] T. Greiner,[6] J. Chan,[6] D. Weisenburger[7].

[1]Department of Pathology, Creighton University Medical Center - Alegent Creighton Health, Omaha, NE, USA; [2]Human Genetics Laboratory, University of Nebraska Medical Center, Munroe-Meyer Institute, Omaha, NE, USA; [3]Department of Biostatistics, University of Nebraska Medical Center, Omaha, NE, USA; [4]Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA; [5]Department of Pathology, Southern Arizona VA Health Care System, Tucson, AZ, USA; [6]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA; [7]Department of Pathology, City of Hope National Medical Center, Duarte, CA, USA.

Introduction: The highly-variable clinical outcome seen in patients with diffuse large B-cell lymphoma (DLBCL) may be due, at least in part, to various genetic abnormalities. BCL2 and BCL6 are often rearranged in DLBCL, and about 10% of cases have rearrangement ofMYC. These oncogenes play an important pathogenic role in DLBCL, and a simultaneous rearrangement of MYC with BCL2 and/or BCL6 (double hit) may have a synergistic effect leading to poor survival. To test this hypothesis, we performed a retrospective analysis of DLBCL cases tested for translocations involving MYC, BCL2 and BCL6, and treated with CHOP-like or R-CHOP-like therapies.

Methods: We searched the database of the Nebraska Lymphoma Study Group for cases of *de novo* DLBCL with available clinical data, patient consent, no prior treatment and available conventional cytogenetics (CC) and/or fluorescence *in situ* hybridization (FISH) results. Patients with a history of HIV infection or organ transplantation were excluded. CC and/or FISH reports were reviewed to identify rearrangements of MYC, BCL2 and BCL6. Cases were classified into three groups according to the rearrangement status of these three genes: (ii) MYC− (with or without BCL2 or BCL6 rearrangement); (ii) MYC/BCL2−/BCL6− or BCL6 unknown; and (iii) MYC/BCL2 or BCL6. The 5-year overall survival (OS) and event-free survival (EFS) of the three groups were then compared.

Results: We identified 208 cases of DLBCL that met the study criteria, with a male to female ratio of 1:1 and a median age of 67 years at diagnosis (range, 20–90 years). On the basis of the rearrangement status of the three genes, 178 cases (85%) that were classified as MYC− had a 5-year OS of 47% and EFS of 38%; 14 cases (7%) that were MYC/BCL2−/BCL6− or BCL6 unknown showed a 5-year OS of 71% and EFS of 64%; and 16 cases (8%) with a double hit (MYC /BCL2 or BCL6) had a 5-year OS of 31% and EFS of only 12%. No significant differences in clinical characteristics were identified among these groups. However, a decreased complete remission rate ($p = 0.14$) and worse OS ($p = 0.11$) and EFS ($p = 0.029$) were identified among double hit cases with MYC/BCL2 or BCL6.

Conclusions: MYC DLBCL cases without BCL2 or BCL6 rearrangements do not have a worse survival when compared with cases without MYC rearrangement. However, MYC DLBCL cases with either BCL2 or BCL6 rearrangements have a poor clinical outcome and should be treated with aggressive or novel therapies.

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PROGNOSTIC SIGNIFICANCE AND PHENOTYPIC MANIFISTATIONS OF MYC/BCL2 PTOTEIN CO-EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WITH EXTRANODAL ORGAN INVOLVEMENT: A REPORT OF THE INTERNATIONAL DLBCL RITUXIMAB-CHOP CONSORTIUM PROGRAM STUDY

A. R. Madadi,[1] M. Raghavendra,[1] A. Tzankov,[2] C. Visco,[3] S. M. Moreno,[4] K. Dybkar,[5] A. Chiu,[6] A. Orazi,[7] G. Bhagat,[8] E. D. Hsi,[9] J. V. Krieken,[10] M. Ponzoni,[11] A. J. Ferreri,[11] J. N. Winter,[12] M. A. Piris,[4] M. B. Moller,[13] . Medeiros,[14] R. S. Go,[1] K. H. Young.[14]

[1]Department of Hematology, Center for Cancer and Blood Disorders, La Crosse, USA; [2]Department of Pathology, University Hospital, Basel, Switzerland; [3]Department of Hematology, San Bortolo Hospital, Vicenza, Italy; [4]Department of Pathology, Hospital Universitario Marques de Valdecilla, Santander, Spain; [5]Department of Hematology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [6]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA; [7]Department of Pathology, Weill Medical College of Cornell University, New York, NY, USA; [8]Department of Pathology, Columbia University Medical Center and New York Presbyterian Hospital, New York, NY, USA; [9]Department of Pathology, Cleveland Clinic, Cleveland, USA; [10]Department of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [11]Department of Hematology, San Raffaele H. Scientific Institute, Milan, Italy; [12]Department of Hematology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [13]Department of Pathology, Odense University Hospital, Odense, Denmark; [14]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Introduction: In diffuse large B-cell lymphoma (DLBCL), multiple extranodal sites of involvement and MYC/BCL2 translocations (double-hit lymphoma) are associated with a poor clinical outcome. The association between the pattern of extranodal involvement and MYC/BCL2 protein co-expression, as well as their prognostic significance, is unknown.

Methods: We analyzed the clinical data of 487 DLBCL patients treated with R-CHOP. Immunohistochemical (IHC) studies were performed for MYC and BCL2. A double-hit score (DHS) was assigned to all patients based on expression of MYC and BCL2. Those with both MYC and BCL2 were DHS 2, with MYC or BCL2 was DHS 1, and neither was DHS 0. Cell-of-origin (COO) classification was achieved by combining gene expression profiling (GEP) and IHC data with GEP as the gold standard.

Results: Approximately half of the patients ($n = 251$; 51.5%) had at least one extranodal site of involvement. In this group, the clinical features were median age of 63 years (range, 12–88), male (58.6%), stage III/IV (63.2%), elevated serum LDH (66.8%), and international prognostic index (IPI) of 3–5 (48.5%). IHC features were MYC (64.9%), BCL2 (49.8%), MYC−/BCL2− (DHS 0; 20.3%), MYC/BCL2− or MYC−/BCL2

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(DHS 1; 44.6%), and MYC/BCL2 (DHS 2; 35.1%).The common extranodal sites of involvement were genitourinary, gastrointestinal, and sinonasal tracts. MYC expression was associated with bone marrow ($p = 0.01$) and skin ($p = 0.04$) involvement, BCL2 with sinonasal ($p = 0.03$) involvement, and MYC/BCL2 with skin/soft tissue ($p = 0.04$) and lung ($p = 0.04$) involvement. Non-germinal centre B-cell like (GCB) subtype was associated with genitourinary tract ($p < 0.01$) and bone marrow involvement ($p = 0.03$). The DHS 2 subgroup was significantly associated with lower complete response rate (62.5% vs 76.1%; $p = 0.02$), shorter progression-free survival (PFS) (median 23.1 vs 80.7 months; $p < 0.001$), and overall survival (OS) (median 25.0 vs 94.5 months; $p < 0.001$) compared with the DHS 0–1 subgroups. By using multivariable analysis, DHS 2 was independently associated with a worse outcome.

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	OS		PFS	
Variable	**HR**	***p***	**HR**	***p***
IPI (3–5 vs 0–2)	1.5	0.01	1.43	0.01
COO (non-GCB vs GCB)	1.0	0.81	1.07	0.70
DHS (2 vs 0–1)	2.9	<0.001	2.7	<0.001

Conclusions: In DLBCL with extranodal disease, MYC/BCL2 co-expression and COO are associated with distinct patterns of organ involvement. Patients with DLBCL with MYC and DHS of 2 (double hit biology) have a poor outcome independent of IPI and COO.

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MICRORNAS OF THE MIR-17–92 CLUSTER PREDICT FOR OUTCOME IN R-CHOP-TREATED DIFFUSE LARGE B-CELL LYMPHOMA AND ARE SIGNIFICANTLY ASSOCIATED WITH EXPRESSION OF MYC PROTEIN

R. E. Culpin,[1] M. Sieniawski,[1] J. J. Anderson,[1] B. Angus,[2] S. J. Proctor,[1] S. T. Kometa,[3] K. Pearce,[4] G. K. Menon,[2] K. McCabe,[1] P. Milne,[1] S. Crosier,[1] T. Mainou-Fowler.[1]

[1]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK; [2]Cellular Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, UK; [3]Information Systems and Services, Newcastle University, Newcastle upon Tyne, UK; [4]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.

Introduction: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease, and current prognostic indicators are not reliably applicable to the clinic or unambiguously predictive. MicroRNAs can be assessed using robust technology, and in B-cell lymphoma, the miR-17–92 cluster is upregulated. Myc has been shown to cooperate with miR-17–92 in a mouse model to enhance lymphomagenesis, by binding upstream of the cluster to upregulate its expression.
Methods: Mature miR-17–92 microRNA expression was assessed in 118 R-CHOP-treated DLBCL patients using qRT-PCR and related to disease outcome and clinical factors. Myc protein and gene expression was also assessed using immunohistochemistry and qRT-PCR, respectively, and related to outcome and expression of miR-17–92.
Results: The cohort included a range of ages (21–91, mean 67 years), stages and international prognostic index (IPI) groups (low 40%, low-intermediate 24%, high-intermediate 27% and high 10%). Mean overall survival (OS) was 64% (44.9 months), and progression-free survival (PFS) was 58.5% (41.7 months). High expression of miR-17-5p ($p = 0.001$), miR-18 ($p = 0.045$), miR-19a ($p = 0.009$), miR-20 ($p = 0.007$) or miR-92 ($p = 0.045$) predicted for poor OS and high miR-17-5p ($p = 0.005$) or miR-20 ($p = 0.033$) for shorter PFS. In multivariate analysis, miR-17-5p, albumin and IPI were independent factors for OS ($p = 0.001$, $p = 0.002$ and $p = 0.003$, respectively), and miR-17-5p, urea and IPI were independent for PFS ($p = 0.004$, $p = 0.031$ and $p = 0.001$, respectively). Patients with late stage disease and high miR-17-5p had a poorer OS ($p = 0.002$) and PFS ($p = 0.035$). High miR-92 expression identified patients with early stage and shorter OS ($p = 0.025$). Expression of miRs-17-3p ($p = 0.023$), -18 ($p = 0.007$), -19a ($p = 0.033$), -19b ($p = 0.027$) and -20 (0.007) significantly positively correlated with Myc protein expression, but there was no association between miR-17–92 and Myc gene expression (>0.05). Myc protein or gene expression alone did not predict for outcome ($p > 0.05$).
Conclusions MiR-17–92 microRNAs, and in particular miR-17-5p, may play an important role as new prognostic markers in DLBCL. We have shown in diagnostic samples a significant correlation between expression of miR-17–92 and Myc protein, suggesting these factors may act in synergy in clinical samples to drive aggressive lymphoma.

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GENETIC ABNORMALITIES IN B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA (B-UCL): THE NEBRASKA EXPERIENCE

A. Perry,[1] D. Crockett,[2] B. J. Dave,[3] P. Althof,[3] L. Winkler,[3] L. M. Smith,[4] P. Aoun,[5] W. C. Chan,[6] K. Fu,[6] T. C. Greiner,[6] P. J. Bierman,[2] R. G. Bociek,[2] J. M. Vose,[2] J. O. Armitage,[2] D. D. Weisenburger.[5]

[1]Department of Pathology, Division of Hematopathology, University of Manitoba, Winnipeg, Canada; [2]Department of Internal Medicine, University of Nebraska, Omaha, USA; [3]Human Genetics Laboratory, Munroe-Meyer Institute, University of Nebraska, Omaha, USA; [4]Department of Biostatistics, University of Nebraska, Omaha, USA; [5]Department of Pathology, City of Hope National Medical Center, Duarte, USA; [6]Department of Pathology, University of Nebraska, Omaha, USA.

Introduction: B-UCL is a new category in the 2008 WHO Classification, but few studies have looked systematically at the cytogenetic abnormalities in this lymphoma. Therefore, we investigated the cases of B-UCL treated by the Nebraska Lymphoma Study Group (NLSG).
Materials and Methods: We searched the NLSG registry for years 1985–2010 for cases of B-UCL and identified 39 cases. We performed immunohistochemical stains, and conventional and fluorescence *in situ* hybridization (FISH) cytogenetic studies for rearrangements of BCL2, BCL6 and CMYC genes. We also performed survival analysis to see if any of these parameters predicted survival.
Results: Among the 39 patients, 21 (54%) were male and 18 (46%) were female, with a median age of 69 years. The majority of patients (62%) presented with advanced-stage (III/IV) disease and high international prognostic index scores (3–5; 54%). The median event-free survival (EFS) was 4.8 months, and the 5-year EFS was only 23%. The median overall survival (OS) was 9 months, and the 5-year OS was only 30%. FISH studies detected genetic abnormalities in 80% of the 35 cases tested including 11 (32%) 'double hit' lymphomas; eight had rearrangement of CMYC and BCL2, and three had rearrangement of CMYC and BCL6. Six cases (17%) had only a CMYC rearrangement, five (14%) had only a BCL2 rearrangement and one (3%) had only a BCL6 rearrangement. Among the 17 cases with CMYC rearrangement, CMYC was rearranged with IGH (14q32) in 12 cases. Furthermore, two cases had rearrangement of IGL, and one case had rearrangement of IGK, suggesting that CMYC was rearranged with these genes. Fourteen cases had copy number changes of CMYC, BCL2 or BCL6 in addition to gene rearrangements or as the sole abnormality. By immunohistochemistry, most of the cases (79%) strongly expressed BCL2 protein, but only 44% of these had a BCL2 gene rearrangement. Inversely, of 13 cases with a BCL2gene rearrangement, 12 (92%) expressed BCL2 protein. The median expression of CMYC protein in these cases was 50% of the cells, and of the cases with CMYC rearrangement, 69% had CMYC protein expression in ≥50% of the cells. However, none of the immunohistochemical or genetic findings was predictive of survival.
Conclusion. B-UCL is an aggressive lymphoma with heterogeneous genetic features. Novel drugs and new targeted therapies should be explored in these patients.

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HOST IMMUNE GENE POLYMORPHISMS AND OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED BY IMMUNOCHEMO-THERAPY: RESULTS FROM A META-ANALYSIS BASED ON 1134 PATIENTS FROM TWO INDEPENDENT PROSPECTIVE COHORTS

H. Ghesquieres,[1] B. R. Larrabee,[2] C. Haioun,[3] B. K. Link,[4] A. Verney,[5] S. L. Slager,[2] N. Ketterer,[6] S. M. Ansell,[7] R. Delarue,[8] M. J. Maurer,[2] O. Fitoussi,[9] T. M. Habermann,[7] F. Peyrade,[10] A. Dogan,[11] T. J. Molina,[12] A. J. Novak,[2] H. Tilly,[13] J. R. Cerhan,[2] G. Salles.[14]

[1]Onco-Hematology, UMR5239 CNRS, Centre Leon Berard, Universite Claude Bernard, Lyon, France; [2]Health Sciences Research, Mayo Clinic, Rochester, MN, USA; [3]Unite Hemopathies Lymphoides, Hopital Henri Mondor, Creteil, France; [4]Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA, USA; [5]UMR5239 CNRS, Universite Claude Bernard, Lyon, France; [6]Oncology, University Hospital Lausanne, Lausanne, Switzerland; [7]Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA; [8]Hematology, Hopital Necker, Paris, France; [9]Onco-Hematology, Polyclinique Bordeaux Nord, Bordeaux, France; [10]Medical Oncology, Centre Antoine Lacassagne, Nice, France; [11]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; [12]Pathology, Descartes University, Paris, France; [13]Hematology, Centre Henri Becquerel, Rouen, France; [14]Hematology, UMR5239 CNRS, Hospices Civils, Universite Claude Bernard, Lyon, France.

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Introduction: Some immune single nucleotide polymorphisms (SNPs) were associated with diffuse large B-cell lymphoma (DLBCL) susceptibility and possibly with DLBCL patient's outcome in some retrospective series mostly conducted before rituximab era.
Methods: We investigated whether SNPs in LTA (rs909253), TNFA (rs1800629), IL10 (rs1800890, rs1800896, rs1800871 and rs1800872), IL1A (rs1800587), IL4R (rs2107356), IL8RB (rs1126580) and BAFF (rs1224141, rs12583006 and rs12428930) were associated with progression-free survival (PFS) and overall survival (OS) in two DLBCL cohorts treated with immunochemotherapy from the Groupe d'Etude des Lymphomes de l'Adulte (GELA) LNH03B trials ($N = 554$) and Iowa/Mayo Specialized Program Of Research Excellence (SPORE) ($N = 580$). In order to increase power, the results of the two series were combined in a meta-analysis ($N = 1134$).
Results: In GELA, the median age was 61 years (range, 18–93) with 50% of age-adjusted international prognostic index (aaIPI) 2–3. In the SPORE, the median age was 62 years (range, 18–92) with 41% of aaIPI 2–3. With a median follow-up of 39 and 59 months, the 3-year PFS rates were 69.3% and 66.5% and the 3-year OS rates were 75.3% and 79.8% in the GELA and SPORE cohorts, respectively. In the meta-analysis after age and aaIPI adjustment, IL10-3575T>A (rs1800890) was associated with PFS ($HR_{AA} = 1.43$; 95% CI, 1.10–1.85; $p = 0.01$) and OS ($HR_{AA} = 1.48$; 95% CI, 1.09–2.00; $p = 0.01$); IL10-1082A>G (rs1800896) was also associated with PFS ($HR_{GG} = 1.26$; 95% CI, 1.01–1.58; $p = 0.04$) with a trend for OS ($HR_{GG} = 1.29$; 95% CI, 1.00–1.68; $p = 0.05$). Finally, the meta-analysis showed that the subset of patients ($N = 168$, 15%) who carried the two deleterious genotypes IL10-3575AA and IL10-1082GG had an increased risk of relapse (HR = 1.43; 95% CI, 1.10–1.85; $p = 0.01$) and death (HR = 1.48; 95% CI, 1.09–2.00; $p = 0.01$).
Conclusions: The results of this meta-analysis based on 1134 patients showed that IL10-3575 and IL10-1082 SNPs were associated with DLBCL patient's outcome treated by immunochemotherapy.

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CLINICOPATHOLOGIC AND HISTOLOGIC CHARACTERISTICS OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA: ANALYSIS OF 231 PATIENTS OF THE TENOMIC PROJECT FROM THE LYSA

F. Le Bras,[1] M. Parrens,[2] L. de Leval,[3] F. Lemonnier,[4] L. Couronne,[5] O. A. Bernard,[5] C. Bastard,[6] R. A. Cairns,[7] T. W. Mak,[7] J. Jais,[8] M. Faron,[9] B. Petit,[10] A. Martin,[11] M. Copin,[12] O. Tournillac,[13] R. Delarue,[4] R. Gressin,[14] J. Bruneau,[15] P. Gaulard,[16] C. Haioun[1].
[1]UF Lymphoide, APHP Hopital Henri Mondor et UPEC, Paris, France; [2]Departement de Pathologie, Hopital Haut-Leveque, CHU et Universite, Bordeaux, France; [3]Department of Laboratories, Institute of Pathology, C.H.U.V. Lausanne, Lausanne, Switzerland; [4]Service D'Hematologie, APHP Hopital Necker et Universite Paris 5, Paris, France; [5]U985, INSERM, Institut Gustave Roussy, Universite Paris Sud, Villejuif, France; [6]Laboratoire de Genetique Oncologique, Centre Henri-Becquerel, Rouen, France; [7]University Health Network, Campbell Family Institute for Breast Cancer Research at Princess Margaret Hospital, Toronto, Canada; [8]Service de Biostatistique, APHP Hopital Necker Universite Paris 5, Paris, France; [9]UFR de Medecine, UPMC - Universite Paris 6, Paris, France; [10]Unite Mixte De Recherche (UMR) 6101, Centre Hospitalier Universitaire (CHU) Limoges, Limoges, France; [11]Service de Pathologie, Hopital Avicenne, Universite Paris 13, Bobigny, France; [12]Pole De Pathologie, Centre de Biologie-Pathologie, CHRU De Lille, Lille, France; [13]Service De Therapie Cellulaire et d'Hematologie Clinique Adulte, Universite d'Auvergne, Inserm CIC-501, Centre Hospitalier Universitaire Clermont-Ferrand Hopital Estaing, Clermont-Ferrand, France; [14]Department of Haematology, Grenoble University Hospital, Grenoble, France; [15]Departement de Pathologie, APHP Hopital Necker et Universite Paris 5, Paris, France; [16]Departement de Pathologie, APHP Hopital Henri Mondor et UPEC, Creteil, France.

Introduction: Peripheral T cell lymphomas (PTCL) constitute a heterogeneous group of uncommon diseases. Recent data indicate that AITL is the most prevalent entity at least in some western countries. The purpose of this work was to describe the clinical, pathological and biological characteristics of AITL.
Materials and Methods: Two hundred thirty-one patients with AITL retrospectively collected in the framework of a multicentre T-cell lymphoma consortium 'TENOMIC' were histologically reviewed and investigated for expression of CD10, TFH markers, follicular dendritic cells (FDC) and EBV and for the presence of TET2 ($n = 112$) and IDH2 ($n = 86$) gene mutations. Clinical features were retrospectively collected.
Results: Of the 231 cases diagnosed with AITL, 198 had been morphologically and phenotypically reassessed, and clinical data were available for 192 patients. Median age at diagnosis was 67 years (27–88), and median follow-up was 65 months. At presentation, 97% of patients had advanced-stage disease, and B symptoms were present in 64% of the patients. Elevated LDH were observed in 72% of patients. Positive Coombs test was seen in 55% and hypergammaglobulinemia in 53% of patients. Overall, 54% of patients were treated with CHOP/CHOP-like regimens, 29% with attenuated chemotherapy and 15% with dose dense/dose intense regimens providing a 5-year overall survival of 33%, 24% and 46%, respectively. International prognostic index (IPI) score was predictive of outcome unlike more recently PTCL-specific published scores (i.e. PIT and PIAI). Furthermore, IDH2 and TET2 mutations in AITL were found in 11.5%and 49% of the patients analyzed, respectively, without prognostic impact. Finally, the level of expression of histological or phenotypic markers associated with tumour cells (CD10, TFH markers) or microenvironment (FDC, eosinophilia) did not correlate with outcome.
Conclusion: We hereby confirm the peculiar clinical and phenotypical characteristics of AITL and its poor prognosis with predictive value of IPI score. These results emphasize the need of new histologically driven targeted therapies for these patients.

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HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AT DIAGNOSIS IN PERIPHERAL T-CELL LYMPHOMA DISPLAYS A DISTINCT CLINICAL PATTERN

T. Clozel,[1] J. Depaus,[1] J. Dupuis,[1] F. Le Bras,[1] K. Belhadj,[1] C. Copie-Bergman,[2] C. Benet,[3] J. Briere,[3] H. Monjanel,[4] C. Thieblemont,[4] P. Gaulard,[2] C. Haioun[1].
[1]Hematologie, CHU Henri Mondor, Creteil, France; [2]Anatomo-Pathologie, CHU Henri Mondor, Creteil, France; [3]Anatomo-Pathologie, Hopital Saint-Louis, Paris, France; [4]Hematologie Clinique, Hopital Saint-Louis, Paris, France.

Peripheral T-cell lymphoma (PTCL) is a rare malignancy among aggressive lymphomas and represents an important therapeutic challenge because of the poor prognosis with 5-year overall survival (OS) rates around 30%. Absence of cell lines and histological heterogeneity make the translational approach difficult so that clinical identification of subgroups of patients and subsequent prognosis factors to individualize therapies seems essential. Hence, we study here the clinical patterns of hemophagocytic lymphohistiocytosis (HLH) at diagnosis in a cohort of 186 patients with PTCL in two French haematology departments. All patients treated for a PTCL between1994 and 2012 in both centres were retrieved from the centre's databases even if the diagnosis was made in intensive care unit. Hundred and eighty six patients were thus studied; median age was 55 years (20–84) and 62% were male. Fourteen patients (7%) had HLH at diagnosis. The most frequent histopathological subtype was NOS (36% for HLH+, 35% for HLH−), and the second one was NK (28%) for patients with HLH (versus 6% for patients without) and AITL (25%) for patients without (versus 14% for patients with). All patients with HLH displayed aggressive clinical and biological features: stage III or IV disease, elevated LDH levels, an IPIaa greater than 2 and bone marrow involvement for all of them. Only two patients were EBV positive per EBER *in situ* hybridization. Thirty-six per cent of them achieved a complete remission after an anthracyclin-based treatment with or without etoposide, and the 64% others were primary refractory. With a median follow-up for alive patients of 34 months, OS at 2 years was 21% (median reached at 6.6 months). Presence of HLH at diagnosis in PTCL, however not common, is not a marginal entity in our study (7%). Such patients are rarely studied, as excluded from most clinical trials. Prognosis seems catastrophic, as more of these patients are primary refractory. Better molecular identification to understand pathways involved in these patients will help to rationalize better treatments in the future.

INDOLENT ENTITIES

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PROGNOSTIC ROLE OF HISTOLOGICAL GRADE 3A IN FOLLICULAR LYMPHOMA IN THE RITUXIMAB ERA. RESULTS OF AN F2-STUDY DATABASE

M. Bellei,[1] L. Marcheselli,[1] M. Ghielmini,[2] P. Solal-Celigny,[3] A. Lopez-Guillermo,[4] P. McLaughlin,[5] S. A. Pileri,[6] M. Cabras,[7] S. Franceschetti,[8] A. Lopez-Hernandez,[9] G. Martinelli,[10] F. Merli,[11] A. Pavlovsky,[12] A. Pulsoni,[13] L. Rigacci,[14] D. Rota Scalabrini,[15] C. Stelitano,[16] U. Vitolo,[17] J. M. Vose,[18] M. Federico[1].
[1]Department of Diagnostic, Clinic and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy; [2]Medical Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [3]ICO Rene' Gauducheau, Institut de Cancerologie de l'Ouest, Nantes, France; [4]Hematology, Hospital Clinic, Barcelona, Spain; [5]Lymphoma-Myeloma, MD Anderson Cancer Center, Houston, USA; [6]Unit of Haematopathology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy; [7]SC Ematologia e CTMO, Ospedale Oncologico Businco, Cagliari, Italy; [8]SCDU Ematologia, AOU Maggiore della Carita', Novara, Italy; [9]Hematology, Hospital Universitari Vall d'Hebron, Barcelona, Spain; [10]Division of Clinical Haemato-Oncology, European Institute of Oncology, Milano, Italy; [11]Hematology, Azienda Ospedaliera Arcispedale Santa Maria Nuova-IRCCS, Reggio Emilia, Italy; [12]Hematology, FUNDALEU, Buenos Aires, Argentina; [13]Hematology,

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Sapienza University, Roma, Italy; [14]SOD Ematologia, AOU Careggi, Firenze, Italy; [15]Divisione Universitaria di Oncologia, IRCC Fondazione del Piemonte per l'Oncologia, Candiolo (Torino), Italy; [16]Divisione di Ematologia, AO Bianchi Melacrino Morelli, Reggio Calabria, Italy; [17]Hematology 2 Section, Citta' della Salute e della Scienza Hospital, Torino, Italy; [18]Internal Medicine, University of Nebraska Medical Center, Omaha, USA.

Introduction: Some studies report that follicular lymphoma grade 3a (FL3a) clinically behaves more aggressively than grades 1 and 2 (FL1–2) and thus needs to be treated accordingly. We explored the possible prognostic role of histological grade 3a in a cohort of patients with FL who were treated in the rituximab era.
Materials and Methods: Out of the 1093 patients prospectively registered in the F2-study between January 2003 and May 2005, we selected a sample of 543 patients with FL1–3a who received a rituximab-containing regimen as first line therapy. FL grade 3b cases were advisely excluded from the population examined. Moreover, for the purpose of the analysis, both doxorubicin and mitoxantrone were considered as anthracyclines. Central pathology review was performed for the first 406 patients and then stopped because of an excellent agreement (98.3%). We analyzed the prognostic role of grade 3a in terms of progression-free survival (PFS); the analysis of the survival curves was performed by using the log-rank test and the Cox proportional hazard (PH) regression.
Results: Out of the 543 patients, 393 (72%) were classified as FL1–2 and 150 (28%) as FL3a, respectively; 112 (30%) FL1–2 and 55 (40%) FL3a patients were at high risk according to the FLIPI2 ($p = 0.1$). With respect to the chemotherapy administered, the majority of patients (451, 83%) received rituximab plus an anthracycline-based regimens regardless of the grade (310, 79% FL1–2 and 141, 94% FL3a); however, a statistically significant difference was recorded in favour of the use of anthracyclines for FL3a versus FL1–2 ($p < 0.001$). No significant 3-year PFS differences were observed, being 67% (95% CI 62–72%) for FL1–2 and 73% (95% CI 63–79%) for FL3a, respectively ($p = 0.3$). Anthracyclines did not improve PFS in any patients subset ($p = 0.3$ for both FL1–2 and FL3a). A Cox PH regression performed stratifying by type of therapy, adjusting by FLIPI2 and taking FL1–2 as reference showed no impact for grade, resulting in a hazard ratio for FL3a of 0.85 (95% CI 0.56–1.23, $p = 0.4$).
Conclusions: With all the limits related to the observational nature of the F2-study and taking into account that this subgroup analysis was unscheduled, the results obtained strongly suggest that histological grade 3a in FL has no prognostic impact compared with grades 1–2 in patients treated with rituximab-containing regimens.

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PATTERNS OF CARE AND OUTCOMES OF FRONT-LINE CHEMOIMMUNOTHERAPY FOR ADVANCE STAGE, GRADE 3 FOLLICULAR LYMPHOMA: DATA FRPM THE NATIONAL LYMPHOCARE STUDY

L. J. Nastoupil,[1] M. Byrtek,[2] R. Ziemiecki,[3] J. Friedberg,[4] B. K. Link,[5] J. R. Cerhan,[6] K. Dawson,[2] C. R. Flowers.[1]
[1]Winship Cancer Institute, Emory University, Atlanta, USA; [2]Medical Affairs, Genentech, Inc, South San Francisco, USA; [3]Biostatistics, RTI Health Solutions, Research Triangle Park, USA; [4]Lymphoma Program, James P. Wilmot Cancer Center, University of Rochester, Rochester, USA; [5]Department of Internal Medicine, Hematology, Oncology and Blood & Marrow Transplantation, University of Iowa, Iowa City, USA; [6]Health Sciences Research, Mayo Clinic, Rochester, USA.

Introduction: The patterns of care and outcomes of therapy for grade 3 (G3) follicular lymphoma (FL) have not been clearly established. We examined outcomes for patients (pts) with G3, stage III/IV FL receiving rituximab (R) with cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP), R with cyclophosphamide, vincristine, and prednisone (RCVP), or R with a fludarabine-based regimen (RFlu), as first-line therapy to examine anthracycline use and outcomes in G3 FL.
Methods: The National Lymphocare Study (NLCS) is a prospective, multicentre, observational study collecting data on >2700 previously untreated pts with FL diagnosed from 2004 to 2007 at 265 sites in the USA. Kaplan–Meier estimation was used to evaluate progression-free survival (PFS), transformation free survival (TFS), and overall survival (OS). To compare treatment regimen impact on outcomes, Cox proportional hazards models were used, controlling for FLIPI components, practice setting, R-maintenance or observation following treatment, bone marrow involvement, sex, and geographic region.
Results: Two hundred ten pts were identified for analysis on the basis of G3 FL (per treating physician without central pathology review), stage III/IV FL, and the following regimens: 79% received RCHOP ($n = 165$), 12% received RCVP ($n = 26$), and 9% received RFlu ($n = 19$) including four with anthracycline.
Results: Results of the Kaplan–Meier estimation and adjusted Cox proportional hazards models for PFS, TFS, and OS comparing anthracycline with non-anthracycline R-chemo are presented in the table. Although the number of G3 pts treated with RFlu was small, they appeared to have inferior PFS, TFS, and OS compared with those treated with RCHOP or RCVP. Anthracycline use, age, and LDH > ULN were significant predictors of OS.
Conclusions: In the USA, anthracycline-based R-chemo was the most commonly used approach for stage III/IV G3 FL and was associated with significantly better TFS and OS even after controlling for FLIPI.

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VIROLOGIC RESPONSE IMPROVES PROGNOSIS IN PATIENTS WITH HEPATITIS C ASSOCIATED B-CELL NON-HODGKIN LYMPHOMAS, NATIONAL ANRS HC-13 LYMPHO-C STUDY RESULTS

J. Michot,[1] D. Canioni,[2] C. Settegrana,[3] P. Rabiega,[4] H. Driss,[4] Y. Barthe,[4] L. Alric,[5] F. Suarez,[6] P. Cacoub,[7] D. Sibon,[6] C. Thieblemont,[8] H. Tilly,[9] C. Haioun,[10] A. Felim,[4] F. N. Guyen Khac,[3] H. Merle-Beral,[3] Y. Taoufik,[11] F. Carrat,[4] F. Davi,[3] C. Besson,[11] O. Hermine.[6]
[1]Internal Medicine, Assistance Publique Hopitaux de Paris, Le Kremlin Bicetre, France; [2]Anatomopathologie, Service d' Anatomopathologie, Assistance Publique Hopitaux de Paris, Hopital Necker, Paris, France; [3]Hematologie Biologique, Assistance Publique des hopitaux de Paris, Pitie Salpetriere, Paris, France; [4]Unite INSERM U707, Faculte de Medecine Paris Saint Antoine, Paris, France; [5]Medecine Interne, CHU Toulouse, Toulouse, France; [6]Hematologie Adulte, Assistance Publique des Hopitaux de Paris, Necker, Paris, France; [7]Medecine Interne, Assistance Publique des Hopitaux de Paris, Pitie Salpetriere, Paris, France; [8]Hematologie Adulte, Assistance Publique des Hopitaux de Paris, Saint Louis, Paris, France; [9]Hematologie Adulte, Centre Henri Becquerel, Rouen, France; [10]Hematologie Adulte, Assistance Publique des Hopitaux de Paris, Henri Mondor, Creteil, France; [11]Immuno-Hematologie Biologique, Assistance Publique des Hopitaux de Paris, Kremlin Bicetre, Kremlin Bicetre, France.

Introduction: Hepatitis C virus (HCV) associated B-cell non-Hodgkin lymphoma (B-NHL) treatment management remains matter of debate. The Lympho-C study collected data from HCV associated B-NHL aiming to better understand lymphomagenesis, treatment and outcome.
Methods: Adult patients with B-NHL and active HCV infection were included in this observational multicentre study. Data were collected from patients with either ongoing (prospective) or past (retrospective) HCV-associated B-NHL. Cyto-histological samples were collected for centralized review.
Results: Between 2006 and 2012, 138 consecutive patients were enrolled. Subsequently,

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3-year survival probabilities for G3 FL								
3-year PFS			3-year TFS			3-year OS		
Anthracyline	Other		Anthracyline	Other		Anthracyline	Other	
70%	53%	0.067	87%	61%	0.002	91%	73%	0.015
G3 FL, hazard ratio (95% CI)								
Anthracyline versus other								
PFS			TFS			OS		
0.49 (0.29, 0.84)			0.40 (0.21, 0.77)			0.45 (0.22, 0.91)		

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13 patients were excluded from analysis. Fifty-nine of 125 (48%) patients were included at B-NHL diagnosis. At lymphoma diagnosis, median age was 61 $years_{IQ25–75}$[50–71], and gender ratio was 0.9. Genotype HCV distribution was genotype 1 (51%), 2 (19%), 3 (9%), 4 (10%), 5 (1%) or unknown (10%). Median delay between HCV infection and B-NHL diagnosis was 25 $years_{IQ25–75}$[21–31] (unknown in 41/125 patients). Histological analysis showed diffuse large B-cell lymphoma (DLBCL): 49/125 (39%) among 17/49 (35%) were transformed from underlying indolent lymphoma, marginal zone lymphoma (MZL): 48/125 (38%), follicular lymphoma: 16/125 (13%) and other types: 13/125 (10%). The MZL patients had higher levels of rheumatoid factors ($p = 0.001$) and more frequent mixed cryoglobulinemia vasculitis (11/48; 23%) ($p = 0.042$). Median follow-up since lymphoma diagnosis was 31 $months_{IQ25–75}$[19–71]. At the end of follow-up, 79/125 (63%) had received antiviral therapy since lymphoma diagnosis, and sustained virological response (SVR) was obtained in 48/79 (61%). Significant association was shown between SVR and haematological response in the MZL group ($p < 0.001$) but not in the DLBCL group ($p = 0.83$). However, in the overall population, patients with SVR had better event free survival ($p = 0.06$) and overall survival ($p = 0.006$) than patients without SVR. During the follow-up, 16 (13%) deaths were recorded, related to lymphoma progression ($n = 7$), infection ($n = 5$), cirrhosis ($n = 3$) or cardiovascular disease ($n = 1$).

Conclusions: This study confirms the predominance of DLBCL and MZL in HCV-infected patients. Virological success appears to improve prognosis of patients with B-NHL lymphoma.

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INFLUENCE OF LIFESTYLE FACTORS ON THE EXPRESSION OF TUMOUR-RELATED MICROENVIRONMENT T-CELLS AND THE IMPACT ON SURVIVAL IN FOLLICULAR LYMPHOMA

A. M. Evens,[1] A. Chadburn,[2] N. Ollberding,[3] D. D. Weisenburger,[4] B. C. Chiu.[5]
[1]Division of Hematology/Oncology, The University of Massachusetts Medical School, Worcester, USA; [2]Pathology, Northwestern University Feinberg School of Medicine, Chicago, USA; [3]Division of Biostatistics and Epidemiology, Cincinnati Children, Cincinnati, USA; [4]Pathology, City of Hope, Duarte, USA; [5]Department of Health Studies, University of Chicago, Chicago, USA.

Background: Several lifestyle factors (e.g. obesity, alcohol, and diet) have been shown to affect the incidence of the development of non-Hodgkin lymphoma (NHL). However, there are minimal data regarding the impact of these factors on outcomes of NHL or regarding potential interactions or added prognostic value of lifestyle factors with established tumour markers.

Methods: From a population-based prospective study on 123 FL patients (pts) diagnosed between 1999 and 2002, we assessed the association between lifestyle factors and tumour markers (CD68, CD7, FOXP3, CD10, and Ki67) and examined the impact on overall survival (OS). Scoring of TMAs was performed by expert pathologists (AC, DDW). Lifestyle habits (i.e. smoking, diet, alcohol use, and smoking) were assessed through validated comprehensive questionnaires (e.g. Health Habits and History Questionnaire).

Results: The median age of pts was 60 years (48–70), and 89% had stage III/IV disease. The median pt follow-up was 8.1 years. High microenvironment expression of CD7 was strongly associated with a dietary pattern high in fruits, vegetables, and starch ($p = 0.04$), as well as more specifically a higher intake of carotene-rich vegetables ($p = 0.005$). Additionally, 'never smokers' more frequently had increased microenvironment expression of CD7 compared with ever smokers (89% vs 58%, respectively, $p = 0.02$). Survival analyses showed that FL pts with high CD7 expression had a significantly better OS [HR = 0.34 (95% CIs 0.14–0.84), $p = 0.02$] for all cause mortality versus pts with low CD7 expression. The impact of of CD7 on all cause mortality was decreased after adjustment for smoking status (HR = 0.4; CI = 0.1–1.30). In addition, we found that FL pts who were above the median intake for fruit were more likely to have CD10 tumour cell expression [OR = 5.3 (95% CIs 1.0–26.5), $p = 0.04$] compared with those below the median intake. The other FL tumour markers examined (i.e. CD68, FOXP3, and Ki67) had no impact on OS nor did they associate with the other lifestyle factors evaluated.

Conclusion: We identified several novel lifestyle/tumour associations including the association of CD7 microenvironment with diets high in fruits and vegetables and with never smokers. Furthermore, we found that CD7 expression was associated with improved OS independent of age and sex; however, its predictive value was slightly attenuated after adjustment for smoking.

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COMPARISON OF CHOP VERSUS CVP TREATMENT IN 835 US VETERANS WITH UNTREATED FOLLICULAR LYMPHOMA

K. Carson,[1] L. Gordon,[2] K. O'Brian,[3] S. Luo,[3] G. Colditz.[4]
[1]Medical Oncology, Washington University School of Medicine, St. Louis, MO, USA; [2]Division of Hematology/Oncology, Northwestern University, Chicago, IL, USA; [3]Research Service, St. Louis VA Medical Center, St. Louis, MO, USA; [4]Division of Public Health Sciences, Washington University School of Medicine, St. Louis, MO, USA.

Introduction: There is no standard of care treatment for follicular lymphoma (FL), although study of treatment patterns suggests that most US oncologists use R-CHOP. Randomized trials demonstrate higher response rates to R-CHOP compared with R-CVP, although overall survival (OS) data are not yet available. We compared CHOP with CVP chemotherapy [+/− rituximab(R)] and assessed progression-free survival (PFS) and OS outcomes in a cohort of US veterans with untreated FL.

Methods: Patients diagnosed with FL between 1998 and 2009 were identified in the Veterans Health Administration central cancer registry. Of 2136 FL diagnoses, 17 were HIV+, 685 had no documentation of treatment, 517 received alternate treatment regimens and 82 received radiotherapy. These 1301 patients were excluded. Thus, 835 patients met inclusion criteria (486 CHOP, 349 CVP). Additional data obtained included age, stage, grade, comorbidities, R use and use of myeloid growth factors.

Results: Baseline comparison showed CHOP patients were significantly younger than CVP patients (mean, 61 vs 66 years), had lower Charlson comorbidity score (mean, 2 vs 2.6), had lower stage (70% vs 77%, stage III/IV), had higher use of myeloid growth factors (55% vs 26%) and were more likely to have grade 3 disease (40% vs 10%). There was no difference between groups in R induction (68% vs 67%) or maintenance (21% vs 23%). In Cox analysis, age (HR 1.04, CI 1.02–1.05), stage (HR 1.8, CI 1.3–2.4) and Charlson score (HR 1.16, CI 1.09–1.22) were associated with higher mortality. R was associated with lower mortality (HR 0.68, CI 0.53–0.88), whereas CHOP chemotherapy was not significantly associated (HR 0.99, CI 0.76–1.3) with OS. Cox analysis evaluating PFS while controlling for the same covariates also demonstrated no benefit from CHOP (HR 0.99, CI 0.79–1.24).

Conclusions: In this real-world cohort of FL patients, CHOP did not improve PFS or OS compared with CVP. Any modest benefit from CHOP must be balanced against the higher treatment costs related to increased growth factor use and increased toxicity. Residual confounding from unmeasured factors may have blunted the effect of doxorubicin, although CVP patients generally had poorer baseline disease and health characteristics. Overall, these results suggest that R-CHOP represents over-treatment of many patients with FL and perhaps should not be considered the standard of care or control arm in randomized trials evaluating FL therapy.

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A MULTICENTRE PHASE II TRIAL OF BORTEZOMIB COMBINED WITH RITUXIMAB THERAPY FOR UNTREATED 'HIGH TUMOUR BURDEN' INDOLENT NON-HODGKIN LYMPHOMA

A. M. Evens,[1] M. R. Smith,[2] I. S. Lossos,[3] J. N. Winter,[4] S. R. Rosen,[5] L. I. Gordon.[4]
[1]Division of Hematology/Oncology, The University of Massachusetts Medical School, Worcester, USA; [2]Division of Hematology/Oncology, Cleveland Clinic, Cleveland, USA; [3]Division of Hematology/Oncology, The University of Miami, Miami, USA; [4]Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, USA; [5]Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, USA.

Background: There remains a deficiency of data examining non-chemotherapeutic options for patients (pts) with untreated high tumour burden (HTB) indolent non-Hodgkin lymphoma (NHL).

Methods: We completed a multicentre phase II study for untreated indolent NHL (NCT00369707). All pts were required to have HTB as defined by GELF/ECOG criteria. Induction therapy consisted of three cycles: rituximab (R) 375 mg/m (2) × 4 weeks, then one dose R given q35 days × two cycles combined with bortezomib (B) 1.6 mg/m(2) days 1, 8, 15 and 22 given q35 days for all three cycles. This was followed by maintenance with R + B each given once q2 months × 8 months. The primary objective was overall response rate (ORR).

Results: Forty-two pts (41 response evaluable) were enrolled. Histologies were follicular lymphoma (FL) ($n = 32$), marginal zone lymphoma ($n = 5$), small lymphocytic lymphoma ($n = 3$) and Waldenstrom's ($n = 1$). Median age was 61 years (40–86), 91% had stage III/IV disease (67% stage IV) and the median FLIPI was 3 (1–5). Therapy was well tolerated. Total grade 3 adverse events (AEs) were fever (5%), infection (5%), cardiac (5%), fatigue (5%), and diarrhoea, hypokalemia and ileus (2% each). The only grade 4 AEs were neutropenia (5%) and thrombocytopenia (2%). After cycle 1, a reduction in measurable tumour size was observed in 100% of pts, although the ORR was only 22% with no complete responders (CR). Following cycle 3, the ORR was 59% (FL ORR 62%, CR 9%). Notably, from cycle 1 to 3, 63% of FL pts improved their response (17 stable disease to partial remission (PR); and 3 PR to CR). The best ORR was 71% with a 39% CR rate (FL: ORR 75%, CR 50%). There were several early 'treatment failures' due to toxicity or physician discretion as well as several early disease progressions (mainly during maintenance) that resulted in a 3-year time to treatment failure rate of 33%. However, with a median follow-up of 41 months (10–59), the 3-year progression-

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free survival (PFS) was 53% (FL 59%), and the 3-year overall survival (OS) was 88% (FL OS: 94%).
Conclusions: In untreated HTB indolent NHL, therapy with R + B was well tolerated and resulted in successive conversions of responses. Further, PFS and OS rates appeared comparable with historical R-chemotherapy series, although early treatment failures were seen in this study. Continued strategies to incorporate novel therapeutic agents into frontline FL are warranted, whereas more protracted dosing schedules should be considered in HTB populations in order to maintain adequate duration of response.

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FLUDARABINE–MITOXANTRONE–RITUXIMAB REGIMEN IN UNTREATED INTERMEDIATE/HIGH-RISK FOLLICULAR NON-HODGKIN

P. Zinzani, B. Casadei, C. Pellegrini, A. Broccoli, E. Derenzini, L. Gandolfi, F. Quirini, G. Stefani, R. Narducci, M. Tschon, L. Argnani, V. Stefoni.
Institute of Haematology, University of Bologna, Bologna, Italy

Introduction: There is no international consensus on optimal frontline chemotherapy regimen for advanced stage follicular lymphoma patients or a clear definition of cure for this disease. Aim of this study was to test the degree of effectiveness and the safety of the regimen containing fludarabine, mitoxantrone and rituximab (FMR) in a subset of poor prognosis follicular lymphoma patients with particular focus on the long-term disease-free survival.
Patients and Methods: An observational retrospective study was conducted on 142 intermediate/high-risk follicular lymphoma patients treated in first line with six-cycle FMR regimen. From September 2000 to March 2010 in our institution, patients aged 18 years or older with biopsy-proven, bidimensionally measurable, stage III or IV untreated indolent follicular lymphoma expressing the CD20 antigen were deemed eligible. The prognostic value of PET was also investigated in a 56-patients subset.
Results: Overall response rate was 95.5% with 88% of complete responses: 18% of patients had disease relapse, yielding an estimated 12-year disease-free survival of 72% (median follow-up 48 months). All cases showed the lymphoma recurrence within 40 months: after this timing, the disease-free survival curve presented a plateau. Overall survival was 73% at 12 years. Post-treatment PET positivity remained a highly significant predictor of disease progression with a 5-year progression-free survival rate of 42% in PET-positive versus 75.5% in PET-negative patients ($p = 0.0024$). The FMR regimen was globally well tolerated, and reversible haematological toxicities were the most common adverse events. Six patients developed secondary malignancies; in particular, only one (0.7%) of them was a haematological neoplasia after 8 months from end of treatment.
Conclusions: The observed high rate of complete responses following the use of FMR regimen in intermediate/high-risk patients seems to be the first step to improve disease-free survival. Our study could be the starting point to consider disease-free survival as a potential alternative endpoint of future clinical trial on follicular lymphoma patients.

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PROGNOSTIC ROLE OF BCL-2 MOLECULAR MONITORING IN PATIENTS WITH EARLY STAGE FOLLICULAR LYMPHOMA

A. Pulsoni,[1] I. Della Starza,[1] G. Annechini,[1] F. De Angelis,[1] G. D'Elia,[1] P. D'Urso,[1] S. Panfilio,[1] M. Cavalli,[1] L. Grapulin,[2] R. Foà[1].
[1]*Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Rome, Italy;* [2]*Department of Radiology and Radiotherapy, Sapienza University of Rome, Rome, Italy.*

Introduction: Stage I/II follicular lymphoma (FL) is an uncommon disease, representing only 20% of FL; despite the negative bone marrow biopsy in all cases, most of the patients present positive Bcl-2 rearranged cells in bone marrow (BM) and peripheral blood (PB). The aim of this study was to analyze the prognostic role of Bcl-2 molecular monitoring in 57 consecutive cases of stage I–II FL in a single centre experience.
Methods: Fifty-seven consecutive patients with a confirmed diagnosis of stage I/II FL were investigated by PCR in order to identify the presence of Bcl-2 rearranged cells in the BM and/or PB. All patients were treated with involved field RT (30–36 Gy). Subsequently, minimal residual disease (MRD) was evaluated every 6 months after RT in patients positive at baseline; patients negative at basal evaluation were not retested.
Results: PCR analysis revealed Bcl-2 rearranged cells in 38/57 patients (66.7%) at presentation in PB and BM. After irradiation of the sole site of the disease, Bcl-2 rearranged cells disappeared in 19 of the 38 (50%) patients positive at baseline from PB and/or BM; in 17/38 (44.7%), MRDpersisted positive, wheres two patients refused to perform test. After a median follow-up of 73 months, 10 patients (17.5%) had a clinical relapse; of them, nine belonged to the group with positive PCR at baseline. Only one patient experienced a clinical relapse with basal negative Bcl2. Among relapsed patients, 6/10 presented a positive Bcl-2 at relapse; one patient was negative at baseline, and three were not evaluable. Thirteen positive Bcl-2 patients were treated with rituximab 375 mg/mq, four weekly administrations: of them, 8/13 (61%) patients became negative; no clinical relapses were observed in Bcl-2 negative after rituximab.
Conclusions: Viable Bcl-2+ cells can be demonstrated in the BM and/or PB of the majority of stage I–II FL patients. Basal presence of Bcl-2 had a prognostic role: no clinical relapses were observed in Bcl-2 negative cases at baseline except for one patient. Irradiation of the sole nodal/extranodal disease sites allows disappearance of Bcl-2+ cells in the 50% of cases (19/38). Rituximab therapy induced negativization of BCL2 in MRD positive patients. A larger population is necessary to confirm the prognostic value of rituximab purging in MRD positive patients.

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PHARMACOKINETICS, SAFETY, AND OVERALL RESPONSE RATE ACHIEVED WITH SUBCU-TANEOUS RITUXIMAB PLUS CHEMOTHERAPY WERE COMPARABLE TO THOSE WITH INTRAVENOUS ADMINISTRATION IN THE FIRST-LINE TREATMENT OF PATIENTS WITH FOLLICULAR LYMPHOMA : STAGE 1 RESULTS OF THE PHASE 3 SABRINA STUDY (BO22334)

A. Davies,[1] F. Merli,[2] B. Mihaljević,[3] N. Siritanaratkul,[4] P. Solal-Celigny,[5] A. Boehnke,[6] C. Berge,[6] C. McIntyre,[7] M. Barrett,[7] D. MacDonald.[8]
[1]*Cancer Research UK Centre, University of Southampton Faculty of Medicine, Southampton, UK;* [2]*Hematology Unit, Azienda Ospedaliera Santa Maria Nuova, Reggio Emilia, Italy;* [3]*Clinic of Hematology, Clinical Center Serbia School of Medicine, Belgrade, Serbia and Montenegro;* [4]*Division of Hematology, Department of Medicine, Siriraj Hospital, Bangkok, Thailand;* [5]*Department of Medicine, Centre Jean Bernard, Le Mans, France;* [6]*Clinical Development, F. Hoffmann-La Roche Ltd, Basel, Switzerland;* [7]*Clinical Pharmacology, Roche Products Ltd., Welwyn Garden City, UK;* [8]*Department of Medicine, QEII Health Sciences Centre, Halifax, Canada.*

Introduction: Rituximab (R) plus chemotherapy (CT) induction followed by maintenance R is the standard frontline follicular lymphoma (FL) therapy. R^{SC} could shorten R^{IV} administration times and improve patient (pt) convenience. Achieving non-inferior R serum C_{trough} levels with R^{SC} compared with R^{IV} dosing is expected to provide similar efficacy.
Methods: BO22334 (NCT01200758) is a two-stage phase 3 study of R^{SC}(seven cycles at 1400 mg after one cycle IV) versus R^{IV} (375 mg/m^2; eight cycles) plus CHOP (≤eight cycles) or CVP (eight cycles) followed by R maintenance by the same administration mode. The stage 1 objective was to estimate the mean R serum $C_{trough,SC} : C_{trough,IV}$ ratio at day 21 of induction cycle 7. The non-inferiority limit was 0.8. Stage 1 end-of-induction outcomes follow.
Results: One hundred twenty-seven pts with confirmed CD20+ grades 1–3a previously untreated FL were randomized to R^{SC} ($n = 63$) or R^{IV} ($n = 64$). At induction cycle 7, geometric mean serum R C_{trough} was 134.6 µg/mL for R^{SC} ($n = 48$) and 83.1 µg/mL for R^{IV} ($n = 54$). The C_{trough} ratio was 1.62 (90% CI: 1.36–1.94); subcutaneous (SC) non-inferiority was shown by the lower limit of the CI> 0.8. The geometric mean AUC ratio ($AUC_{SC} : AUC_{IV}$)was 1.38 (90% CI: 1.24–1.53). Overall adverse event (AE) rates were similar. Gr 3/4 AEs occurred in 47% and 46% of pts in the $R^{SC}$and R^{IV} arms, respectively. Any-Gr administration-related reactions (ARRs) occurred in 31 (50%) R^{SC} and 21 (32%) R^{IV} pts; most were Gr 1/2. Investigator-assessed overall response rates (ORRs): 90.5% (95% CI: 80.4–96.4) in R^{SC}pts and 84.4% (95% CI: 73.1–92.2) in R^{IV} pts. Complete response (CR/CRu) rates: 46.0% (95% CI: 33.4–59.1) for R^{SC}pts and 29.7% (95% CI: 18.9–42.4) for R^{IV} pts. Consistent with this assessment, independent response review indicated that SC administration did not impair the anti-lymphoma activity of R.
Conclusions: These data demonstrate serum R C_{trough} non-inferiority and comparable investigator-assessed ORRs that were in line with those obtained upon independent review for pts treated with R^{SC} (1400 mg) versus R^{IV} (375 mg/m^2). AE profiles were similar; ARRs were mostly mild or moderate. Stage 2 has begun recruitment.

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FINAL RESULTS OF THE BP22333 STUDY DEMONSTRATE NONINFERIOR PHARMACOKINETICS AND SAFETY OF SUBCUTANEOUS VERSUS INTRAVENOUS RITUXIMAB AS MAINTENANCE THERAPY IN FOLLICULAR LYMPHOMA

A. Salar,[1] I. Avivi,[2] J. Larouche,[3] A. Janikova,[4] J. Pereira,[5] M. Brewster,[6] O. Catalani,[7] C. McIntyre,[6] P. Sayyed,[8] A. Haynes.[9]
[1]*Hematology Department, Hospital del Mar, Barcelona, Spain;* [2]*Hematology and Bone Marrow Transplantation, Rambam Medical Center, Haifa, Israel;* [3]*Unite de Recherche en Hematologie et Oncologie, CHAUQ Hopital Enfant-Jesus, Quebec, Canada;* [4]*Department of Hematology and Oncology, University Hospital Brno, Brno, Czech*

Republic; [5]Servico de Hematologia, Hospital das Clinicas da FMUSP, Sao Paulo, Brazil; [6]Clinical Pharmacology, Roche Products Ltd, Welwyn Garden City, UK; [7]Pharmaceutical Development, F. Hoffmann-La Roche, Basel, Switzerland; [8]Clinical Development, F. Hoffmann-La Roche, Basel, Switzerland; [9]Department of Haematology, Nottingham City Hospital, Nottingham, UK.

Introduction: BP22333 (NCT00930514) is a two-stage phase 1b study assessing pharmacokinetics (PK) and tolerability of subcutaneous (SC) versus intravenous (IV) maintenance rituximab in patients with first-line or relapsed follicular lymphoma (FL). Stage 1 dose-finding results (Salar et al., ASH 2010, abs 2858; Salar et al, EHA 2012, abs 0794) identified a fixed dose of 1400 mg for formal C_{trough} noninferiority testing in stage 2. We report stage 2 data for which the objective was to demonstrate noninferior C_{trough} of rituximab SC 1400 mg vs IV 375 mg/m^2, using a noninferiority test with a lower boundary of 0.8 for the 90% confidence interval (CI).
Methods: Patients aged ≥18 years with CD20$^+$ grade 1, 2, or 3a FL who responded to a rituximab induction regimen and received at least one dose of rituximab IV maintenance therapy were eligible for randomization in stage 2.
Results: Patients ($n = 154$) were randomized 1:1 to rituximab SC 1400 mg ($n = 77$) or IV 375 mg/m^2 ($n = 77$) for their remaining maintenance cycles and stratified by two-monthly (q2m) versus three-monthly (q3m) regimen. Baseline characteristics were similar between SC and IV arms. Median treatment duration on study was 14.8 months (range, 0–19) in the SC group and 13.8 months (range, 0–19) in the IV group. The primary endpoint was met; rituximab SC 1400 mg C_{trough} was noninferior to rituximab IV 375 mg/m^2 C_{trough}. Geometric mean $C_{trough,SC}/C_{trough,IV}$ ratios were 1.24 for q2m and 1.12 for q3m. The lower limits of the two-sided 90% CI were 1.02 (q2m) and 0.86 (q3m), exceeding the protocol-specified noninferiority limit ($C_{trough,SC}/C_{trough,IV}$ ratio of 0.8). Adverse event (AE) incidence and intensity were generally balanced; 79% of patients in each group had AEs, and grade 3/4 AEs occurred in 18% and 17% of SC and IV patients, respectively. Administration-related reactions (ARRs) were the most frequent AEs, with a higher incidence in the SC group (31% SC vs 4% IV), reflecting the expected ARR profile when switching to SC administration. ARRs were mostly local reactions and mild in intensity; the most common in the SC group was erythema (13%).
Conclusions: Rituximab SC 1400 mg C_{trough} was noninferior to rituximab IV C_{trough} following standard dosing during maintenance therapy in FL. Rituximab SC was well tolerated. As expected, local ARRs occurred more frequently in the SC group, reflecting the method of administration.

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FCGR3A AND FCGR2A POLYMORPHISMS DO NOT PREDICT RESPONSE RATE OR DURATION IN FOLLICULAR LYMPHOMA PATIENTS TREATED WITH SINGLE-AGENT RITUXIMAB: A PROSPECTIVE CORRELATIVE ANALYSIS FROM THE RESORT STUDY (ECOG E4402)

V. P. Kenkre,[1] F. Hong,[2] J. R. Cerhan,[3] M. E. Williams,[4] R. D. Gascoyne,[5] S. J. Horning,[6] B. S. Kahl.[1]
[1]Medicine, University of Wisconsin, Madison, USA; [2]Biostatistics, Dana Farber Cancer Institute, Boston, USA; [3]Health Sciences Research, Mayo Clinic, Rochester, USA; [4]Medicine, University of Virginia, Charlottesville, USA; [5]Pathology, BC Cancer Agency, Vancouver, Canada; [6]Clinical Development Hematology/Oncology, Genentech, South San Francisco, USA.

Introduction: Pre-clinical studies suggest that single nucleotide polymorphisms (SNPs) in the FcG receptor (FcγR) genes influence the response to rituximab, via potentiation of antibody-dependent cellular cytotoxicity. Because the relationship of these SNPs to rituximab efficacy is not well defined in the clinic, we prospectively obtained tissue for genotyping in the RESORT study.
Methods: Three hundred eighty-four untreated, low tumour burden follicular lymphoma (FL) patients were enrolled in the RESORT study. Patients received single-agent rituximab in four weekly doses, and responders were randomized to rituximab re-treatment (RR) upon progression versus maintenance rituximab (MR). SNP genotyping was performed using pyrosequencing in 320 consenting patients. Response rates to initial therapy and response duration were correlated with the FCGR3A SNP rs396991 [valine (V) or phenylalanine (F) at position 158] and the FCGR2A SNP rs1801274 [histidine (H) or arginine (R) at position 131].
Results: Successful genotyping was achieved in 314 patients for FCGR3A and 303 patients for FCGR2A. Allele frequencies were as expected: VV 13%, VF 42%, and FF 45% for FCGR3A; HH 29%, HR 49%, and RR 22% for FCGR2A. The overall response rate to initial rituximab was 71%. No FcγR genotypes (VV vs any F, HH vs any R) or groupings of genotypes (VV/HH vs FF/RR) were predictive of initial response to rituximab. Two hundred seventy-four patients were randomized to RR ($n = 134$) or to MR ($N = 140$). With a median follow-up of 3.8 years, the 3-year response duration in the RR arm and the MR arm was 53% and 77%, respectively. Genotyping was performed in 221/274 randomized patients. In both the RR arm and MR arm, response duration was not associated with any FcγR genotypes or genotype combinations.
Conclusion: On the basis of this analysis of a treatment-naïve, low tumour burden FL, we conclude that the FCGR3A rs396991 and FCGR2A rs1801274 SNPs do not confer a differential response to rituximab. These data indicate that these two SNPs should not be used to select patients for single agent rituximab and raise additional questions regarding the mechanism of rituximab cytotoxicity.

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SIGNIFICANCE OF THE QUANTITATIVE BCL2/IGH@ REARRANGEMENT EVALUATION IN PATIENTS AFFECTED BY FOLLICULAR LYMPHOMA AFTER CONVENTIONAL TREATMENT: THE ANCILLARY STUDY OF THE FIL-FOLL05 TRIAL

S. Galimberti,[1] S. Luminari,[2] E. Ciabatti,[1] A. Dondi,[2] M. Ladetto,[3] P. Piccaluga,[4] I. Del Giudice,[5] A. Gazzola,[4] L. Monitillo,[3] B. Mantoan,[3] I. Della Starza,[5] M. Cavalli,[5] C. Mannu,[4] G. A. Palumbo,[6] L. Marcheselli,[2] U. Vitolo,[3] G. Gaidano,[7] A. M. Carella,[8] M. Petrini,[1] M. Federico.[2]
[1]Clinical and Experimental Medicine, Hematology, Pisa, Italy; [2]University of Modena, Oncology, Modena, Italy; [3]Città della Scienza e della Salute, Hematology, Torino, Italy; [4]Department of Experimental, Diagnostic, and Specialty Medicine, Hemato-pathology, Bologna, Italy; [5]Department of Cellular Biotechnologies and Hematology, Hematology, Roma, Italy; [6]Azienda Ospedaliera "Ferrarotto", Hematology, Catania, Italy; [7]Azienda Ospedaliero-Universitaria della Carità, Novara, Italy; [8]Azienda Ospedaliera Universitaria San Martino, Hematology, Genova, Italy.

The role of the quantitative assessment of BCL2/IGH@ in patients affected by follicular lymphoma is still a matter of debate. Other authors previously reported that patients with low amount of BCL2/IGH@ had a significant advantage in event-free survival (Rambaldi A, 2005). In the present study, we report about the significance of the quantitative BCL2/IGH@ assessment at diagnosis and after treatment. Results from the qualitative assessment have been already reported at ASH 2012 (Galimberti S, 2012). In the FOLL05 trial, conducted by the Fondazione Italiana Linfomi (FIL), 504 untreated patients were randomized to receive R-CHOP, R-CVP, or R-FM (Clinical trial.gov NCT00774826). Quantitative PCR was performed by the four laboratories of the 'FIL MRD NETWORK' (Bologna, Pisa, Roma, and Torino, Italy), according to European guidelines (Ladetto M, 2001, van der Velden, 2007). The sensitivity was $1{:}10^{-5}$. At the enrollment, quantitative PCR was performed in 105 cases; the median value was 3×10^{-3} copies (range: $<1 \times 10^{-5}$ to 6). The copy number did correlate only with high FLIPI/FLIPI2. For patients with high BCL2/IGH@copies, overall response rate was significantly lower (76.6% vs 38.9%; $p = 0.006$). When the receiver operating characteristic curve was performed, 22% of cases showing $<1 \times 10^{-4}$copies relapsed versus 78% of patients with $>\times 10^{-4}$copies ($p = 0.033$). Moreover, cases displaying values $<1 \times 10^{-4}$copies showed a clear advantage also in terms of progression-free survival (PFS) (3-year PFS 80% vs 59% for cases with higher tumour burden; $p = 0.015$). On the contrary, the qualitative PCR results at diagnosis did not influence quality of response nor PFS. Concerning the impact of treatment on the BCL2/IGH@ tumour burden, the mean observed reduction was about two logarithms. Seventy-one per cent of cases achieved PCR-negativity; no differences in this effect were observed according to the arm of treatment. Nevertheless, an inferior tumour burden reduction in the R-CVP arm was observed, even not statistically significant. In conclusion, this study shows that patients with $<1 \times 10^{-4}$copies of BCL2/IGH@ at diagnosis would have longer PFS, thus supporting the usefulness of the quantitative PCR monitoring of these patients by the diagnosis.

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MINIMAL RESIDUAL DISEASE AT MULTIPLE TIMEPOINTS IS A STRONG OUTCOME PREDICTOR IN FOLLICULAR LYMPHOMA PATIENTS: RESULTS OF THE ML17638 TRIAL OF THE FONDAZIONE ITALIANA LINFOMI

M. Ladetto,[1] C. Lobetti-Bodoni,[1] B. Mantoan,[1] A. Evangelista,[2] C. Boccomini,[3] E. Genuardi,[1] A. Chiappella,[3] L. Baldini,[4] G. Rossi,[5] A. Pulsoni,[6] F. Di Raimondo,[7] L. Rigacci,[8] A. Pinto,[9] S. Galimberti,[10] S. Sacchi,[11] D. Rota-Scalabrini,[12] F. Merli,[13] G. Parvis,[14] E. Gamba,[15] U. Vitolo.[3]
[1]Division of Hematology, Department of Experimental Medicine and Oncology, University of Turin, Torino, Italy; [2]Unit of Cancer Epidemiology, Azienda Ospedaliera Citta della Salute e della Scienza, Torino, Italy; [3]Division of Hematology 2, Azienda Ospedaliera Citta della Salute e della Scienza, Torino, Italy; [4]Division of Hematology, Fondazione IRCCS Ca Granda OM Policlinico, Milano, Italy; [5]Division of Hematology, Spedali Civili, Brescia, Italy; [6]Institute of Hematology, University Sapienza, Roma, Italy; [7]Division of Hematology, Oncology,

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and Clinical Pathology, Department of Clinical and Molecular Biomedicine, University of Catania, Catania, Italy; [8]Hematology Functional Unit, Azienda Ospedaliera Universitaria Careggi, Firenze, Italy; [9]Istituto Di Ricovero e Cura a Carattere Scientifico IRCCS, Istituto Nazionale Tumori, Fondazione G. Pascale, Napoli, Italy; [10]Department of Oncology, Transplants and Advanced Technologies, Section of Hematology, Pisa University, Pisa, Italy; [11]Program of Innovative Therapies in Oncology and Hematology, Department of Oncology and Hematology, University of Modena and Reggio Emilia, Modena, Italy; [12]Division of Medical Oncology, Institute for Cancer Research and Treatment, Candiolo, Torino, Italy; [13]Division of Hematology L. e A. Seragnoli, St. Orsola-Malpighi Hospital, Bologna, Italy; [14]Internal Medicine and Hematology, S. Luigi Hospital, University of Torino, Orbassano, Italy, [15]Roche, S. p.A., Monza, Italy.

Introduction: PCR-based minimal residual disease (MRD) detection is a strong outcome predictor in follicular lymphoma (FL), but its role is not fully investigated in Rtx intensive programmes with/without maintenance (maint). We present MRD results of the ML17638 study.
Methods: Clinical results of the study have been already reported (Vitolo ASH '11). Two hundred thirty-four patients (pts) (60–75 years) achieving ≥ PR were enrolled and randomized to Rtx maint (Arm A) or observation (obs, Arm B). Pts with a molecular marker at diagnosis (bcl-2/IgH MBR or mcr) underwent nested PCR (n-PCR) and real-time PCR (RQ-PCR) on bone marrow (BM) cells at eight timepoints (TP): mid-treatment (M5), end of induction (M8), maint/obs and f up (M12, M18, M24, M30, M36 and M42) or until relapse. The blind analysis was centralized at a Euro-MRD lab. Log-rank tests, Cox and time-varying covariate models were used.

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	2-year PF					
	N-PCR			RQ-PCR		
TP	Pos (%)	Neg %	*p*	Pos %	Neg %	*p*
M5	62	69	0.34	54	72	0.11
M8	39	72	0.007	0	74	<0.001
M12	54	78	0.042	0	78	<0.001
M18	40	81	0.013	34	78	0.079
M24	44	90	<0.001	20	88	<0.001

Results: Ninety-seven per cent of pts were screened: a marker was found in 116 (51%). Five hundred fifty-nine f up samples (83% of exp) were analyzed. Pts with/without a marker had identical progression-free survival (PFS) (61% at M42). At M8, PCR-positivity rate was similar in the two arms, while at later TPs, except M5, predicted for a better 2-year PFS (Table 1). This occurred in CR and PR pts (p 0.023, HR 0.33) (p 0.074, HR = 0.28) and in both arms (A: 83% vs 60%, p 0.007, HR 0.37; B: 71% vs 50% ($p < 0.001$, HR 0.27). PCR-positivities at M8 and CR were the only indipendent predictors of 2-year PFS (HR 3.1, p 0.007, and HR 2.96, p 0.030). The time-varying covariate model showed that accumulation of PCR-negativities reduced the risk of relapse (multiple imputation for missing data p 0.01, HR 0.30).
Conclusion: MRD is a strong outcome predictor in FL in Rtx intensive programmes. Fondazione Italiana Linfomi trials are investigating MRD as a decision-making tool.

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THE USE OF RITUXIMAB AT FIRST LINE DOES NOT IMPAIR THE EFFICACY OF SECOND-LINE THERAPY IN PATIENTS WITH FOLLICULAR LYMPHOMA. RESULTS OF THE REFOLL STUDY BY THE FONDAZIONE ITALIANA LINFOMI

G. Rossi,[1] L. Marcheselli,[2] C. Bottelli,[1] A. Tucci,[1] A. Dondi,[2] S. Luminari,[2] L. Arcaini,[3] M. Merli,[4] A. Pulsoni,[5] C. Boccomini,[6] B. Puccini,[7] M. Micheletti,[1] G. Martinelli,[8] A. Rossi,[9] V. R. Zilioli,[10] V. Bozzoli,[11] M. Balzarotti,[12] S. Bolis,[13] M. G. Cabras,[14] M. Federico.[2]
[1]Hematology, Spedali Civili Hospital, Brescia, Italy; [2]Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy; [3]Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [4]Internal Medicine, Division of Hematology, Circolo e Fondazione Macchi Hospital, Varese, Italy; [5]Cellular Biotechnology and Hematology, Sapienza University, Roma, Italy; [6]Hematology 2, Citta' della Salute e della Scienza Hospital, Torino, Italy; [7]Hematology, Azienda Ospedaliera Universitaria Careggi, Firenze, Italy; [8]Division of Clinical Haemato-Oncology, European Institute of Oncology, Milano, Italy; [9]Hematology, Ospedali Riuniti, Bergamo, Italy; [10]Oncology, Division of Hematology, Niguarda Ca' Granda Hospital, Milano, Italy; [11]Hematology, Catholic University Sacro Cuore, Roma, Italy; [12]Hematology Unit, Humanitas Cancer Center, Rozzano, Milano, Italy; [13]Hematology Unit, San Gerardo Hospital, Monza, Italy; [14]Division of Hematology, Businco Hospital, Cagliari, Italy.

Introduction: Follicular lymphoma (FL) patients (pts) commonly receive multiple lines of treatment. The multicentre, retrospective study REFOLL evaluated the potential reciprocal effects of different types of first-line and salvage treatments. We analyzed the effects of prior rituximab (RTX) exposure on patient's outcome after relapse.
Methods: In 512 FL pts registered from 25 institutions relapsing between 2000 and 2008, RTX was used at first line in 258 (50%), with either alkylators-based (46%) or anthracyclin-based (48%) or nucleoside analogue-based (65%) chemotherapy (CHT), and was then given to 100 pts (20%) as maintenance. Salvage treatments included either the same first-line CHT in 271 (53%) or autologous stem cell transplant (ASCT) in 151 (29%) or only RTX/ibritumomab in 90 pts (18%). RTX was given with CHT to 198/271 pts (73%) and to 36/53 (68%) evaluable ASCT pts. RTX maintenance after salvage was used in 99 pts (19%). Endpoints were time to next treatment after relapse (TTNT) and overall survival (OS).
Results: After a median of 41 months, the median TTNT was 41 months (CI95% 35–47). TTNT was slightly worse for pts who had received first-line CHT + RTX (34.1%) versus CHT only (40.2%, $p = 0.022$); however, statistical significance was lost after adjustment by age and stage (HR = 1.20; $p = 0.167$). RTX maintenance after first-line treatment did not influence TTNT (HR = 0.99, $p = 0.988$). On the whole, either use of RTX at first line did not adversely affect the outcome of second-line therapy (HR = 1.11; $p = 0.444$). OS was 88% at 5 years (CI95% 85–91); it was not influenced by RTX use at first line. After relapse, compared with pts receiving CHT only as salvage, OS was significantly better in pts receiving either ASCT or CHT + RTX (HR = 0.58; $p = 0.041$), and it was further improved by RTX maintenance (HR = 0.20; $p = 0.001$, adjusted by stage, age and first-response duration).
Conclusions: With the limitations of its retrospective nature, REFOLL study shows that in FL pts, prior exposure to RTX, either with CHT or as maintenance, does not adversely affect the efficacy of subsequent treatments, including RTX retreatment.

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A SINGLE CENTRE PHASE II TRIAL OF ^{90}YTTRIUM–IBRITUMOMAB–TIUXETAN PRODUCES HIGH RESPONSE RATES AS FIRST-LINE THERAPY FOR EARLY STAGE B-CELL INDOLENT LYMPHOMA, INCLUDING BULKY DISEASE

F. Samaniego,[1] J. Romaguera,[1] N. Fowler,[1] M. Fanale,[1] B. Pro,[2] J. Shah,[1] P. McLaughlin,[1] V. Selvaraj,[1] F. K. Braun,[1] R. Mathur,[1] L. Feng,[1] S. Neelapu,[1] L. Kwak.[1]
[1]Lymphoma/Myeloma, U.T. MD Anderson Cancer Center, Houston, USA; [2]Immune Cell Development and Host Defense, Fox Chase Cancer Center, Philadelphia, USA.

Background: ^{90}Yttrium–ibritumomab–tiuxetan (^{90}YIT) is approved for the treatment of relapsed follicular lymphoma or as consolidation therapy after first-line chemotherapy. Its role as therapy for the front-line treatment of early stage B-cell indolent lymphoma is under investigation.
Patient and Methods: Thirty-one patients with B-cell lymphoma: grade 1 or 2 follicular lymphoma (FL) or marginal zone lymphoma (MZL) with stage I or II were enrolled for treatment with Zevalin (^{90}YIT). Patients received ^{90}YIT according to standard procedure. Primary end points were overall response rates and progression-free survival (PFS). Initial evaluation included CT scans, PET-CT, CBC and bone marrow biopsies. Assessment of response was made at 4 months and monitored every 3, 6 and 12 months during years 1, 2 and 3 of follow-up, respectively. CBC was analyzed every 2 weeks until recovery of counts. Median and range for quantitative, absolute and relative frequencies for categorical variables were calculated. PFS was estimated by using Kaplan–Meier method.
Results: Twenty patients had FL and 11 had MZL. The median age was 57 years (range 28–87) with 6/31 (19%) having bulky lymphoma (greater than 5 cm diameter). The overall response rate was 100% with CR 87% and Cru 13% with a median follow-up of 56 months for the censored observations. The median PFS time has not been reached. Ten patients relapsed; 8/20 (40%) with FL and 2/11 (18%) with MZL. The rate of relapses was 33% in bulky and 32% in nonbulky lymphoma. Adverse events were primarily haematologic; the incidences of grade 3 and 4 neutropenia, thrombocytopenia, and anaemia were 45% and 16%, 19% and 16%, and 0% and 3%, respectively. Non-haematologic toxicity never exceeded grade 2 according to CTCAE v3.0.
Conclusion: ^{90}YIT is well tolerated and has achieved high response rates in patients with early stage B-cell indolent lymphoma. Bulky disease did not adversely affect tumour responses.

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TREATMENT OF FOLLICULAR LYMPHOMA WITH RADIO-IMMUNOTHERAPY—DATA FROM THE RADIOIMMUNOTHERAPY NETWORK, AN INTERNATIONAL DATA BASE ON DAILY CLINICAL PRACTICE

C. W. Scholz,[1] K. Hohloch,[2] A. Bischof Delaloye,[3] C. Windemuth-Kieselbach,[4] J. Gomez-Codina,[5] W. Jurczak,[6] C. Suh,[7] P. L. Zinzani,[8] L. Truemper.[9]
[1]Hematology and Oncology, Charite University Medical Center, Berlin, Germany; [2]Hematology and Oncology, Goettingen Comprehensive Cancer Center, Goettingen, Germany; [3]Service of Nuclear Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [4]Statistics, Alcedis GmbH, Giessen, Germany; [5]Servicio de Oncologia Medica, Hospital Universitario y Politecnico La Fe, Valencia, Spain; [6]Department of Haematology, Jagiellonian University, Cracow, Poland; [7]Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [8]Istituto di Ematologia e Oncologia Medica, Universita di Bologna, Bologna, Italy; [9]Hematology and Oncology, Goettingen Comprehensive Cancer Center, Goettingen, Germany.

Radioimmunotherapy (RIT) has successfully been tested in distinct clinical trials as relapse, consolidation, and first line treatment. To assess the efficacy of RIT in daily clinical practice, data on 1105 lymphoma patients, treated with RIT between December 2006 and November 2009, were collected in an international, web-based RIT network.

Results from an analysis substantially extending previously published data (J Nucl Med. 2011; 52:1354–60) are presented, that is, data from 479 follicular lymphoma patients are shown, whereas formerly, data from 271 patients were reported. Of the 479 individuals with follicular lymphoma, 219 had relapsed or refractory disease. For the whole cohort of follicular lymphoma patients ($n = 479$), overall response to RIT was 84% (CR 67%, PR 17%). At the time of analysis, 73 patients had progressed and 43 individuals had died. For the subgroup of 220 refractory or relapsed follicular lymphoma patients, overall response after a median of three prior therapies was 80% (CR 56%, PR 24%). Thirty-nine patients had progressed and 27 patients had died. For the whole group of follicular lymphoma patients, median overall survival has not been reached. In contrast, median overall survival for patients with refractory and relapsed disease was 1280 days. Additional data on toxicity, time to progression, and overall survival will be presented.

Conclusion: Data from the RIT network underscore the role of RIT as a safe and efficient treatment option for relapsed follicular lymphoma.

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UPFRONT AUTOLOGOUS STEM-CELL TRANSPLANTATION IN TRANSFORMED INDOLENT NON-HODGKINS LYMPHOMA: AN OUTCOME ANALYSIS

C. Madsen,[1] M. B. Pedersen,[1] E. K. Segel,[1] K. Bendix,[2] B. A. Jensen,[3] P. Jensen,[4] M. B. Moeller,[5] P. Johansen,[6] L. Munksgaard,[7] P. D. Brown,[8] F. A. D'Amore.[1]
[1]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark; [3]Department of Hemathology, Odense University Hospital, Odense, Denmark; [4]Department of Hemathology, Aalborg University Hospital, Aalborg, Denmark; [5]Department of Pathology, Odense University Hospital, Odense, Denmark; [6]Department of Pathology, Aalborg University Hospital, Aalborg, Denmark; [7]Department of Hemathology, Roskilde University Hospital, Roskilde, Denmark; [8]Department of Hemathology, Rigshospitalet, Copenhagen Univesity Hospital, Copenhagen, Denmark.

Introduction: When indolent non-Hodgkins lymphomas (IL) transform to a more aggressive histology (TRIL), the result is often a rapid clinical course and a shortened survival. The purpose of this study was to determine whether the outcome of these patients improves if treated upfront with autologous stem-cell transplantation (ASCT) compared with rituximab-containing chemotherapy alone.

Methods: Ninety-five patients (<65 years) with IL and histologically proven transformation diagnosed from 2002 to 2012 were identified from three Danish centres using the Danish Pathology Registry. Clinico-pathological parameters as well as treatment and outcome data were collected through the Danish Lymphoma Registry LYFO and patient records from each centre. Patient characteristics and response rates were compared using χ^2 test, Fisher's exact test or t test. Treatment outcomes were described by 5-year overall survival (OS) and progression-free survival (PFS) according to the Kaplan–Meier and compared using the log-rank test. The analyses were conducted on the cohort as a whole and on subdevisions of patients primarily diagnosed with TRIL (composite lymphoma) and patients where the transformation process occurred over time (sequential lymphoma).

Results: Sixty-five patients (67%) were treated with ASCT and 31 patients (33%) with rituximab-containing chemotherapy alone. No significant differences in clinico-pathological parameters were found between the two groups. Comparing the outcome of patients receiving ASCT with patients receiving chemotherapy alone shows an OS of 65% vs 48% ($p = 0.11$) and a PFS of 57% vs 30% ($p = 0.02$). When futher subdividing the cohort, the composite lymfomas have an OS of 80% vs 67% ($p = 0.51$) and a PFS of 75% vs 61% ($p = 0.35$) in the ASCT/non-ASCT-treated groups, respectively, whereas the sequential lymphomas have an OS of 57% vs 36% ($p = 0.09$) and a PFS of 47% vs 6% ($p = 0.003$).

Conclusions: Looking at the entire cohort, PFS but not OS was significantly improved in patients treated with upfront ASCT as compared with rituximab-containing conventional chemotherapy alone. Interestingly, both PFS and OS (borderline significant) were found to be improved when adopting upfront ASCT in patients with sequential as opposed to those with composite lymphomas. Futher studies are warranted in order to demonstrate the optimal treatment strategy for these patients.

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A POINT SCORE SIMPLIFICATION FOR PRACTICAL USE OF THE RISK STRATIFICATION FOR SPLENIC MARGINAL ZONE LYMPHOMA BASED ON HEMOGLOBIN, PLATELET COUNT, HIGH LDH LEVEL AND EXTRAHILAR LYMPHADENOPATHY

C. Montalban,[1] V. Abraira,[2,3] L. Arcaini,[4,5] E. Domingo-Domenech,[6] P. Guisado-Vasco,[1] E. Iannitto,[7] M. Mollejo,[8] E. Matutes,[9] A. J. Ferreri,[10] A. Salar,[11] S. Rattotti,[12] A. Carpaneto,[13] R. Perez,[14] J. Bello,[15] M. Hernandez,[16] D. Caballero,[17] F. Carbonell,[18] M. A. Piris.[19]
[1]Internal Medicine, Hospital Ramon y Cajal, Madrid, Spain; [2]Department of Clinical Biostatistics Unit, Hospital Ramon y Cajal, IRYCIS, Madrid, Spain; [3]CIBERESP, Madrid, Spain; [4]Department of Molecular Medicine, University of Pavia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [5]Department of Hematology Oncology, University of Pavia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [6]Hematology, Hospital Duran i Reynals, ICO, Barcelona, Spain; [7]Hematology Oncology, Ospedale S.G.Moscati, ASL, Taranto, Italy; [8]Pathology, Hospital Virgen de la Salud, Toledo, Spain; [9]Haemato Oncology, Royal Marsden Hospital/Institute of Cancer, London, UK; [10]Unit of Lymphoid Malignancies, San Raffaele Scientific Institute, Milan, Italy; [11]Hematology, Hospital del Mar, Barcelona, Spain; [12]Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [13]UOC Ematologia, Ospedale Sant'Anna, Viterbo, Italy; [14]Oncology, Hospital Gregorio Maranon, Madrid, Spain; [15]Hematology, Hospital Universitario de Santiago de Compostela, Santiago de Compostela, Spain; [16]Hematology, Hospital Universitario de Canarias, Tenerife, Spain; [17]Hematology, Hospital Clinico, Salamanca, Spain; [18]Hematology, Hospital General Universitario, Valencia, Spain; [19]Pathology, Hospital Marques de Valdecilla, Santander, Spain.

Introduction: In a large series, the Splenic Marginal Zone Lymphoma Study Group (SMZLSG) proposed a risk stratification based in the combination of haemoglobin, platelet count, high LDH and extrahilar lymphadenopathy, the HPLL/ABC scoring, that resulted in three risk groups (A, B and C) with different 5-year lymphoma specific survival (LSS) (BJH 2012;159:164); hsemoglobin level and platelet counts were used as continuous variables to provide the best fit, but this required a formula that may complicate its everyday use. To solve this, the SMZLSG decided to explore a simplification of the HPLL/ABC in order to obtain an easier score based on points for practical use.

Methods: Five hundred fifty of the 593 SMZL patients of the original series with data of all the four variables (haemoglobin, platelet count, LDH and extrahilar lymphadenopathy) were used. Clinically acceptable cut points were established for haemoglobin level (9.5 g/dL) and for platelet count ($80 \times 10^3/\mu L$). One point was given to each adverse factor, and LSS was calculated for the groups of patients having 0, 1 or 2 and ≥ 3 factors. The Kaplan–Meier method was used to estimate LSS and the log-rank test to compare curves. The extension of net reclassification improvement and the Hosmer–Lemeshow test were used to compare the accuracy and discrimination, respectively, of this scoring with the original HPLL/ABC.

Results: The three groups, (A) with 198 patients (36%) and 0 points (no adverse factors), (B) with 311 patients (56.5%) and 1–2 factors and (C) with 41 patients (7.5%) and ≥ 3 factors had, respectively, a 5-year LSS of 95%, 87% and 68%, which was significantly different in the overall ($p = 0.000$) and pairwise comparisons. This stratification, referred as HPLLs/ABC to denote the simplification due to the point-based scoring, is of easy practical use, despite an acceptable loss of accuracy with respect to the original HPLL/ABC.

Conclusions: This simplified HPLL/ABC risk stratification using cut points for haemoglobin and platelet count results in an easier point-based approach suitable for clinical practice and to use in eventual risk-adapted trials.

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PERFORMANCE OF THE MONOCLONAL ASSAY FOR CIRCULATING FREE LIGHT CHAINS IN AL AMYLOIDOSIS

G. Palladini,[1] A. Jaccard,[2] T. Bosoni,[3] D. Lavergne,[2] P. Milani,[1] S. Bender,[2] L. Pirolini,[3] M. Cogne,[2] A. Foli,[1] F. Lavatelli,[1] V. Valentini,[1] R. Albertini,[3] G. Merlini.[1]
[1]Department of Molecular Medicine and Amyloidosis Research and Treatment Center, University of Pavia and Foundation "IRCCS Policlinico San Matteo", Pavia, Italy; [2]Centre de Reference des Amyloses Primitives, CHU de Limoges, Limoges, France; [3]Clinical Chemistry Laboratory, Foundation "IRCCS Policlinico San Matteo", Pavia, Italy.

Introduction: Measurement of free light chains (FLC) is fundamental in AL amyloidosis. A novel method based on monoclonal antibodies has been marketed in Europe. We evaluated its performance in diagnosis and prognostication of survival in 353 consecutive patients diagnosed at the Amyloidosis Research and Treatment Center (ARTC, Pavia, Italy). Response was evaluated in 226 ARTC patients and 73 subjects enrolled at the Centre National de Référence pour l'Amylose AL (Limoges, France) who had data at 0 and 3 months.
Methods: FLC concentration was measured by a polyclonal [binding site (BS)] and a monoclonal [Siemens (S)] immunoassay on a Behring BNII nephelometer.
Results: The two assays had similar diagnostic sensitivity (82% for the BS and 84% for the S assay; 98% for both tests combined with serum and urine immunofixation). However, the agreement between the two methods was not optimal ($r^2 = 0.85$ for k FLC, and $r^2 = 0.60$ for l FLC). Patients in whom the difference between involved (amyloidogenic) and uninvolved FLC concentration (dFLC) was greater than the median value had a worse survival (41% vs 65% at 2 years, $p = 0.001$, with both methods). The dFLC median value by S (165 mg/L) was incorporated in a staging system including N terminal pro natriuretic peptide type B (cut-off 1800 ng/L) and troponin I (cut-off 0.07 ng/mL), providing survival discrimination between patients with 0, 1, 2, and 3 markers above median value (95%, 65%, 38%, and 20% surviving 2 years, respectively, $p < 0.001$). The median dFLC decrease in patients surviving 1 year was 67% with the BS assay and 39% with the S assay. A receiver operating characteristic analysis showed that the dFLC decrease best predicting survival at 1 year was 50% as previously reported with the BS assay and 30% with the S assay. A ≥30% dFLC decrease by the S assay resulted in a survival advantage (78% vs 64% at 2 years, $p = 0.003$).
Conclusion: The two assays have comparable diagnostic sensitivity but are not interchangeable. The S test can be used for prognostic stratification. The current haematologic response criteria designed for the BS are not applicable to the S assay, and specific criteria need to be designed and validated for the S assay.

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B-CELL NON-HODGKINS LYMPHOMA AMONG ISRAELIS AND PALESTINIANS: DIVERSE DISEASE PATTERNS AND RISK FACTORS IN POPULATIONS IN GEOGRAPHIC PROXIMITY

O. Paltiel,[1] R. Samman,[2] G. Kleinstern,[1] R. Perlman,[3] Z. Abdeen,[4] A. Khatib,[5] A. Ramlawi,[6] H. Elyan,[7] L. Shaheen,[8] F. Sabatine,[9] G. Amir,[10] Y. Hamamreh,[9] N. Salim,[9] F. Rawashdeh,[9] M. Ghoul,[4] K. Halahle,[9] D. Ben Yehuda.[3]
[1]Department of Hematology and School of Public Health, Hadassah Medical Organization, Jerusalem, Israel; [2]Hadassah-Hebrew University Medical Center, and Al Quds University, Department of Hematology, Jerusalem, Israel; [3]Department of Hematology, Hadassah Medical Center, Jerusalem, Israel; [4]Nutrition and Health Research Institute, Al Quds University, Abu Dis, Occupied Palestinian Territory; [5]Pathology, Augusta Victoria Hospital, Jerusalem, Israel; [6]Department of Primary Health Care, Ministry of Health, Ramallah, Occupied Palestinian Territory; [7]Hematology, Beit Jalla Hospital, Beit Jalla, Occupied Palestinian Territory; [8]Oncohematology, National Hospital Nablus, Nablus, Occupied Palestinian Territory; [9]Cancer Care Center, Augusta Victoria Hospital, Jerusalem, Israel; [10]Hadassah Medical Center, Department of Pathology, Jerusalem, Israel.

Background and Methods: Israelis and Palestinians represent genetically and culturally diverse populations living in geographic proximity. We have undertaken a unique collaborative case–control study of demographic, environmental, infectious and genetic determinants of B-cell non-Hodgkin lymphoma (B-NHL) in our region focusing on innate immunity, infection history, family history, exposures and disease characteristics among Palestinian Arabs (PA) and Israeli Jews (IJ).
Results: We report herein preliminary results on 679 B-NHL patients and 641 controls recruited to date. Among 310 PA cases, the mean age at disease onset was 50.3 years, SD = 16.2, whereas for IJ, it was 59.2 years, SD = 14.7 ($p < 0.0001$). Diffuse large B-cell lymphoma comprised 62% and 48% among PA and IJ cases, whereas follicular lymphoma comprised 11% and 26% ($p < 0.0001$), respectively. In terms of risk factors, after adjusting for age and sex, high birth order was protective for B-NHL [odds ratio (OR): 0.56, 95% confidence interval (0.36–0.88)] among PA, whereas the opposite pattern emerged among IJ [OR 1.48 (0.95–2.3)]. Gardening as a hobby was associated with B-NHL among PA [OR 1.48 (1.65–2.07)], but not IJ, whereas frequent (>once per week) household pesticide use was positively associated in both [pooled OR 1.8 (1.4–2.8)]. Self-reports of herpes infection [pooled OR 1.7 (1.26–2.3)] and hepatitis B or C [pooled OR 2.09 (1.21–3.6)] were more common among cases than controls in both populations. Similarly, a positive family history of cancer in a first degree relative was consistently associated with B-NHL [pooled OR 1.37 (1.07–1.75)].
Conclusions: Comparing PA and IJ cases, distinctive histologic patterns of B-NHL emerge. Age at disease onset, disease is 9 years younger among PA cases. Some demographic factors and personal exposures differ among the two populations, whereas personal history of specific viral infections, family history of cancer and household pesticide use represent common risk factors. Further planned assessment of single nucleotide variation and its relation to B-NHL occurrence will further clarify whether these differential results have a genetic basis and will enable the analysis of gene–environment interactions.

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EFFECT OF PLACE OF RESIDENCE AND CARE ON SURVIVAL OUTCOMES IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

D. Villa,[1] B. Lee,[2] O. Goktepe,[1] K. Hay,[2] J. Connors,[1] L. Sehn,[1] K. Savage,[1] T. Shenkier,[1] R. Klasa,[1] T. Shenkier.[1]
[1]Centre for Lymphoid Cancer, British Columbia Cancer Agency, Vancouver, Canada; [2]Faculty of Medicine, University of British Columbia, Vancouver, Canada.

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma and is potentially curable with chemoimmunotherapy. Timely administration of therapy at appropriate dose intensity is considered vital for optimizing outcome. We examined the relationship between location of residence at the time of diagnosis of DLBCL and health outcomes in a geographically large Canadian province with publicly funded, universally available medical care.
Patients and Methods: The BC Cancer Registry was used to identify all patients 18–80 years of age diagnosed with DLBCL in British Columbia (BC) between Jan 2003 and Dec 2008. Home and treatment centre postal codes were used to determine urban versus rural status, as well as driving distances to access treatment. Information on treatement received, including chemotherapy, radiation, and stem cell transplantation, was obtained using the centralized pharmacy database and electronic medical records. Urban areas were defined as having a population of at least 1000 and a population density of >400 per square kilometer, and further subdivided into small (population 1000–29 000), medium (population (30 000–99 999), and large (population >100 000). All other areas were considered rural.
Results: One thousand one hundred forty patients were identified, with median age 64 years (range 18–80), 60% male, 47% stage III/IV, 34% received radiotherapy, and 4% underwent stem cell transplantation. One thousand three hundred fifty-five (94%) had DLBCL-NOS, of which 1101 (81%) received R-CHOP, whereas the rest received palliative chemo or radiotherapy. The remaining 85 (6%) patients had primary CNS lymphoma, of which 36 received high-dose methotrexate. One hundred ninety-seven (14%) resided in a rural area and 1243 (88%) in an urban area at diagnosis: 151 (11%) small, 194 (14%) medium, and 898 (62%) large urban. Patients from medium urban areas presented with more B symptoms, and patients in large urban areas had worse performance status and greater involvement of extranodal sites. About 80% were referred to BCCA for management, although those in rural and small urban areas were less likely to be referred ($p = 0.03$). Patients in small urban locations were less likely to receive chemotherapy and radiotherapy compared with those residing in other areas ($p < 0.001$). The 5-year overall survival (OS) was 59% for patients in rural, 57% in large urban, 51% in medium urban, and 43% in small urban areas ($p = 0.027$). In a multivariate analysis controlling for prognostic factors, geographic region, driving distances to access treatments, and referral to BCCA, there was no difference in OS between rural and large urban patients (HR 1.0, 95% CI 0.8, 1.4), although patients in small (HR 1.4, 95% CI 1.0, 1.8) and medium (HR 1.4, 95% CI 1.1, 1.8) had statistically significant worse OS compared with those in large urban areas.
Conclusion: Place of residence at diagnosis is associated with survival of patients with DLBCL in BC. Rural patients have similar survival to those in large urban areas, whereas patients living in small and medium urban areas experience worse outcomes. Differential access to curative and palliative treatments across different urban and rural areas may account for this effect, although there may be

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other associated factors. Further investigation is needed to confirm whether this is a real finding that can be improved.

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AN ENHANCED INTERNATIONAL PROGNOSTIC INDEX (NCCN-IPI) FOR RISK STRATIFICATION OF DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS IN THE RITUXIMAB ERA: VALIDATION USING THE BRITISH COLUMBIA CANCER AGENCY DATABASE

Z. Zhou,[1] L. H. Sehn,[2] A. Rademaker,[3] L. I. Gordon,[1] A. S. LaCasce,[4] A. Crosby-Thompson,, A. Vanderplas,[5] M. A. Rodriguez,[6] A. D. Zelenetz,[7] G. A. Abel,[4] A. Nademanee,[8] M. S. Kaminski,[9] M. Czuczman,[10] M. M. Millenson,[11] J. C. Niland,[5] R. D. Gascoyne,[2] J. M. Connors,[2] J. W. Friedberg,[12] J. N. Winter.[1]

[1]Medicine, Division of Hematology and Oncology, Northwestern University, Chicago, USA; [2]Hematology and Oncology, BC Cancer Agency, Vancouver Clinic, Vancouver, Canada; [3]Preventive Medicine, Northwestern University, Chicago, USA; [4]Hematology and Oncology, Dana-Farber Cancer Institute, Boston, USA; [5]Biostatistics, City of Hope National Medical Center, Duarte, USA; [6]Hematology and Oncology, University of Texas MD Anderson Cancer Center, Houston, USA; [7]Hematology and Oncology, Memorial Sloan-Kettering Cancer Center, New York, USA; [8]Hematology and Oncology, City of Hope National Medical Center, Duarte, USA; [9]Hematology and Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, USA; [10]Hematology and Oncology, Roswell Park Cancer Institute, Bufallo, USA; [11]Hematology and Oncology, Fox Chase Cancer Center, Philadelphia, USA; [12]Hematology and Oncology, James P. Wilmot Cancer Center, University of Rochester, Rochester, USA.

Background: The U.S. National Comprehensive Cancer Network Prognostic Index (NCCN-IPI) was recently developed for initial risk stratification of diffuse large B-cell lymphoma (DLBCL) cases based on 1650 patients treated in the rituximab era (Zhou, ASH'12 #2656). It showed an enhanced capacity to discriminate risk groups, especially a high-risk subset (5-year overall survival [OS] = 33%). The index used an 8-point (pt) scale (pts: age: 1 if 40–60 years; 2 if 61–75; 3 if >75; LDH:1 if 1–3×'s normal; 2 if >3×; stage: 1 if III/IV; extranodal sites: 1 if CNS, bone marrow, lung or liver/GI; performance status: 1 if 2–4). Four risk groups were defined: low (0–1 pt), low-intermediate (2–3), intermediate-high (4–5) and high risk (>6). We report the external validation of the NCCN-IPI compared with the IPI in the fully independent British Columbia Cancer Agency (BCCA) dataset.
Methods: The two cohorts were compared with respect to baseline demographics and disease characteristics. DLBCL patients from both cohorts were risk stratified based on the NCCN-IPI and standard IPI. Validation of NCCN-IPI compared with IPI was assessed for Discrimination measured by the concordance index (95% CI) from the training (NCCN) and validation (BCCA) datasets, respectively. The performance of the two indexes was compared for Akaike's information criteria (AIC) in the two cohorts. Finally, K–M estimates were compared between the two indexes for each risk stratum in the BCCA data.
Results: The NCCN ($n = 1650$) and BCCA ($n = 1138$) cohorts differ in age (median: 57 vs 63), gender (%male: 54 vs 60) and ECOG PS (% 2–4: 11 vs 37), as well as % with extranodal involvement of vital organs (36 vs 25). NCCN-IPI outperformed IPI in both cohorts with higher concordance index in discrimination (0.77 vs 0.74) and better global model fit (AIC: 4349 vs 4378). Within the BCCA cohort, 5-year K–M OS estimates for the NCCN-IPI and IPI differ in the high risk groups, 38% (29–46%) vs 43% (36–49%), as well as the low risk category, 96% (90–99%) vs 84% (80–88%).
Conclusions: The greater capacity of the NCCN-IPI to risk stratify was maintained in the current external data validation, despite differences in patient population. Notably, it demonstrated improved discrimination not only in the high-risk category but also in the low-risk stratum. The NCCN-IPI is a robust and valuable prognostic index for DLBCL patients in the rituximab era.

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IMPACT OF GENDER, BODY MASS INDEX AND BODY SURFACE AREA ON OUTCOMES OF DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH RITUXIMAB: ANALYSIS OF THE U.S. NATIONAL COMPREHENSIVE CANCER NETWORK DATABASE

Z. Zhou,[1] A. Rademaker,[2] L. I. Gordon,[1] A. S. LaCasce,[3] A. Crosby-Thompson,[3] A. Vanderplas,[4] M. A. Rodriguez,[5] A. D. Zelenetz,[6] G. A. Abel,[3] A. Nademanee,[7] M. S. Kaminski,[8] M. Czuczman,[9] M. M. Millenson,[10] J. C. Niland,[4] J. W. Friedberg,[11] J. N. Winter.[1]

[1]Hematology and Oncology, Northwestern University Feinberg School of Medicine, Chicago, USA; [2]Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, USA; [3]Hematology and Oncology, Dana-Farber Cancer Institute, Boston, USA; [4]Biostatistics, City of Hope National Medical Center, Duarte, USA; [5]Hematology and Oncology, University of Texas MD Anderson Cancer Center, Houston, USA; [6]Hematology and Oncology, Memorial Sloan-Kettering Cancer Center, New York, USA; [7]Hematology and Oncology, City of Hope National Medical Center, Duarte, USA; [8]Hematology and Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, USA; [9]Hematology and Oncology, Roswell Park Cancer Institute, Buffalo, USA; [10]Hematology and Oncology, Fox Chase Cancer Center, Philadelphia, USA; [11]Hematology and Oncology, James P. Wilmot Cancer Center, University of Rochester, Rochester, USA.

Background: Previous reports from prospective clinical trials suggested that gender and weight influence rituximab (R) metabolism and, consequently, clinical outcomes for diffuse large B-cell lymphoma (DLBCL) patients after R-containing therapy. To further investigate these associations, we studied the effect of gender, body mass index (BMI) and body surface area (BSA), and potential interactions between these factors on treatment outcomes in DLBCL patients from the U.S. National Comprehensive Cancer Network database.
Methods: *De novo* DLBCL cases diagnosed between 6/2000 and 12/2010, treated with R as part of first-line therapy, were assessed for progression-free survival (PFS) and overall survival (OS) at 3 and 5 years as a function of gender, BMI and BSA at presentation by Cox regression. Effect modifications of gender by BMI (≤18.5, >18.5–25 or >25) or BSA (≤2 or >2) were tested using model interaction terms. BSA was viewed as an index for R dosing in this context. The effect of these factors was also adjusted for the international prognostic index (IPI) for survival outcomes. Finally, analyses were repeated specifically in the elderly cohort (age >60 years).
Results: Among 1420 DLBCL patients confirmed to have received R, males (54%) were less likely to survive (OS: 3-year HR 1.6, $p < 0.01$; 5-year HR 1.4, $p = 0.01$) and were more likely to progress (PFS: 5-year HR 1.3, $p = 0.02$). The adverse risk associated with male gender did not change after adjusting for BMI or BSA, and there was no significant interaction with either factor ($p > 0.05$). Underweight males (BMI ≤18.5) had the lowest 5-year OS (HR: 4.3, $p < 0.01$) and PFS (HR: 2.5, $p < 0.02$) compared with females with normal or high BMIs. Risk remained largely unchanged after adjusting for IPI. Low versus high BMI independently predicted poor outcomes (5-year OS, HR: 2.5, $p < 0.01$). In either gender, higher BSA (≥2) correlated with lower risk of 5-year mortality (HR: 0.65, $p < 0.01$). Analysis of the elderly subset ($n = 624$) yielded similar results.
Conclusions: Male gender was associated with worse outcomes in DLBCL patients treated with R-containing therapy. Higher BMI or BSA favourably impacted outcomes after considering the effect of gender as well as IPI. The magnitude of the gender effect was not impacted by either BMI or BSA. The benefit associated with higher BSA underscores the importance of adequate R dosing.

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MALE SEX EVOLVES AS A SIGNIFICANT RISK FACTOR IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB ONLY IN ELDERLY, BUT NOT IN YOUNG PATIENTS: CORRELATION WITH RITUXIMAB PHARMACOKINETICS

M. Pfreundschuh,[1] C. Mueller,[2] S. Zeynalova,[3] G. Held,[1] V. Poeschel,[1] C. Zwick,[1] T. Rixecker,[1] N. Schmitz,[4] N. Murawski.[1]

[1]Internal Medicine I, Saarland University Medical School, Homburg, Germany; [2]Department of Pharmacology, University Hospital Cologne, Cologne, Germany; [3]IMISE, University Leipzig, Leipzig, Germany; [4]Hematology/Oncology, Asklepios Hospital St. Georg, Hamburg, Germany.

Introduction: Sex and weight influence rituximab (R) clearance in elderly diffuse large B-cell lymphoma (DLBCL) patients (pts) (Mueller et al., Blood 2012).
Methods: We analyzed the impact of sex on R pharmacokinetics and outcome of 1222 elderly pts of the RICOVER-60, 823 young (18 to 60 years) age-adjusted international prognostic index (aaIPI) = 0.1 pts of the MInT, and 275 aaIPI = 2.3 pts of the Mega-CHOEP trials. R pharmacokinetics was determined in 33 young and 49 elderly pts. Population pharmacokinetic modeling was performed with nonlinear mixed-effect modeling software (NONMEM VI).
Results: R clearance was independent of tumour mass (IPI) but weakly correlated (0.2, R^2 linear = 0.045) with increasing age in male and moderately inversely correlated (−0.5, R^2 linear = 0.207) with age in female DLBCL patients, resulting in similar R clearances in young female and male pts (9.88 vs 10.38 mL/h; $p = 0.238$), but a significantly slower R clearance in elderly males compared with females (10.50 vs 8.25 mL/h; $p = 0.006$). In the RICOVER-60 trial, elderly females had a higher 3-year progression-free survival (PFS) (68% vs 61%) and overall survival (OS) (74% vs 68%) than male pts because of a greater outcome improvement by the addition of R in females. In a multivariable analysis adjusting for IPI, the male hazard for progression was not significantly increased after CHOP (HR = 1.1; $p = 0.348$) but was significantly higher after R-CHOP (OR = 1.6; $p = 0.004$). In contrast, young males

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treated in the MInT and Mega-CHOEP trials benefitted as much as females from the addition of R, with a similar male hazard after CHOP and R-CHOP (HR = 1.2) with no significant difference to female pts ($HR_{PFS} = 1.2$, $p = 0.552$; $HR_{OS} = 1.0$; $p = 0.898$).
Conclusions: While no differences in R clearance and benefit from rituximab were found in young female compared with male pts, the reduced benefit of adding R to CHOP in elderly male DLBCL pts who have a shorter R serum half life and hence lower serum levels suggests that this subpopulation is suboptimally dosed when R is given based on body surface area at 375 mg/m^2. Appropriately designed prospective DSHNHL studies investigate whether higher R doses for pts with a shorter R serum half life can improve the outcome of the respective patients.

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BCL2 PROTEIN EXPRESSION COMBINED WITH EARLY [^{18}F]FLUORO-DEOXYGLUCOSE-POSITRON EMISSION TOMOGRAPHY RESPONSE ALLOWS IMPROVED STRATIFICATION OF LARGE B-CELL LYMPHOMA PATIENTS

C. Copie-Bergman,[1] E. Itti,[2] J. Moroch,[1] V. Safar,[3] M. Baia,[1] J. Dupuis,[4] K. Belhadj,[4] O. Casanovas,[5] T. Petrella,[6] C. Fruchart,[7] P. Vera,[8] A. Berriolo-Riedinger,[9] J. Picquenot,[10] S. Bardet,[11] E. Lechapt,[12] H. Tilly,[13] M. Meignan,[2] C. Haioun.[4]
[1]Departement de Pathologie, Hopital Henri Mondor, Creteil, France; [2]Service de Medecine Nucleaire, Hopital Henri Mondor, Creteil, France; [3]Hematology Department, CHLS, Lyon, France; [4]Unite Hemopathies Lymphoides, Hopital Henri Mondor, Creteil, France; [5]Hematology Department, CHU Dijon, Dijon, France; [6]Departement de Pathologie, CHU Dijon, Dijon, France; [7]Hematology Department, Centre F. Baclesse, Caen, France; [8]Medecine Nucleaire, Centre Henri Becquerel, Rouen, France; [9]Medecine Nucleaire, Centre Georges-Francois Leclerc, Dijon, France; [10]Departement de Pathologie, Centre Henri Becquerel, Rouen, France; [11]Medecine Nucleaire, Centre F. Baclesse, Caen, France; [12]Departement de Pathologie, CHU de Caen, Caen, France; [13]Service d'Hematologie, Rouen, France.

Introduction: Diffuse large B-cell lymphoma (DLBCL) represents a heterogeneous group of disease with variable clinical, morphological and molecular features. Early interim [^{18}F]fluoro-deoxyglucose-positron emission tomography (FDG-PET) is a powerful tool to predict outcome in patients treated with rituximab-based chemotherapy. BCL2, MYC gene alterations and/or protein overexpression represent additional interesting biomarkers that have been associated with inferior survival.
Methods: Ninety-one patients with *de novo* DLBCL treated with rituximab-CHOP/CHOP-like regimens were included in the study. All patients underwent baseline and interim PET after two cycles of treatment. Tumour specimens were centrally reviewed by expert pathologists and evaluated for BCL2 and MYC protein expression by immunohistochemistry and BCL2, BCL6 and MYC gene alterations using interphase fluorescent *in situ* hybridization.
Results: Mean age was 55 years (18–78) with a male predominance (M/F = 56/35); 84% had stage III–IV Ann Arbor disease and 35% bone marrow involvement. BCL2 protein expression >50% and MYC protein expression >40% were observed in 68% (59/86) and 33% (30/89) of cases, respectively. BCL2, BCL6 and MYC gene rearrangement and/or amplification were shown in 30% (25/83), 20% (17/83) and 13% (11/83) of cases, respectively. Only BCL2 gene alterations were predictive of poor progression-free survival (PFS) ($p = 0.03$). Patients with positive (pos) interim PET ($n = 26$), evaluated with Deauville criteria, showed lower PFS ($p = 0.0003$) and overall survival (OS) compared with negative (neg) patients ($n = 65$) ($p = 0.002$). BCL2 protein expression combined with early PET response allowed better stratification of patients, with 3-year PFS estimates of 84% (neg–neg, $n = 20$) vs 71% (neg/pos, $n = 49$) vs 43% (pos–pos, $n = 17$), respectively ($p = 0.007$). OS prediction was less significant ($p = 0.04$). Similarly, BCL2 gene alterations combined with early PET response was predictive of PFS ($p = 0.0002$) and OS ($p = 0.001$).
Conclusion: Combined evaluation of BCL2 expression with rapidity of metabolic response by PET identifies patients with good outcome (BCL2 neg/rapid metabolic response) and poor outcome (BCL2 pos/slow metabolic response).

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FCGR3A/FCGR2A POLYMORPHISMS AND PROGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH IMMUNO-CHEMOTHERAPY: RESULTS FROM A META-ANALYSIS BASED ON 1134 PATIENTS FROM TWO INDEPENDENT PROSPECTIVE COHORTS

H. Ghesquieres,[1] B. R. Larrabee,[2] C. Haioun,[3] B. K. Link,[4] A. Verney,[5] S. L. Slager,[2] N. Ketterer,[6] S. M. Ansell,[7] R. Delarue,[8] M. J. Maurer,[2] O. Fitoussi,[9] T. M. Habermann,[7] F. Peyrade,[10] A. Dogan,[11] T. J. Molina,[12] A. J. Novak,[2] H. Tilly,[13] J. R. Cerhan,[2] G. Salles.[14]
[1]Onco-Hematology UMR5239 CNRS, Centre Leon Berard Universite Claude Bernard, Lyon, France; [2]Health Sciences Research, Mayo Clinic, Rochester, MN, USA; [3]Unite Hemopathies Lymphoides, Hopital Henri Mondor, Creteil, France; [4]Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA, USA; [5]UMR5239 CNRS, Universite Claude Bernard, Lyon, France; [6]Medical Oncology, Lausanne University Hospital, Lausanne, Switzerland; [7]Division of Hematology, department of Internal Medicine, Mayo Clinic, Rochester, MN, USA; [8]Hematology, APHP, Hopital Necker, Paris, France; [9]Onco-Hematology, Polyclinique Bordeaux-Nord, Bordeaux, France; [10]Medical Oncology, Centre Antoine Lacassagne, Nice, France; [11]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; [12]Pathology, Descartes University, Paris, France; [13]Hematology, Centre Henri Becquerel, Rouen, France; [14]Hematology, UMR5239 CNRS, Hospices Civils, Universite Claude Bernard, Lyon, France.

Introduction: Single nucleotide polymorphisms (SNPs) in FCγ-receptor genes FCGR3A (rs396991) and FCGR2A (rs1801274) influence the binding affinity of the Fc portion of anti-CD20 monoclonal antibody. Their roles in diffuse large B-cell lymphoma (DLBCL) treated with rituximab in combination with chemotherapy remain controversial.
Methods: FCGR2A and FCGR3A SNPs were genotyped in two prospective DLBCL cohorts from Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials ($N = 554$) and Iowa/Mayo Specialized Program Of Research Excellence (SPORE) ($N = 580$). Correlation with treatment response and haematological toxicity were assessed in GELA. Correlation with progression-free survival (PFS) and overall survival (OS) was performed in the two cohorts. In order to increase power, the results of the two series were combined in a meta-analysis.
Results: No correlation between these SNPs with treatment response was observed. At least one grade 3–4 febrile neutropenia during treatment was more frequently observed in FCGR3A VV (39%) than VF (29%) and FF (32%) carriers ($p = 0.04$). In the two series and in combined analysis, FCGR3A was not associated with outcome. In the meta-analysis using an ordinal model, FCGR2A was associated with PFS (HR = 0.87; 95% CI, 0.76–0.99; $p = 0.04$) and OS (HR = 0.86; 95% CI, 0.73–1.00; $p = 0.05$) and remained significant after international prognostic index adjustment for PFS (HR = 0.85; 95% CI, 0.74–0.97; $p = 0.02$) and OS (HR = 0.83; 95% CI, 0.71–0.97; $p = 0.02$). We observed that the 3-year PFS was 75.4% vs 67.4% ($p = 0.08$) and 72.4% vs 64.8% ($p = 0.06$) for FCGR2A RR versus FCGR2A H in GELA and the SPORE, respectively. A better OS in GELA (81.5% vs 73.4, $p = 0.04$) and a trend in the SPORE (85.9% vs 78%, $p = 0.10$) were observed for FCGR2A RR versus FCGR2A H patients. No significant heterogeneity was observed in sensitivity analyses by sex, tumour burden and absolute lymphocyte count.
Conclusions: The meta-analysis showed that DLBCL patients with the low affinity FCγRIIA RR had a better outcome than FCγRIIA H carriers. Whether rituximab efficacy is improved in FCγRIIA RR DLBCL patients because of a clearance reduction or other functions of FCγRIIA should be investigated.

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R-CHOEP-14 IMPROVES OVERALL SURVIVAL IN YOUNG HIGH-RISK PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA COMPARED WITH R-CHOP-14. AN UPDATED ANALYSIS WITH 5 YEARS MEDIAN FOLLOW-UP OF THE POPULATION-BASED INVESTIGATION FROM THE DANISH LYMPHOMA GROUP

A. O. Gang,[1] M. Pedersen,[1] F. D'Amore,[2] L. M. Pedersen,[3] A. Bukh,[4] B. B. Pedersen,[5] M. B. Moeller,[6] T. Mourits-Andersen,[7] S. Pulczynski,[8] P. D. Brown.[9]
[1]Hematology, Herlev Hospital, Herlev, Denmark; [2]Hematology, Aarhus, Denmark; [3]Hematology, Roskilde Hospital, Roskilde, Denmark; [4]Hematology, Aarhus Hospital, Aarhus, Denmark; [5]Hematology, Aarhus Hospital, Aarhus, Denmark; [6]Pathology, Odense, Denmark; [7]Hematology, Sydvestjydsk Hospital, Esbjerg, Denmark; [8]Hematology, Holstebro Hospital, Holstebro, Denmark; [9]Hematology, Rigshospitalet, Copenhagen, Denmark.

Background: Optimal treatment of young patients with high-risk diffuse large B-cell lymphoma (DLBCL) remains a matter of debate. Improvement has been achieved with the use of rituximab, possibly in combination with dose-dense regimens given every 2 weeks (R-CHOP-14, R-CHOEP-14). In 2010, we reported a retrospective population-based analysis, where we compared R-CHOP-14 with R-CHOEP-14 in a cohort of high-risk DLBCL patients aged 18–60 years with two or more additional risk factors (advanced stage, elevated s-LDH, and performance status >1) (Ann Oncol 2012;23:147–53). This is an updated outcome report of the same cohort based on an extended 5-year median follow-up.
Methods: We obtained data for the period 2004–2009 from the Danish Lymphoma Group's Database. One hundred and fifty-nine patients were included in the study. Analyzed end-points wereoverall response rate, overall survival (OS), and progression-free survival (PFS). Median follow-up for all alive patients was 70 months.

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Results: The updated results confirmed a superior 5-year OS in the R-CHOEP-14 group, that is, 79% compared with 63% for R-CHOP-14-treated patients ($p = 0.046$). This superiority is confirmed by an updated5-year PFS of 75% for the R-CHOEP-14 group compared with 53% for the R-CHOP-14 group ($p = 0.006$).
Conclusion: The present study update with extended median follow-up confirms the impression of a higher efficacy of R-CHOEP-14 for young patients with high-risk DLBCL with improved OS and PFS compared with R-CHOP-14. The superiority especially in PFS possibly reflects rescue treatment with HDT. Our results are in line with data from the German MegaCHOEP trial, in which the control arm with R-CHOEP-14 remains an efficient treatment option for these patients.

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COMBINATION OF LENALIDOMIDE WITH RCHOP (R2CHOP) OVERCOMES NEGATIVE PROGNOSTIC IMPACT OF NON-GCB PHENOTYPE IN DLBCL—A PHASE 2 STUDY

G. Nowakowski, C. B. Reeder, J. M. Foran, L. F. Porrata, W. R. Macon, P. B. Johnston, C. E. Rivera, T. M. Habermann, D. J. Inwards, I. N. Micallef, B. LaPlant, G. D. Nelson, S. M. Ansell, T. E. Witzig.
Hematology, Mayo Clinic, Rochester, USA.

Background: The non-germinal centre B-cell (non-GCB) subtype of diffuse large B-cell lymphoma (DLBCL) is associated with an inferior outcome in patients (pts) treated with RCHOP. Lenalidomide has significant single agent activity in relapsed DLBCL, particularly in the non-GCB subtype. Lenalidomide 25 mg days 1–10 of the cycle can be safely combined with RCHOP21 (R2CHOP) (Leukemia 2011 Dec; 25(12): 1877). We now present phase 2 results of R2CHOP with outcomes based on the phenotypic subtype of DLBCL.
Methods: Eligible pts were adults with newly diagnosed DLBCL or follicular lymphoma grade 3 (FLIII). The DLBCL subtype was assessed by Hans algorithm (Blood 2004;103:275–82). All pts received R2CHOP × six cycles with response assessment using PET/CT at end of therapy. The progression-free survival (PFS) was defined as time from diagnosis to disease progression or death.
Results: Sixty-seven pts have been enrolled—94% (63/67) DLBCL and 6% (4/67) FLIII. The median age was 65 years (range, 19–87); 66% were males. International prognostic index (IPI) was low, low-interm., high-interm. and high in 15%, 40%, 33% and 12% pts respectively. Haematological toxicities were thrombocytopenia and neutropenia grades 3 and 4 (21% and 18%, and 15% and 73% of pts, respectively). The most frequent non-haematologic toxicities were febrile neutropenia (8%) and fatigue (5%). There was one death (2%) due to bowel perforation. For 63 pts evaluable for response, the overall (ORR) and complete response (CR) rates were 98% and 74%, respectively. PFS was 66% (95% CI: 55–80%) at 18 months (mo). Because the enrolled pts represented a relatively high-risk group, the PFS was compared a contemporary cohort of 87 consecutive DLBCL pts with similar characteristics in the Mayo Clinic Lymphoma Database and treated with standard RCHOP. The PFS at 18 mo in the RCHOP cohort was 57% (95% CI: 48–69%). The non-GCB pts treated with RCHOP alone, as expected, had a significantly worse PFS when compared with GCB patients—18-mo PFS of 32% (95% CI: 19–55%) and 70% (95% CI: 59–82%), respectively, ($p = 0.002$). In contrast, the non-GCB pts treated with R2CHOP had an 18-mo PFS of 73% (95% CI: 55–97%), not significantly different than the 18-mo PFS of 55% (95% CI: 37–83%) of GCB pts, ($p = 0.78$).
Conclusion: R2CHOP is well tolerated with ORR, CR and PFS rates in elderly high IPI pts that compare favourably with RCHOP-treated patients. The addition of lenalidomide to RCHOP appears to overcome the negative prognostic impact of the non-GCB phenotype on outcome.

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PHASE 2 STUDY OF RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, AND PREDNISONE WITH BORTEZOMIB (VCR-CAP) OR VINCRISTINE (R-CHOP) IN PATIENTS WITH NEWLY DIAGNOSED NON-GERMINAL CENTRE B-CELL-LIKE DIFFUSE LARGE B-CELL LYMPHOMA

F. Offner,[1] O. Samoilova,[2] E. Osmanov,[3] H. Eom,[4] M. Topp,[5] J. Raposo,[6] V. Pavlov,[7] D. Ricci,[8] E. Zhu,[8] H. van de Velde,[9] C. Enny,[8] A. Rizo,[9] B. Ferhanoglu.[10]
[1]Department of Hematology, University Hospital Gent, Gent, Belgium; [2]Hematology Department, Nizhniy Novgorod Region Clinical Hospital, Nizhniy Novgorod, Russia; [3]Hematology Department, N. N. Blokhin Cancer Research Center, Russian Academy of National Sciences, Moscow, Russia; [4]Hematology-Oncology Clinic, National Cancer Center, Goyang Gyeonggi, Korea; [5]Hematology Department, University of Wuerzburg, Wuerzburg, Germany; [6]Hematology Department, Santa Maria Hospital, Lisbon, Portugal; [7]Hematology Department, Medical Scientific Radiology Center, Moscow, Russia; [8]Oncology, Janssen R&D, Raritan, USA; [9]Oncology, Janssen R&D, Beerse, Belgium; [10]Internal Medicine, Division of Hematology, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey.

Introduction: The constitutive overexpression of NFkB in non-germinal centre B-cell-like diffuse large B-cell lymphoma (non-GCB DLBCL) makes it a potential target for therapeutic intervention. Patients (pts) with non-GCB versus GCB DLBCL show inferior outcomes with standard frontline R-CHOP therapy, and more efficacious combination regimens are needed. Bortezomib (Vc) has shown specific benefit in non-GCB DLBCL consistent with Vc-mediated NFkB inhibition. Substituting Vc for vincristine in R-CHOP may improve efficacy in non-GCB DLBCL.
Methods: Pts with newly diagnosed non-GCB DLBCL centrally confirmed by immunohistochemistry (Hans method) were randomized 1:1 to receive up to six 21-day cycles of rituximab 375 mg/m^2 IV, cyclophosphamide 750 mg/m^2 IV, doxorubicin 50 mg/m^2 IV, all on day 1, and prednisone 100 mg/m^2 PO on days 1–5, plus Vc 1.3 mg/m^2 IV, days 1, 4, 8, and 11 (VcR-CAP), or vincristine 1.4 mg/m^2 IV (max 2 mg), day 1 (R-CHOP). The primary efficacy endpoint was complete response (CR) rate by central radiology. Secondary endpoints included overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and safety.
Results: One hundred sixty-four pts were randomized [R-CHOP $n = 80$, VcR-CAP $n = 84$; 54% male; median age 59 years (range 20–84); 51% high/high-intermediate international prognostic index]. Baseline characteristics were similar between arms. Ninety-two per cent (R-CHOP) vs 87% (VcR-CAP) of pts completed six cycles. Median follow-up was 12.9 months. There were no significant differences in response rates, 1-year PFS, or 1-year OS between arms (table). Rates of grade ≥3 adverse events (AEs) (89% R-CHOP, 88% VcR-CAP), serious AEs (34%, 38%), and deaths due to AEs (5%, 2%) were similar between arms. Common grade ≥3 AEs were neutropenia (81%, 78%), leukopenia (23%, 22%), thrombocytopenia (3%, 37%), and febrile neutropenia (20%, 9%). Peripheral neuropathy was seen in 22% vs 32% (3% vs 6% grade ≥3).
Conclusions: VcR-CAP did not improve CR rate versus R-CHOP in newly diagnosed non-GCB DLBCL; safety profiles were similar. The impact on PFS and OS requires extended follow-up.

Abstract 214 Table

	R-CHOP	VcR-CAP		
Response, %	***n* = 74**	***n* = 76**	**OR**	***p*-value**
CR	64	65	1.038	0.915
ORR	99	93	0.208	0.110
Outcome, %	*n* = 80	*n* = 84	HR	
1-year PFS	84	79	1.42	0.413
1-year OS	84	94	0.66	0.417

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RESULTS OF A PHASE 2 MULTICENTRE STUDY OF THE ADDITION OF RITUXIMAB TO CODOX-M/IVAC FOR UNTREATED BURKITTS'S LYMPHOMA: IMPACT OF PLASMA AND CEREBROSPINAL FLUID RITUXIMUB LEVELS

A. M. Evens,[1] K. R. Carson,[2] J. M. Kolesar,[3] C. Nabhan,[4] P. F. Caimi,[5] S. A. Gregory,[6] L. I. Gordon,[7] P. Barr.[8]
[1]Division of Hematology/Oncology, The University of Massachusetts Medical School, Worcester, USA; [2]Division of Hematology/Oncology, Washington University School of Medicine, St. Louis, USA; [3]University of Wisconsin Carbone Cancer Center, School of Pharmacy, University of Wisconsin-Madison, Madison, USA; [4]Division of Hematology/Oncology, Advocate Lutheran General Hospital, Park Ridge, USA; [5]Division of Hematology/Oncology, Case Western University, Cleveland, USA; [6]Division of Hematology/Oncology, Rush University Medical Center, Chicago, USA; [7]Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, USA; [8]Division of Hematology/Oncology, University of Rochester Medical Center, Rochester, USA.

Background: Despite improvement with intensive multi-agent chemotherapy, the 2-year survival rates for adult Burkitt's lymphoma (BL) remains <65%. Furthermore, there remains a lack of prospective data examining the addition of rituximab (R) to CODOX-M/IVAC in BL.

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Methods: Twenty-five patients (pts) with classic BL were enrolled (NCT00392990). Low risk (LR) pts received three CODOX-M cycles, whereas high risk (HR) pts had four alternating CODOX-M/IVAC cycles. Liposomal doxorubicin (40 mg/m^2) was used in lieu of doxorubicin, and intravenous R (500 mg/m^2) was given days 0 + 8 of CODOX-M and days 0 + 6 of IVAC cycles. Correlative analyses of paired plasma and cerebrospinal fluid (CSF) R levels were obtained from the first 10 pts for cycles 1 + 3 at 24 and 72 h after day 0 R infusions.
Results: Median age was 44 years (23–70). There were 20 HR and 5 LR pts; three HR pts and one LR pt were HIV+. Fifiteen per cent of HR pts had + CNS disease. Therapy was completed at a median of 13 weeks (11–20) for HR pts and 10 weeks for LR pts (9–12). Myelosuppression and mucositis were comparable with prior CODOX-M/IVAC data. Notably, no grade 3/4 neuropathy occurred. The objective response rate after two cycles was 100% (67% complete remission). At a median follow-up of 34 months, the 2-year progression-free survival (PFS) and overall survival (OS) rates for all pts were 86% and 86%, respectively (LR 2-year PFS and OS: both 100%; and HR 2-year PFS and OS: both 82%). Further, 2-year PFS and OS for HR, HIV− pts were both 91% (disease-specific survival 100%). Two pts died from progressive disease; both were HIV+ HR pts. For correlative analyses, median plasma R levels for cycle 1 at 24 and 72 h were 228 820 and 126 365 ng/mL, respectively, whereas levels at cycle 3 (24 and 72 h) were 246 200 and 199 700 ng/mL, respectively. For paired CSF samples, R levels were 104 and 227 ng/mL, respectively (cycle 1) and 260 and 248 ng/mL, respectively, at cycle 3. This equates to plasma : CSF ratios of 0.04% and 0.18%, respectively, for cycle 1 and 0.10% and 0.20%, respectively, for cycle 3. Interestingly, cycle 1, 24-h plasma R levels were significantly higher among pts without relapse compared with the two pts who relapsed/died ($p = 0.042$). Cycle 3, 24-h serum R levels were of borderline significance ($p = 0.06$). There were no differences identified regarding tumour burden and R levels.
Conclusions: The integration of R into CODOX-M/IVAC for adult BL was feasible and associated with similar tolerability compared with prior reports. This regimen was associated with excellent survival rates, especially for HIV-negative BL. Further investigation of the predictive value of plasma R levels is warranted.

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TUMOUR EPSTEIN–BARR VIRUS REPLICATION IS ASSOCIATED WITH EARLY-ONSET AND AGGRESSIVE BEHAVIOR OF POST-TRANSPLANTT LYMPHOPROLIFERATIVE DISORDER

J. Rovira Sole,[1] B. Gonzalez,[2] B. Gonzalez,[2] G. Gutierrez Garcia,[1] A. Martinez Trillos,[1] E. Gine,[1] N. Martinez Cibrian,[1] L. Colomo,[1] N. Villamor,[1] E. Campo,[2] A. Lopez Guillermo,[1] A. Martinez Pozo.[2]
[1]Hematology, Hospital Clinic, Barcelona, Spain, [2]Pathology, Hospital Clinic, Barcelona, Spain.

Introduction: Post-transplant lymphoproliferative disorder (PTLD) represents a spectrum of lymphoplasmacytic proliferations in the setting of immunodeficiency associated with allograft. Although the involvement of Epstein–Barr virus (EBV) is widely known, the role of tumour replication of EBV remains to be determined, and it is the aim of this study.
Methods: Thirty-five patients (26M:9F, median age 54 years) were diagnosed with PTLD in a single institution between 2000 and 2011 using WHO criteria. EBV latency genes (EBER1–2, LMP1 and EBNA2) and two EBV replication genes (BZLF1/ZEBRA and EAD11) were assessed by immunohistochemistry or *in situ* hybridization. Main clinical, biological and evolution characteristics were collected and analyzed.

Abstract 216 Table

	EBV−	EBV+/ ZEBRA−	EBV+/ ZEBRA+
		(Non-replicating)	(Replicating)
	(*n* = 7)	(*n* = 13)	(*n* = 15)
Median time transplant—PTLD (mo)	140	34	7*
SOT/HSCT	7/0	8/5	8/7
Polymorphic histological subtype	0	1	5**
EBV latency pattern I/II/III	–	5/5/3	1/4/10*
OS (from PTLD) at 1 year (%)	57	46	22

*$p < 0.05$;
**$p = 0.06$.

Results: Twenty-three patients (66%) had received solid organ transplantation (SOT) and 12 hematopoietic stem-cell transplantation (HSCT). Median time from transplantation to PTLD was 24 months (2–252). Twelve patients (34%) were diagnosed with early-onset PTLD (<1 year). Patients with early-onset corresponded more frequentlyto HSCT than SOT (9/12 vs 3/23; $p = 0.0005$). Histological distribution was 77% diffuse large cell lymphoma, 17% polymorphic, 3% plasma cell myeloma and 3% Hodgkin lymphoma. Polymorphic PTLD showed more frequently early-onset (5/6 vs 7/29; $p = 0.01$). EBV infection (EBER1–2+) was observed in 28 cases (80%), including virus replication (ZEBRA+) in 15. EBV latency pattern was latency I, 6 cases; II, 9; and III, 13. The time from transplantation to PTLD, type of transplant (SOT and HSCT), histological subtype, EBV latency pattern and OS according to the EBV replication pattern (EBV−, EBV+ with no replication (ZEBRA−) and EBV+ with replication (ZEBRA+) are detailed in the table.
Conclusions: EBV can replicate in tumour cells of PTLD, which is associated with early-onset lymphoma and poor prognosis. Such determination could select a group of high-risk patients.

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EPSTEIN BARR VIRUS PRIMO-INFECTIONS AND REACTIVATION TREATMENT AFTER HEART TRANSPLANTATION, IN PREVENTION OF POST-TRANSPLANTATION LYMPHOPROLIFERATION DISEASE. RESULTS OF A MONOCENTRIC PROSPECTIVE STUDY ON 299 PATIENTS AND HISTORICAL COMPARISON WITH 820 PATIENTS

S. Choquet,[1] V. Leblond,[1] C. Deback,[2] S. Varnous.[3]
[1]Clinical Haematology, APHP, Pitie-Salpetriere, Paris, France; [L]Laboratory of Virology, APHP, Pitie-Salpetriere, Paris, France; [3]Cardiovascular Surgery, APHP, Pitie-Salpetriere, Paris, France.

Background: Post-transplantation lymphoproliferation diseases (PTLD) represent a rare but severe complication of heart transplantation. The vast majority of early PTLD (<1 year after transplantation) are Epstein Barr virus (EBV) positive, and high EBV viral load (EBVvl) is predictive of PTLD. After solid organ transplantation (SOT), several questions remain for EBV PI/ ReA treatment: what is the best attitude, the risk of graft rejection, the effect on PTLD incidence? Use of rituximab (R), in EBV PI/ReA in SOT, is not published.
Methods: From January 2004 to May 2009, all new heart transplanted patients in Pitie-Salpetriere Hospital, Paris, France, still alive at 1 month, have been included. Every month, for 1 year, patients had an EBVvl and an endomyocardic biopsy to detect rejection. Immunosuppressive treatment was ciclosporine, mycophenolate mofetyl (MMF) and prednisone. Primo-infection (PI) is defined as a positive EBVvl in seronegative patient. For ReA, if EBVvl > 105/mL, a CT scan or PET scan is carried out to detect potential PTLD, and decrease of immunosuppression (DIS) is carried out (MMF removed). One R injection (375 mg/m^2) is used if EBVvl do not decrease or if EBVvl > 106/mL. PTLD incidence is compared with an historical cohort of 820 heart transplantations made in the same unit, from January 1987 to December 2003.
Results: Two hundred ninety-nine patients have been included, 226 males and 73 females, mean 47 years (16–72). Six were EBV negative with a positive donor. Thirty-seven patients (12%) had an EBVvl >105/mL and five >106/mL. All EBV negative patients developed a PI and all responded to DIS; one had a possible liver PTLD (to small for biopsy) that disappeared after DIS and is still in CR at 5 years. R has been used in five cases in first line and three after DIS failure. All EBV ReA responded with only two relapses that responded again to DIS for one and DIS + R in the other. Heart rejection did not increase compared with other patients. With a median follow-up of 4 years, one PTLD arise in a patient lost because of an early hospitalization in an intensive care unit and not followed by EBVvl. In comparison with the historical cohort (13 EBV+ PTLD, 1.8/year), decrease of PTLD incidence is significant ($p = 0.03$).
Conclusion: This is the largest prospective study in the field. Systematic preemptive treatment in case of EBV PI/ReA after heart transplantation efficiently normalizes EBVvl and significantly decreases PTLD incidence, without major toxicity nor graft rejection risk.

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RISK-TAILORED CNS PROPHYLAXIS IN A MONOINSTITUTIONAL SERIES OF 194 PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED IN THE RITUXIMAB ERA

M. Bruno Ventre, G. Citterio, G. Donadoni, M. Foppoli, S. Govi, L. Scarfo, F. Caligaris-Cappio, A. J. Ferreri.
Department of OncoHematology, Ospedale San Raffaele, Milano, Italy.

Background: Predictors of CNS relapse in diffuse large b-cell lymphoma (DLBCL) and the most effective prophylaxis strategy remain to be defined in the rituximab era.

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Methods: Risk-tailored intrathecal (IT) and intravenous (IV) CNS prophylaxis was addressed in a retrospective series of 194 HIV− pts with DLBCL treated with first-line R-CHOP or similar ± RT. Primary CNS, mediastinal and leg-type lymphomas were excluded. High risk of CNS relapse was defined by the involvement of the testis, spine, base of the skull, kidney, and/or breast or by IPI ≥2 (including two among extranodal sites ≥2, advanced stage and high serum LDH).
Results: Risk of CNS relapse was low in 89 patients (pts) and high in 105. No low-risk pt received prophylaxis, whereas 40 (38%) high-risk pts received 3–4 c. of methotrexate 3 g/m^2 ± IT liposomal cytarabine (n = 30), cytarabine 16 g/m^2 in 4 days (n = 2) or IT chemotherapy (n = 8).
After first-line treatment, 160 pts achieved a CR (82%; 95% CI = 77–87%) and 34 pts had PD. At a median f-up of 52 months (12–150), a single low-risk pt and nine high-risk pts (1% vs 9%; p = 0.02) experienced CNS relapse (exclusive site; brain in five pts and meninges in five), with a median TTP of 12 months (7–55). Among high-risk pts, CNS relapse occurred in six pts with extranodal disease (kidney 3; testis 2, orbit) and in three pts with IPI ≥2. In the high-risk group, CNS relapses occurred in 7/65 (11%) pts who did not receive prophylaxis and in 2/8 (25%) pts who received only IT chemotherapy, whereas no CNS relapses were detected in the 32 pts treated with IV prophylaxis. CNS relapse rate was 12% for pts treated with inadequate prophylaxis (none or IT only) and 0% (p = 0.05) for pts managed with IV prophylaxis. Eight pts with CNS relapses died of PD after 7–37 months (median 14), which represented one third of all deaths (n = 27) in the high-risk group. Pts treated with IV CNS prophylaxis had a significantly better overall survival than the other high-risk pts (5 years: 92 ± 7% vs 41 ± 6%; p = 0.001).
Conclusions: Stratification by specific extranodal disease and IPI distinguishes two risk groups in DLBCL. CNS relapse affects 1% of low-risk pts and 12% of high-risk pts managed with inadequate prophylaxis. IT chemotherapy seems to be insufficient to prevent CNS failure, whereas high IV doses of methotrexate or cytarabine significantly reduce CNS failures in high-risk pts.

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OUTCOMES IN DIFFUSE LARGE B-CELL LYMPHOMA AFTER FAILURE TO SECOND-LINE CHEMOTHERAPY: ANALYSIS OF PATIENTS INCLUDED IN THE INTERNATIONAL CORAL STUDY

E. Van Den Neste,[1] C. Gisselbrecht,[2] N. Schmitz,[3] N. Mounier,[4] D. Gill,[5] D. Lynch,[6] M. Trneny,[7] N. Milpied,[8] J. Radford,[9] N. Ketterer,[10] O. Shpilberg,[11] U. Duehrsen,[12] D. Ma,[13] J. Briere,[14] C. Thieblemont,[15] G. Salles,[16] C. Moskowitz,[17] B. Glass.[18]
[1]Hematology, Cliniques Universitaires UCL Saint-Luc, Brussels, Belgium; [2]Hematology, Hopital Saint Louis, Paris, France; [3]Abteilung Hematologie und Stammzelltransplantation, Asklepios Klinik St. Georg, Hamburg, Germany; [4]Hematology, CHU de l'Archet, Nice, France; [5]Hematology, Princess Alexandra Hospital, Woodville, Australia; [6]Haematology, University College London Medical School, Cancer Institute, London, UK; [7]Hematology, Charles Univ. General Hosp, Praha, Czech Republic; [8]Hematologie Clinique et Therapie Cellulaire, Hopital Haut-Leveque, Pessac, France; [9]Department of Medical Oncology, University of Manchester, Manchester, UK; [10]Hematology, Clinique Bois-Cerf, Lausanne, Switzerland; [11]Hematology, Davidoff Center Rabin Medical Center, Petah Tikva, Israel; [12]Klinik fur Hamatologie, Universitatsklinikum Essen, Essen, Germany; [13]Hematology, St Vincent, Darlinghurst, Australia; [14]Anatomie Pathologique, Hopital Saint Louis, Paris, France; [15]Hematology, Hopital Saint Louis, Paris, France; [16]Service Clinique Hematologie, Chu Lyon Sud/Hospices Civils De Lyon, Pierre Benite, France; [17]Hematology, Memorial Sloan Kettering Cancer Center, New York, USA; [18]Abteilung Hamatologie und Stammzelltransplantation, Asklepios Klinik St. Georg, Hamburg, Germany.

Introduction and Methods: Salvage chemotherapy followed by high-dose therapy and autologous stem cell transplantation (ASCT) is the standard of care for relapsing/refractory diffuse large b-cell lymphoma (DLBCL). The CORAL study found no difference between two randomized salvage regimens (R-DHAP or R-ICE) and no benefit from post-ASCT rituximab maintenance (Gisselbrecht et al, JCO 2010 & 2012). Because outcome data are limited in DLBCL pts failing second-line strategy, 220 pts included in the CORAL study who were either refractory to R-DHAP/ICE before scheduled ASCT (REF, n = 145) or who relapsed after BEAM/ASCT (REL, n = 75) were herein reviewed.
Results: Median age at relapse was 56 years (range 20–68), M/F ratio 142/78, international prognostic index: 0–1 in35%, 2–3 in50%, 4–5 in15%. MYC was rearranged in 18/75 (24%) pts. Fifty-one of 122 (42%) were non-GC. Third-line therapy consisted of ICE-type (24%), DHAP-type (20.5%), gemcitabine-containing (20%), CHOP-like (9.5%), dexaBEAM (5.5%), and miscellaneous (26%) regimens. overall response rate was 43% (CR 24%, CRu 6%, PR 14%) and was similar between REF and REL pts. In the REF group, median time between CORAL inclusion and failure was 2.3 months (m). Median overall survival (OS) from progression (FU 32.8 m) was 6 m in REF and not influenced by the type of third-line regimen. Sixty-four REF pts were transplanted: median OS after ASCT (n = 56) was 11.5 m, and 7.9 m after alloSCT (n = 8). In the REL group, median time between CORAL inclusion and failure was 10.3 m (40% > 1 year). Median OS from progression was 10 m. Sixteenof them received a second transplantation (alloSCT: 13; ASCT: 3). Their median OS after alloSCT was 17.4 m. Overall, OS for the 80 transplanted pts was significantly longer (11.8 m, 95% CI: 8.5–19.5) as compared with pts treated with chemo alone (5.8 m, 95% CI: 5–7.2, p = 0.0004). Median OS was 11.5 m, after ASCT (n = 59), and 15.4 mafter alloSCT (n = 21). Age >50 years independently predicted for OS (HR 1.4, p = 0.04).
Conclusions: Outcome of REF or REL DLBCL pts after third line is overall poor. However, response is non-negligible, and selected patients who can be further transplanted can enjoy long-term disease control. This approach should then be encouraged, although there is obviously an urgent need for new strategies in this setting.

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INFLUENCE OF PRIOR RITUXIMAB ON THE RESULTS OF AUTOLOGOUS STEM-CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE B-CELL LYMPHOMA: A GELTAMO STUDY

A. M. Redondo,[1] A. Martin,[1] H. Pomares,[2] M. J. Vidal,[3] M. J. Pascual,[4] B. Quereda,[5] J. Sancho,[6] M. Polo,[7] J. Lopez-Jimenez,[8] E. Conde,[9] I. Jarque,[10] N. Alonso,[11] M. J. Ramirez,[12] P. Fernandez,[13] M. Juan,[14] M. J. Requena,[15] A. Salar,[16] J. D. Gonzalez,[17] E. Gonzalez-Barca,[2] D. Caballero.[1]
[1]Hematology, Hospital Clinico Universitario De Salamanca And IBSAL, Salamanca, Spain; [2]Institut Catalano D'Oncologia Duran I Reynals, L'Hospitalet De Llobregat, Barcelona, Spain; [3]Hematology, Hospital Donostia, San Sebastian, Spain; [4]Hematology, Hospital Carlos Haya, Malaga, Spain; [5]Hematology, Hospital De La Princesa, Madrid, Spain; [6]Hematology, Ico-Hospital Germans Trias I Pujol, Badalona, Spain; [7]Hematology, Hospital Clinico San Carlos, Madrid, Spain; [8]Servicio De Hematologia Y Hemoterapia, Hospital Universitario Ramon y Cajal, Madrid, Spain; [9]Hematology, Hospital Marques De Valdecilla, Santander, Spain; [10]Hematology, Hospital Universitario La Fe, Valencia, Spain; [11]Hematology, Hospital Universitario De Santiago De Compostela, Santiago De Compostela, Spain; [12]Hematology, Hospital De Jerez De La Frontera, Jerez De La Frontera, Spain; [13]Hematology, Hospital General De Alicante, Alicante, Spain; [14]Hematology, Hospital Universitario Dr. Peset, Valencia, Spain; [15]Hematology, Hospital Universitario Severo Ochoa, Leganes, Spain; [16]Hematology, Hospital Del Mar, Barcelona, Spain; [17]Hematology, Hospital Universitario Insular De Gran Canarias, Las Palmas, Spain.

Introduction: Recent studies indicate that the use of highly effective rituximab (R)-containing primary therapy in diffuse large B-cell lymphoma (DLBCL) makes it more difficult to salvage patients who are refractory or relapse. To date, peripheral-blood autologous stem-cell transplantation (PBASCT) is the reference treatment for these patients, but the impact of previous exposure to R on the results is still unknown.
Patients and Methods: We have retrospectively analyzed 248 patients with DLBCL or grade 3B follicular lymphoma with relapsed or refractory disease after at least one R-containing regimen ('R+' group) who received PBASCT in 17 GELTAMO centres, in comparison with a control group of 127 patients who received PBSCT without previous exposure to R ('R−' group).
Results: No significant differences between R+ and R− groups were found with respect to age-adjusted international prognostic index (IPI) at transplant, disease status at salvage therapy and at transplant, or number or prior chemotherapy regimens. More patients in the R+ group were ≥60 years (30% vs 19%, p = 0.02). Complete response (CR) (71% v 71%) and overall response (82% v 79%) rates to PBASCT were similar in R+ and R− groups. In multivariate analysis, factors with significant influence on CR rates were age-adjusted IPI at diagnosis (<2), number of prior chemotherapy lines (<3), and disease status at transplant (CR). Median follow-up was 35 (1–130) and 122 (2–214) months in the R+ and R− groups, respectively. Patients in the R+ group had a significantly better progression-free survival (PFS) (63% vs 48% at 5 years, p = 0.041) and OS (72% vs 61% at 5 years, p = 0.02) as compared with patients in the R− group. In multivariate analysis, independent factors with negative influence on both PFS and OS were disease status at transplant (partial response), disease status at salvage therapy (primary refractory disease or early relapse, in comparison with first partial response or late relapse), age >60 years, and no previous exposure to R.
Conclusions: In patients with chemosensitive relapsed or refractory aggressive B-cell lymphoma, PBASCT is at least as effective in patients pre-treated with R-containing therapy as compared with R-naive patients.

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COMPREHENSIVE GERIATRIC ASSESSMENT IS AN ESSENTIAL TOOL TO SUPPORT TREATMENT DECISIONS IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA. RESULTS OF A MULTICENTRE STUDY BY THE FONDAZIONE ITALIANA LINFOMI

A. Tucci,[1] M. Martelli,[2] L. Rigacci,[3] P. Riccomagno,[4] M. G. Cabras,[5] F. Salvi,[6] C. Stelitano,[7] A. Fabbri,[8] S. Storti,[9] S. Fogazzi,[1] S. Mancuso,[10] M. Brugiatelli,[11] A. Fama,[2] B. Puccini,[3] P. Paesano,[2] C. Bottelli,[1] F. Bertagna,[12] G. Rossi,[1] M. Spina.[13]
[1]Department of Hematology, Spedali Civili, Brescia, Italy; [2]Department of Cellular Biotecnologies and Hematology, Sapienza University, Roma, Italy; [3]Department of Hematology, Careggi University Hospital, Firenze, Italy; [4]Hematology 2, Citta' della Salute e della Scienza Hospital, Torino, Italy; [5]Department of Hematology, Oncologic Hospital Armando Businco, Cagliari, Italy; [6]Hematology Division, SS Antonio e Biagio Hospital, Alessandria, Italy; [7]Hematology Division, Bianchi, Melacrino, Morelli Hospital, Reggio Calabria, Italy; [8]Division of Hematology, University Hospital of Siena, Siena, Italy; [9]Onco-Hematology Division, Catholic University - Giovanni Paolo II Foundation, Campobasso, Italy; [10]Unit of Hematology and Transplantation, Paolo Giaccone University Hospital, Palermo, Italy; [11]Department of Hematology, Papardo Hospital, Messina, Italy; [12]Nuclear Medicine, Brescia University, Brescia, Italy; [13]Department of Medical Oncology, National Cancer Institute, Aviano (PN), Italy.

Introduction: Following the observation that a simple comprehensive geriatric assessment (CGA) can identify elderly diffuse large B-cell lymphoma (DLBCL) non-fit patients (pts) in whom curative treatment is not better than palliation, we performed a prospective multicentre study in order to validate this finding in a larger population and to verify the utility to further divide non-fit pts in two subgroups able to benefit of treatments of different intensity.
Methods: During 1 year, all consecutive pts aged >69 years with DLBCL from 13 Institutions of the Fondazione Italiana Linfomi (FIL) were classified according to CGA as fit, unfit or frail. Treatment with curative or palliative intent was driven by clinical judgment only. The outcome of pts was analyzed according to the treatment received and to the results of CGA.
Results: Out of 177 pts recorded, 162 had fully evaluable data and were considered for the analysis. According to CGA, 46% of them were 'fit', 17% 'unfit' and 37% 'frail'. Fit pts were younger ($p < 0.0001$), and all but four received treatment with curative intent (R-CHOP or R-CHOP like regimens). Their 2-year overall survival (OS) was significantly better than in pts considered non-fit by CGA (85% vs 49%; $p < 0.0001$); conversely, 2-year OS in unfit and frail pts was 54% and 43%, respectively (p 0.12). Among unfit and frail pts, 63% and 29% had received intensive treatment, whereas the remaining received palliative therapy. These subgroups did not differ in any clinical characteristic except for younger age and more advanced stage in the group treated intensively. Within different CGA categories, the 2-year OS of pts treated with curative or palliative intent was 88% vs 25% (p 0.0001) in fit, 75% vs 50% (p 0.52) in unfit and 43% vs 42% (p 0.89) in frail pts, respectively. Lymphoma rather than toxicity was the main cause of failure even among non-fit pts treated aggressively.
Conclusions: CGA was confirmed as an efficient method to identify elderly DLBCL pts who can benefit from a curative approach with anthracycline-containing regimens. The better trend of survival in unfit compared with frail pts, especially when treated intensively, suggests that any effort to better tailor treatment in this intermediate CGA category could further improve their outcome.

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ELDERLY INTERNATIONAL PROGNOSTIC INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS AGE >60 YEARS TREATED WITH RCHOP: INTERNATIONAL VALIDATION STUDY USING DATA FROM RICOVER-60 (GERMAN HIGH-GRADE NON-HODGKIN LYMPHOMA STUDY GROUP) AND LNH 98-5 (GROUPE D'ETUDE DE LYMPHOME D'ADULTES)

R. H. Advani,[1] H. Li,[2] F. Hong,[2] B. S. Kahl,[3] G. Varma,[1] S. Boussetta,[4] M. Pfreundschuh,[5] M. Ziepert,[6] B. Coiffier.[7]
[1]Medicine/Oncology, Stanford University Medical Center, Stanford, USA; [2]Biostatistics and Computational Biology, Dana Farber Cancer Institute, Boston, USA; [3]Hematology/Oncology, University of Wisconsin, Madison, USA; [4]Biostatistics, LYSARC, Centre Hospitalier Lyon Sud, Lyon, France; [5]Medicine, University of Saarland, Homburg, Germany; [6]Statistics and Epidemiology, Institute for Medical Informatics, Leipzig University, Leipzig, Germany; [7]Hematology, Hospices Civiles de Lyon, Centre Hospitalier Lyon Sud, Lyon, France.

Introduction: The international prognostic index (IPI) developed in pre-rituximab era for patients (pts) with diffuse large b-cell lymphoma (DLBCL) dichotomized age at 60 years for eligibility to aggressive therapies. Studies in the RCHOP era have shown improved outcomes for pts >60 years. We have previously reported an alternate index, the elderly (E) IPI, which provided better discrimination of outcome for this group (Advani et al, BJH 2011). This study aims to independently validate the EIPI.
Methods: We compared the IPI and the EIPI with data from RICOVER-60 and LNH 98-5 trials. Pts were stratified into four risk groups: low (L), low intermediate (LI), high intermediate (HI) and high risk (H), per IPI and EIPI and OS estimated using Kaplan–Meier. Performance of indices was compared by measure of global fit [Akaike's information criteria (AIC)], concordance probability estimate (CPE) and area under the receiver operator curve (AUC) over time. A reclassification calibration (Hosmer-Lemeshow test) was used to compare the fit of *predicted* overall survival (OS) by EIPI versus IPI to the *observed* OS over time.
Results: Eight hundred twleve pts were included. The median follow-up was 7.4 years. Pt characteristics were stage III/IV, 58%; >1 EN site, 26%; elevated LDH, 53%; and ECOG PS ≥17%. The median age was 68.5 years with 39% >70 years. On univariate analysis, all characteristics were significant for 5-year OS. Both the IPI and EIPI provided prognostic discrimination for OS of the four groups. The AUC ranked the EIPI higher than the IPI over all event times. Similar rankings were obtained using AIC and CPE. EIPI versus IPI classified more pts as L risk (40% vs 25%) and fewer as HI (18% vs 25%). For patients reclassified (e.g. IPI-LI to EIPI-L), the 5-year EIPI OS (80%) matched the 5-year observed OS (80%) compared with the 5-year IPI OS (70%). Reclassification calibration indicated a significantly better fit for OS over time starting at 2 years for the EIPI versus IPI.
Conclusion: For pts >60 years treated with RCHOP, while both the IPI and EIPI provided prognostic discrimination for OS, the EIPI outperformed the IPI using all measures, thus validating our previous findings. The EIPI is a useful index to incorporate in ongoing and future studies for risk stratification and treatment planning of pts >60 years with DLBCL.

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TREATMENT PATTERNS AND COMPARATIVE EFFECTIVENESS IN AN ELDERLY DIFFUSE LARGE B-CELL LYMPHOMA POPULATION IN THE USA

P. A. Hamlin,[1] S. Satram-Hoang,[2] C. Reyes,[3] K. Q. Hoang,[2] S. R. Guduru,[2] S. Skettino.[4]
[1]Lymphoma Outpatient Service, Memorial Sloan-Kettering Cancer Center, New York, USA; [2]Epidemiology & Health Outcomes, Q.D. Research, Inc., Granite Bay, USA; [3]Health Economics & Outcomes Research, Genentech, Inc., South San Francisco, USA; [4]Medical Affairs, Genentech, Inc., South San Francisco, USA.

Introduction: A disproportionate number of newly diagnosed diffuse large B-cell lymphoma (DLBCL) occurs in elderly patients (pts). We evaluated treatment patterns and outcomes of elderly pts in a real-world population.
Methods: We used a retrospective cohort analysis of 9333 DLBCL pts in the linked Surveillance, Epidemiology, and End results-Medicare database. Pts were diagnosed between 1/1/2000 and 12/31/2007, >66 years, and continuously enrolled in Medicare Part A and B in the year prior to diagnosis. There were 4565 (49%) rituximab (R) + Chemo, 2181 (23%) Chemo-only, and 467 (5%) R-mono pts who initiated treatment within 3 months after diagnosis. The remaining 2120 (23%) did not receive treatment. Statistical comparisons were made between R + Chemo versus Chemo-only, and R-mono versus No Treatment. Cox regression estimated the relative risk of death adjusting for demographic and clinical factors. Date of last follow-up was 12/31/2009.
Results: Pts receiving R + Chemo were slightly younger (≤75 tears: 50% vs 46%; $p < 0.05$), more likely white, and married compared with those receiving Chemo-only. Pts receiving R-mono were older (>80 years: 60% vs 50%; $p < 0.01$) and more likely female compared with those Not treated. Overall, male gender, increasing age (especially >80 years), unmarried, and higher comorbidity increased the odds of not receiving any treatment. In multivariate survival analysis, pts receiving Chemo-only had a twofold increased risk of death (HR = 2.20; 95% CI=2.04–2.37) compared with R + Chemo pts. Increasing age and comorbidity score were associated with significant increases in mortality. A higher mortality risk was noted with receipt of more than six cycles (HR = 1.40; 95% CI=1.22–1.62) compared with six cycles. R-mono pts had a 69% lower risk of death (HR = 0.31; 95% CI=0.28–0.35) compared with Not treated pts.
Conclusions: R + Chemo significantly decreased mortality risk in elderly pts with DLBCL. We observed that suboptimal durations of curative intent therapy (more than six cycles) are frequent and associated with poorer outcomes. Twenty-three per cent of elderly DLBCL pts, more likely >80 years and male, received no therapy at all. In this group, R-mono therapy may be useful to improve survival compared with no therapy.

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TREATMENT APPROACH AND CAUSE SPECIFIC SURVIVAL IN ELDERLY PATIENTS DIAGNOSED WITH DIFFUSE LARGE B-CELL LYMPHOMA

A. M. Dyszkiewicz-Korpanty,[1] J. Kuruvilla,[1] V. Kukreti,[1] R. Tiedemann,[1] M. Maganti,[2] R. Tsang,[3] D. Hodgson,[3] A. Sun,[3] M. Pintilie,[2] M. Gospodarowicz,[3] M. Crump.[1]
[1]Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, Canada; [2]Department of Biostatistics, Princess Margaret Hospital, Toronto, Canada; [3]Radiation Medicine Program, Princess Margaret Hospital, Toronto, Canada

Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoma reported in patients (pts) >60 years of age. Outcomes of very elderly pts aged >80 years are significantly under-represented in clinical trials and reports of cohort data. We wished to explore differences in treatment approach, outcomes and cause specific survival (CCS) in very elderly versus younger pts at our centre.
Methods: This was a retrospective analysis of 331 pts with a new diagnosis of DLBCL aged >70 years treated at Princess Margaret Hospital from 2001to 2010. Data on stage, prognostic factors, treatment and outcome were retrieved from a prospective lymphoma database. Treatment intent was recorded as curative (combination chemo CHOP/R-CHOP + radiation) or palliative. Univariate analysis of categorical data was performed using Chi-square or Fisher's exact test. Probability of death due to lymphoma was estimated using a competing risks approach; Gray's test was used to report significance between groups.
Results: Patient demographics, treatment intent and outcomes are presented in the table.

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Variable	Age 70–80 years N (%)	Age >80 years N (%)	p-value
Number of patients	220	111	
Females	121 (55)	61 (54.9)	0.99
Stages III–IV	80 (36.4)	39 (35.1)	
Extranodal disease	168 (76.4)	84 (76.4)	1
ECOG 0–1	148 (71.8)	72 (66.9)	0.13
Treatment intent			
Palliative	21 (9.6)	40 (36.4)	<0.0001
Curative	197 (90.4)	70 (63.6)	
Number of deaths	107	75	
Death from lymphoma at 5 years: whole cohort	31%	43.5%	0.03
Death from lymphoma at 5 years, curative intent ($n = 267$)	27.9%	29.4%	0.94
Death from lymphoma, CHOP/R-CHOP ($n = 241$)	29%	24.1%	0.83

Conclusions: Pts aged >80 years were more likely to receive palliative treatment compared with younger pts. CCS at 5 years in very elderly was inferior to survival in patients aged 70–80 years in the whole cohort. However, there was no difference in CCS between pts >80 years and younger pts who received chemotherapy with curative intent. Pt characteristics and comorbidities influencing treatment decisions in very elderly pts require further evaluation.

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PATIENT-REPORTED SYMPTOMS ARE STILL THE SINGLE MOST IMPORTANT FACTOR FOR DETECTING LYMPHOMA RELAPSE

T. El-Galaly,[1] K. Mylam,[2] P. Brown,[3] M. Rossing,[3] A. Gang,[4] A. Haglund,[5] B. Arboe,[6] M. Clausen,[7] P. Jensen,[5] M. Pedersen,[4] A. Bukh,[7] B. Amdi Jensen,[2] C. Poulsen,[6] F. D'Amore,[7] M. Hutchings.[3]
[1]Departments of Hematology, Aarhus and Aalborg University Hospitals, Aarhus, Denmark; [2]Department of Hematology, Odense University Hospital, Odense, Denmark; [3]Department of Hematology, Rigshospitalet, Copenhagen University Hospitals, Copenhagen, Denmark; [4]Department of Hematology, Herlev Hospital, Copenhagen University Hospitals, Copenhagen, Denmark; [5]Department of Hematology, Aalborg University Hospital, Aalborg, Denmark; [6]Department of Hematology, Roskilde Hospital, Roskilde, Denmark; [7]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark.

Introduction: After therapy patients with Hodgkin lymphoma (HL) and aggressive non-Hodgkin lymphomas (aNHL) are followed closely for early signs of relapse. The current practice is controversial and warrants a critical evaluation.
Methods: A multicentre retrospective study of HL and aNHL patients diagnosed during the period 2002–2011 was conducted. Only patients in at least a partial remission after first-line therapy and relapsing ≥1 month after completing therapy were included. Follow-up practice included outpatient visits with physical examination, blood tests and CT scans. The physician perceived efficacy of current follow up practice was assessed by a questionnaire mailed to attending lymphoma specialists.
Results: A total of 309 patients (52 HL and 257 NHL) with recurrent disease were included. The median age at relapse was 65 years. Relapse investigations were prompted by patient-reported symptoms (PRS) alone (41%), routine imaging (27%), PRS combined with abnormal blood tests or physical examination (23%), physical examination alone (6%) or blood tests alone (3%). Relapse investigations were initiated at unscheduled visits in 54% of patients. Patients with imaging detected relapse (I-relapse) had an odds ratio of 0.48 (95% CI: 0.28–0.82) for being diagnosed with limited stage disease. The median time to relapse was similar in patients with I-relapse versus non-I-relapse (7 vs 8 months). Palliative treatment was chosen in 39% of patients with I-relapse. In a multiple Cox analysis, I-relapse was associated with better overall survival in B-cell aNHL (HR 0.56, 95% CI 0.37–0.85) but not in T-cell aNHL or HL. The questionnaire concerning physician-perceived efficacy of current follow-up practice was returned by 33 out of 35 lymphoma specialists. PRS were considered the most important cause of relapse detection by 32, wheras 24 estimated the frequency of I-relapse to be 25% or less.
Conclusions: PRS are still the single most important factor for detecting lymphoma relapse and the low number of I-relapses calls for improved criteria for the use of surveillance imaging. Patients with I-relapse had lower disease burden and I-relapse was associated with better outcome for B-cell aNHL, although lead and/or length time bias may in part explain this finding.

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ROLE OF POST-THERAPY SURVEILLANCE SCANS IN THE DETECTION OF DIFFUSE LARGE B-CELL LYMPHOMA RELAPSE: RESULTS OF TWO INDEPENDENT COHORTS

H. Ghesquieres,[1] C. A. Thompson,[2] M. J. Maurer,[3] E. Nicolas-Virelizier,[1] W. R. Macon,[4] C. Sebban[1], T. M. Habermann,[2] P. Biron,[1] P. Rey,[1] A. Belhabri,[1] T. E. Witzig,[2] C. Chassagne-Clement,[5] T. Gargi,[1] J. R. Cerhan,[3] B. K. Link.[6]
[1]Onco-Hematology, Centre Leon Berard, Universite Claude Bernard, Lyon, France; [2]Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA; [3]Health Sciences Research, Mayo Clinic, Rochester, MN, USA; [4]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; [5]Pathology, Centre Leon Berard, Universite Claude Bernard, Lyon, France; [6]Holden Comprehensive Cancer Centre, University of Iowa, Iowa City, IA, USA.

Introduction: The optimal follow-up strategy for diffuse large B-cell lymphoma (DLBCL) patients in remission is not clear. The goal of this study is to determine the utility of surveillance scans in two cohorts of DLBCL patients.
Methods: Patients were from the prospective cohort ($N = 644$) of the Iowa/Mayo Specialized Program of Research Excellence (SPORE) and from consecutive cases ($N = 269$) included in the Leon Berard Cancer Centre registry (CLB, Lyon, France). All patients were treated with immunochemotherapy and followed for relapse, retreatment and death. Patient clinical records were reviewed for details at relapse and relationship to planned follow-up visits and surveillance scans.
Results: Among 644 SPORE patients (median age, 63 years), 538 entered post-treatment observation: 108 (20%) relapsed after a median follow-up of 59 months (8–116). Among 269 CLB patients (median age, 66 years), 230 entered post-treatment observation: 48 (21%) relapsed after a median follow-up of 35 months (13–129). Among relapsed patients in SPORE and CLB cohorts, 63% and 56% presented earlier than a planned follow-up visit. At the time of relapse, 68% and 52% were symptomatic, and 42% and 52% of patients had abnormal physical exam, 55% and 54% had elevated LDH, and 87% and 90% of patients had one or more of these features in SPORE and CLB cohorts, respectively. Among the 38 and 21 patients with relapse detected at a planned visit in SPORE and CLB, 26 (68%) and 14 (66%) had clinical features of relapse, respectively. Finally, 12 (11%) and 5 (10%) lymphoma relapses were detected solely by planned surveillance scan in SPORE and CLB, respectively. Among these 17 patients, 7 had relapse of low-grade or other subtype and 10 had DLBCL relapse (5 had equivocal response at the end of initial treatment). Surveillance scanning detected DLBCL relapse prior to clinical manifestations in only 8/538 (1.5%) and 2/230 (1%) in SPORE and CLB cohorts, respectively.

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Conclusions: Two independent DLBCL cohorts demonstrated similar patterns of relapse detection occurring outside of planned follow-up visits, which are detectable via symptoms, physical exam or laboratory abnormalities. Post-therapy surveillance scans added little to detection of relapse.

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IMMUNOGENETIC ANALYSIS OF THE B-CELL REPERTOIRE OF INDOLENT MANTLE-CELL LYMPHOMA WITH A SPLENIC PRESENTATION

C. Sarkozy,[1] M. Cheminant,[2] M. Boudgrha,[2] J. Lazarovici,[1] S. Le Gouill,[3] F. Berger,[4] L. Baseggio,[5] P. Felman,[5] A. Traverse-Glehen,[4] A. Le Bourgeois,[3] P. Talmant,[6] A. Moreau,[7] S. Lissandre,[8] V. Eclache,[9] J. Briere,[10] R. Houlgatte,[11] F. Davi,[2] C. Thieblemont.[1]
[1]Hematology, Hopital Saint Louis, Paris, France; [2]Hemato-Biology, Hopital De La Pitie Salpetriere, Paris, France; [3]Hematology, CHU de Nantes, Nantes, France; [4]Hemato-Pathology, Centre Hospitalier Lyon Sud, Pierre Benite, France; [5]Hemato-Biology, Centre Hospitalier Lyon Sud, Pierre Benite, France; [6]Cytogenetic, CHU de Nantes, Nantes, France; [7]Hemato-Pathology, CHU de Nantes, Nantes, France; [8]Hematology, CHU de Tours, Tours, France; [9]Hemato-Biology, Hopital Avicennes, Avicennes, France; [10]Hemato-Pathology, Hopital Saint Louis, Paris, France; [11]U954, Inserm, Nancy, France.

Introduction: Mantle-cell lymphoma (MCL) is clinically a heterogeneous disease but well-defined by the presence of the t(11;14)(q13;q32) translocation. Recently, it has been recognized that some patients experience an indolent course with a prolonged survival without therapy. These patients generally present with splenomegaly, blood involvement and minimal lymphadenopathy. The aim of our study was to perform an immunogenetic analysis of IGHV-IGHD-IGHJ gene rearrangements from indolent MCL with splenic presentation (splenic iMCL).
Method: Genomic DNA of 19 patients with splenic iMCL extracted from tissue specimen (blood, bone marrow biopsy and spleen) was used to characterize the IGHV-IGHD-IGHJ gene repertoire, to analyze somatic hypermutation (SHM) features and to look for common CDR3 motifs.
Results: Patient characteristics include a median age of 65 years (range: 48–84), 5F/14M, ECOG-PS < 1 for all patients, MIPI score low in 2, intermediate in 7 and high in 8. Median leucocyte count was 14.4G/L. Anaemia was present in 6 patients, thrombocytopenia in 11 and, LDH > UNL in 4. The median FU was 85 months (15–213). At time of analysis, 10 patients were alive without receiving polychimiotherapy. Immunophenotype was available in 18 patients: Matutes score was ≤ 3 (0–1 $n = 11$, 5 $n = 2$, 3 $n = 2$), CD5 was negative in four patients, IgM (+/−IgD) expression was reported in 12 cases/12 (IgG in 0), and κ and λ in 11 and 7, respectively. The IGHV gene repertoire, available for 15 patients, was remarkably biaised with 3/15 of IGHV3–7 and 3/15 of V4–34. On this small series, V1–2, the major VH used in MZL was not represented, and on V3–21, the major VH used in MCL was present in only one case. DH repertoire had no particularity. The JH repertoire was remarkable by the use of JH4 in 9/15 cases and no use of JH5. On the basis of SHM status, 86.6% (13/15) of the patients had mutated IGHV, and the median germline identity was 96% (from 99.9% to 90% and $n = 10 < 97\%$ and $n = 5 < 95\%$). The median CDR3 length was 13 amino acids (6–20). In this small sample, no CDR3 subset was found.
Conclusion: In this small series of iMCL with splenic presentation, our results suggest a peculiar IGHV-IGHD-IGHJ gene rearrangement with a high level of SHM. These features will be supported by genomic analysis to better characterize the pathophysiology of this entity.

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SOX11 IS A TUMOUR REGULATORY PROTEIN IN MANTLE CELL LYMPHOMA AND INCREASED EXPRESSION LEVELS CORRELATES TO OVERALL SURVIVAL IN A HOMOGENOUSLY TREATED COHORT

L. Nordstrom,[1] V. Kuci,[1] S. Sernbo,[1] M. Jerkeman,[2] S. Ek.[1]
[1]Immunotechnology, Lund University, Lund, Sweden; [2]Oncology, Lund University, Lund, Sweden.

Introduction: The transcription factor SOX11 has during recent years been shown to be a diagnostic, prognostic and functional antigen in mantle cell lymphoma (MCL). We have previously developed a monoclonal antibody to target SOX11 in clinical applications such as IHC and FACS. Using this antibody with improved specificity, we investigated the prognostic use of SOX11 in the homogenously treated Nordic MCL2 and MCL3 cohort. Additionally, we investigated the functional role of SOX11 in MCL cell lines using an inducible knockdown system and gene expression profiling (GEP).
Methods: The Nordic MCL2 ($n = 58$) and MCL3 ($n = 69$) patient cohort, treated with intensive immunochemotherapy, followed by high-dose chemotherapy and ASCT as well as addition of ibritumomab radioimmunotherapy for MCL3 patients, was stained for SOX11 expression in relation to clinicopathological and biological parameters. To evaluate the gene regulatory role of SOX11, an inducible knockdown system was established in MCL cell lines Z138, GRANTA-519 and JEKO-1 and GEP over time (0, 24 and 96 h) was performed.
Results: We show that high protein levels of SOX11 correlate to an increased overall survival (OS) among MCL2/3 patients. The MCL international prognostic index (MIPI) has been extensively used to guide in treatment decisions but has been shown to poorly separate the low and intermediate risk patients within the MCL2 cohort. We could show that SOX11 separates the MIPI low/intermediate MCL2/3 patient cohort into two groups with a significant difference in OS. Furthermore, we analyzed the functional role of SOX11 by GEP knocked cell lines over time. Among identified pathways are Wnt/β-catenin and RB/EF2 pathway, both being previously associated with MCL.
Conclusions: We show that the expression level of SOX11 correlates to improved OS in the MCL2/3 cohort, and this is the first time SOX11 has been correlated to survival in a homogenously treated cohort. Furthermore, the use of SOX11 can separate MIPI low/intermediate patients into two groups with significant difference in OS. Thus, combining SOX11 status and the MIPI index can be used to stratify patients for treatment selection. In addition, GEP data indicate that SOX11 is a key regulatory protein signalling through pathways previously identified as important for MCL biology.

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RESPONSE RATES USING STANDARD CRITERIA, FDG-PET AND MRD MEASUREMENT AFTER FOUR COURSES OF R-DHAP AND AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MANTLE CELL LYMPHOMA, RESULTS FROM THE LYMA TRIAL CONDUCTION ON BEHALF OF THE LYSA GROUP

S. Le Gouill,[1] M. Callanan,[2] E. Macintyre,[3] C. Bodet-Milin,[4] M. Meignan,[5] A. Moreau,[6] A. Traverse-Glehen,[7] M. Bene,[8] E. Van Den Neste,[9] O. Tournilhac,[10] F. Morschhauser,[11] B. Christian,[12] G. Cartron,[13] B. Lioure,[14] M. Delfau-Larue,[15] O. Hermine.[16]
[1]Hematology, CHU de Nantes, Nantes, France; [2]Hematology Laboratory, CHU Grenoble, Grenoble, France; [3]Hematology Laboratory, Hopital Necker APHP, Paris, France; [4]Medical Nuclear Department, CHU de Nantes, Nantes, France; [5]Medical Nuclear Department, Hopital Henry Mondor, APHP, Creteil, France; [6]Anatomo-Pathologie, CHU de Nantes, Nantes, France; [7]Anatomo-Pathologie, CHU de Lyon, Lyon, France; [8]Laboratoire d'Hematologie, Nantes, France; [9]Hematology, Universite Catholic de Louvain, Louvain, Belgium; [10]Hematology, CHU de Clermont-Ferrand, Clermont-Ferrand, France; [11]Hematology, CHU de Lille, Lille, France; [12]Hematology, CHR de Metz, Metz, France; [13]Hematology, CHU de Montpellier, Montpellier, France; [14]Hematology, CHU de Strasbourg, Strasbourg, France; [15]Laboratoire d'Hematologie, Creteil, France, [16]Hematology, Hopital Necker APHP, Paris, France.

Induction chemotherapy followed by autologous stem cell transplantation (ASCT) is the current standard of care in younger patients with mantle cell lymphoma (MCL) (<65 years). Cytarabine-containing chemotherapy regimens prior to ASCT are associated to longer overall survival (OS) (Hermine et al, ASH 2012). The European MCL network has also demonstrated that rituximab maintenance after R-CHOP enhances OS in elderly patients (Kluin-Nelemans et al NEJM, 2012). In 2008, the GOELAMS and GELA groups (now Lysa) launched a prospective, phase III trial addressing the question of rituximab maintenance after ASCT (Lyma trial) in younger MCL patients. Before ASCT, all patients were planned to receive four courses of R-DHAP (without R-CHOP). Transplanted patients were randomized between two study arms (rituximab maintenance versus observation). After four courses of R-DHAP and after ASCT, patients were monitored for response according to the Cheson 99 criteria. At the same time points, minimal residual disease (MRD), assessed by quantitative PCR, was measured in peripheral blood (PB) and bone marrow (BM). FDG-PET analysis was also performed. From September 2008 to August 2012, 299 patients were enrolled. Herein, we present response rates for the first 200 patients enrolled in the trial (response assessment for the 99 remaining patients are ongoing and will be presented at the time of the meeting). Patients presented the following characteristics: median age, 57.2 years; male, 79.5%; MIPI score, low (l-MIPI) 53%, intermediate (i-MIPI) 27% and high (h-MIPI), 20%. Blastoid variant was diagnosed in 12.5% of the patients. One hundred eighty-two patients (91.5%) received four courses of R-DHAP and 164 (82%) proceeded to transplant. One hundred fifty-six (78.5%) patients were randomized. After four courses of R-DHAP, CR/CRu rate was 76.5% (l-MIPI 74.5%, i-MIPI 83.5%, H-MIPI 70%), whereas 13% of the patients reach PR. One hundred sixty-two patients underwent FDG-PET, and no abnormal FDG uptake was observed for 79.6% of the patients (l-MIPI 83%, i-MIPI 81% and H-MIPI 66.6%). Regarding MRD status, 79.2% and 64.4% of patients reached negativity in PB and BM, respectively. After ASCT, CR/CRu rate was 93.5% (l-MIPI 93%, i-MIPI

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98%, H-MIPI 89,5%) and 95.3% of transplanted patients reached MRD negativity in PB versus 78.1% in BM. According to FDG-PET ($n = 121$). CR after ASCT was 89%. These results show for the first time that CR/CRu and MRD negativity rates after only four courses of R-DHAP are similar to those reported after six courses of R-CHOP/R-DHAP. Furthermore, the Lyma trial is the first large phase III trial that prospectively evaluates FDG-PET response in MCL. Because it has been demonstrated that response before and after ASCT can predict response duration after ASCT, response rates after 4xR-DHAP alone are warranted to design next generation clinical trials in MCL. Interim analysis regarding the primary objective (rituximab maintenance) will be done in Q4 2014, and final analysis will be performed in 2016.

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PREEMPTIVE IMMUNOTHERAPY WITH RITUXIMAB SIGNIFICANTLY PROLONGS THE DISEASE-FREE SURVIVAL IN PATIENTS WITH MOLECULAR RELAPSE OF MANTLE CELL LYMPHOMA

D. Salek,[1] B. Tichy,[2] A. Mareckova,[2] A. Janikova,[1] J. Michalka,[1] J. Supitova,[1] L. Smardova,[1] M. Navratil,[1] A. Baumeisterova,[1] L. Kren,[3] S. Pospisilova,[2] J. Mayer.[1]
[1]Department of Internal Medicine, Hematology and Oncology, University Hospital and Masaryk University Brno, Brno, Czech Republic; [2]Center of Molecular Biology and Gene Therapy, University Hospital and Masaryk University Brno, Brno, Czech Republic; [3]Department of Pathology, University Hospital and Masaryk University Brno, Brno, Czech Republic.

Introduction: Mantle cell lymphoma (MCL) is characterized by a specific chromosomal translocation t(11;14)(q13;q32), which can be used for monitoring of minimal residual disease (MRD). MCL has still poor prognosis, namely in elderly patients, where no aggressive treatment can be given. Our project evaluates prospectively the effect of preemptive immunotherapy with rituximab on disease-free survival (DFS) in patients with molecular relapse of MCL detected by polymerase chain reaction (PCR).
Methods: Fifty newly diagnosed MCL patients (pts) were treated with various rituximab-based regimens and monitored for MRD by quantitative PCR in intervals of 3 months. The t (11;14) breakpoint or IGHV clone-specific sequence was used as the molecular targets. Molecular relapse (MRel) was defined as two consecutive positive samples after a period of PCR negativity. Parallel bone marrow samples were also tested. After exclusion of simultaneous clinical relapse, pts were randomly either observed until clinical progression or received four doses of rituximab (375 mg/m^2 weekly). These two subgroups were then statistically compared in terms of DFS (defined as the time from the date of molecular relapse to clinical relapse or last follow-up).
Results: Median follow-up from the end of induction treatment was 35 months (range 1.5–111). MRel without parallel clinical relapse was detected in 15 pts. Seven of 15 pts were observed, and all of them relapsed also clinically (median 4.3 months, range 1.5–21). Eight of 15 pts were treated with rituximab, and 7/8 pts subsequently achieved molecular remission. MRel reappeared in 3/8 pts but was successfully retreated with rituximab again. Finally, 4/8 pts relapsed clinically; 4/8 pts stay in clinical remission (max. 41 months). Pts treated preemptively with rituximab had significantly better DFS compared with the group with observation only (median 30.3 vs 4.3 months, $p = 0.0025$).
Conclusions: Our data show that the detection of molecular relapse in MCL is a sensitive predictor of impending clinical relapse and that the preemptive immunotherapy with rituximab may prolong the period without a clinical progression.

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ZEVALIN (YTTRIUM-90 IBRITUMOMAB TIUXETAN) AND BEAM (Z-BEAM) VERSUS RITUXIMAB AND BEAM (R-BEAM) CONDITIONING CHEMOTHERAPY PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MANTLE CELL LYMPHOMA

M. D. Berger,[1] J. Hoffmann,[1] B. Klaeser,[2] T. Pabst.[1]
[1]Department of Medical Oncology, University Hospital, Bern, Switzerland; [2]Department of Nuclear Medicine, University Hospital, Bern, Switzerland.

Introduction: Mantle cell lymphoma (MCL) is characterized by early relapses and poor response to current treatment strategies. Maintenance treatment with the anti-CD20 antibody rituximab and high-dose chemotherapy (HDCT) followed by autologous stem cell transplantation (ASCT) have improved survival of MCL patients. We investigated the role of ibritumomab tiuxetan (Zevalin), a 90Yttrium labeled CD20 targeting antibody, as part of the conditioning regimen in MCL patients undergoing ASCT.
Methods: This single centre study analyzed 27 consecutive MCL patients receiving HDCT with rituximab and BEAM (R-BEAM) or Zevalin prior to BEAM (Z-BEAM) followed by peripheral ASCT. Zevalin was given at 14.8 MBq/kg on day −14 prior to ASCT, whereas Rituximab was administered on the first day of BEAM conditioning.
Results: Eighteen patients received HDCT with R-BEAM, and nine patients had Z-BEAM. The median age of the patients was 56 years (range 35–67). Eighty-five per cent had advanced-stage disease (stage III or IV), with 74% showing bone marrow infiltration at diagnosis. Sixteen per cent had blastoid variant, and 84% had classical MCL. Before ASCT, the rates of complete remission (CR) in the R-BEAM and Z-BEAM groups were 28% and 33%, and partial remission was seen in 72% and 67%, respectively. The mean number of transplanted CD34+ cells was 3.75 and 4.28×106/kg ($p = 0.319$). The median day of engraftment after ASCT was 11 days for leukocytes in both groups and for platelet recovery 15 days in the R-BEAM and 17 days in the Z-BEAM group. There were no early treatment-related deaths in either group, and no differences in toxicities and infection rates were observed. The median follow-up for MCL patients who received R-BEAM or Z-BEAM was 33.5 and 43 months, respectively. A 3½-year OS in the R-BEAM versus Z-BEAM groups was 52% and 89% ($p = 0.183$), and 3½-year PFS was 32% and 43% ($p = 0.206$), respectively.
Conclusion: This is the first study comparing two cohorts of MCL patients, treated with Z-BEAM and with R-BEAM as conditioning regimen prior to ASCT. High dose chemotherapy with Z-BEAM tended to yield better 3½-year OS and PFS compared with R-BEAM, without increased toxicity. Our data indicate that in MCL patients, Z-BEAM followed by ASCT could possibly be more effective than R-BEAM, without added toxicity. This approach should be tested in a prospective randomized trial.

T-CELL LYMPHOMAS

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PROTEOMIC ANALYSIS IDENTIFIES OUTCOME-PREDICTIVE MARKERS IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

M. Ludvigsen,[1] M. B. Pedersen,[2] S. J. Hamilton-Dutoit,[3] K. Bendix,[3] M. B. Moeller,[4] P. Noergaard,[5] F. D'Amore,[2] B. Honore.[1]
[1]Department of Biomedicine, University of Aarhus, Aarhus, Denmark; [2]Department of Haematology, Aarhus University Hospital, Aarhus, Denmark; [3]Institute of Pathology, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Pathology, Odense University Hospital, Odense, Denmark; [5]Department of Pathology, Herlev Hospital, Herlev, Denmark.

Introduction: Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), is a heterogeneous category of nodal and extranodal lymphomas in the WHO classification accounting for 25–30% of all mature T-/NK-cell malignancies. The clinical outcome is generally poor with a 5-year overall survival (OS) of 30–35% after conventional treatment strategies. Further understanding of the underlying biology of PTCL-NOS is necessary in order to improve the diagnostic, prognostic and therapeutic tools in this lymphoma entity. The aim of the present study is to characterize protein expression in PTCL-NOS according to clinical outcome by a proteomic approach.
Methods: Archival frozen tissue samples from 20 patients diagnosed with PTCL-NOS from 1991 to 2010 were grouped according to clinical outcome and matched according to age, sex, and international prognostic index. All patients were homogeneously treated with curatively intended anthracycline-containing combination regimens. Primary nodal tissue samples from patients with favourable (survival ≥ 2 years, $n = 8$) versus unfavourable (survival < 2 years, $n = 12$) outcome were compared. Clinico-pathological features were obtained from the Danish Lymphoma Registry (LYFO) and from patient records. Hyperplastic tonsils from healthy adults were included as reference tissue ($n = 8$). Tissue samples were subjected to high-resolution two-dimensional gel electrophoresis. Individual protein spots were visualized with fluorescence staining, and the expression profiles in the cohorts were compared. Differentially expressed (twofold or higher, Mann–Whitney *U*-test) proteins were identified by liquid chromatography–tandem mass spectrometry.
Results: Distinct, significant differences in protein expression patterns were identified between the two PTCL-NOS groups (13 downregulated and 3 upregulated) as well as between all the PTCL-NOS samples, taken as one group, and the reference tonsil tissue (53 downregulated and 45 upregulated). The identified proteins are currently under characterization and will be further investigated by immunohistochemistry on a large tissue microarray-based validation set ($n = 196$) and correlated with clinical features and outcome parameters.

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Conclusions: By proteomic analysis, we identified outcome-associated protein markers in PTCL-NOS. A validation of their predictive value based on a larger set of samples is warranted and is currently ongoing.

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T-LGL LEUKAEMIA AND NK-CHRONIC LYMPHOPROLIFERATIVE DISORDER: TWO DISEASES WITH A COMMON ETIOPATHOGENETIC MECHANISM?

R. Zambello,[1] C. Gattazzo,[1] A. Teramo,[2] F. Passeri,[2] E. De March,[1] G. Barila',[1] B. Montini,[1] M. Facco,[1] G. Semenzato.[1]
[1]Medicine, Hematology and Clinical Immunology Unit, Padova University School of Medicine, Padova, Italy; [2]Hematology, Venetian Institute of Molecular Medicine (VIMM), Padova, Italy.

The large granular lymphocyte (LGL) disorders are characterized by expansions of T or NK cells with cytotoxic activity. WHO 2008 classifies these disorders as separate entities, referred to as T-LGL leukaemia (T-LGLL) or NK-chronic lymphocyte proliferation disease (NK-CLPD). The marker of clonality is the rearrangement of TCR for T-LGLL and a restricted pattern of killer immunoglobulin-like receptor (KIR) expression for NK-CLPD. Although these disorders are characterized by the expansion of different cell types, compelling evidence supports the hypothesis that a common pathogenetic mechanism would be involved in both these disorders. In this study, we evaluated whether clonal T-cell populations were detectable in NK-CLPD patients, to corroborate the hypothesis that a common pathogenetic mechanism takes place in both these disorders.
Fourty-six therapy-free patients with NK-CLPD were enrolled in this study. TCR gamma chain rearrangement was investigated by PCR in DNA purified from PBMC. KIR restriction was analyzed in all patients. Twenty-three patients were available for serial measurements over 3 to 14 years.
We found that in 54% of NK-CLPD patients, a discrete clonal T-cell population was detected, despite the fact that all patients presented an NK disorder with a restricted pattern of KIR expression. This surprisingly high percentage of T clones present in NK-cell proliferation can not be explained by concomitant infections, because all patients were asymptomatic. No relevant clinical differences were demonstrated between the two groups of patients. Follow-up analysis confirmed that monoclonal T population, when established, was stable during time. Furthermore, in five patients, we could demonstrate a switch between a dominant KIR restricted NK-CLPD to a monoclonal T-LGLL during a mean follow-up of 5 years (range 3–13 years).
Results of this study support the hypothesis that an antigenic pressure is taking place in patient with NK-CLPD. The demonstration of switch between NK-CLPD and T-LGLL further suggests that a common mechanism is involved in the pathogenesis of these disorders.

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HOW MANY PERIPHERAL T-CELL LYMPHOMAS ARE ELIGIBLE FOR AND ENTERED INTO CLINICAL TRIALS? A POPULATION-BASED ANALYSIS FROM THE DANISH LYMPHOMA REGISTRY

M. B. Pedersen, F. D'Amore.
Department of Hematology, Aarhus University Hospital, Aarhus C, Denmark.

Background: Peripheral T-cell lymphomas (PTCL) accounts for 10–12% of all lymphomas. Although it has been suggested that the frequency of PTCL seems to increase, its rarity and diagnostic complexity, including bone marrow evaluation, still represent a challenge for the enrollment of PTCL patients into clinical trials in general and stem cell transplantation (SCT) trials in particular. The aim of the present study was, on the basis of the Danish population-based lymphoma registry (LYFO), to characterize and quantify the PTCL patient population potentially eligible for inclusion in clinical trials with or without the use of dose-intensified regimens followed by autologous or allogeneic SCT.
Methods: A cohort of 499 cases covering the period 2000–2010 was identified from the LYFO database. Clinico-pathological features were obtained from the LYFO database and compared using Chi^2, Fisher's exact, or *t* test. Time to event end-points were estimated by the Kaplan–Meier method and compared by the log-rank test.
Results: The incidence of PTCL was <1 case per 100 000. The most frequent subtypes were PTCL-NOS (43%), anaplastic large-cell lymphoma (32%), and angioimmunoblastic T-cell lymphoma (18%). Clinico-pathological features showed a median age of 61 years (range 16–90), stages III–IV (67%), high LDH (51%), PS 3–4 (14%), and >1 extranodal site (20%) with bone marrow involvement being the most frequent extranodal site (25%). Both overall survival and progression-free survival were effectively predicted by the prognostic scores IPI and PIT. Overall, approximately 20% of the patients in our cohort were considered to be not eligible to enter any interventional clinical trial. Moreover, almost half of the patients (49%) were not considered eligible (>65 years, PS >2) for enrollment in a clinical trial with upfront SCT. Of the potential 80% trial candidates within the study cohort, only 11% were actually included in a clinical trial.
Conclusion: In a population-based Caucasian PTCL cohort, almost half of all patients were not eligible for dose-intensified chemotherapy and/or autologous or allogeneic SCT. Of all patients, approximately 20% would not have been eligible for any clinical trial. However, only a minor fraction actually entered a clinical trial, emphasizing the need of optimizing recruitment strategies and trial designs for the frailest subset of clinical trial candidates.

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LONG-TERM FOLLOW-UP OF THE PHASE II STUDY OF SMILE CHEMOTHERAPY FOR PATIENTS WITH NEWLY DIAGNOSED STAGE IV, RELAPSED OR REFRACTORY EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE: THE NK-CELL TUMOR STUDY GROUP STUDY

R. Suzuki,[1] Y. Kwong,[2] Y. Maeda,[3] R. Sakai,[4] W. Kim,[5] C. Suh,[6] F. Ishida,[7] K. Izutsu,[8] Y. Isobe,[9] A. Kunitomi,[10] E. Sueoka,[11] Y. Okoshi,[12] J. Takizawa,[13] H. Kobayashi,[14] S. Nakamura,[15] J. Suzumiya,[16] K. Oshimi,[9] M. Yamaguchi.[17]
[1]HSCT Data Management and Biostatistics, Nagoya University, Nagoya, Japan; [2]Hematology, University of Hong Kong, Hong Kong, Hong Kong; [3]Hematology, Okayama University, Okayama, Japan; [4]Medical Oncology, Kanagawa Cancer Center, Yokohama, Japan; [5]Hematology, Samsung Medical Center, Seoul, South Korea; [6]Oncology, Asan Medical Center, Seoul, South Korea; [7]Hematology, Shinshu University, Matsumoto, Japan; [8]Hematology, NTT Medical Center Tokyo, Tokyo, Japan; [9]Hematology, Juntendo University, Tokyo, Japan; [10]Hematology, Kurashiki Central Hospital, Kurashiki, Japan; [11]Hematology, Saga University, Saga, Japan; [12]Hematology, Tsukuba University, Tsukuba, Japan; [13]Hematology, Niigata University, Niigata, Japan; [14]Hematology, Obihiro Kosei Hospital, Obihiro, Japan; [15]Pathology, Nagoya University, Nagoya, Japan; [16]Oncology Center, Shimane University, Izumo, Japan; [17]Hematology, Mie University, Tsu, Japan.

Introduction: Extranodal NK/T-cell lymphoma, nasal type (ENKL), is an uncommon subtype of lymphoma, and its prognosis used to be poor. For ENKL patients in newly diagnosed stage IV, relapsed or refractory disease, we previously showed that the treatment response dramatically improved by using a novel regimen, SMILE (steroid = dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide). The overall response rate of SMILE was 79% [90% confidence interval (CI): 65–89%], but no long-term effect has been indicated (J Clin Oncol 2011; 29: 4410).
Methods: In the SMILE phase II study, patients were treated with two courses of SMILE, accompanied by the physician's choice of additional SMILE, and/or autologous/allogeneic transplant. The study protocol was revised to collect data of long-term follow-up. The data used for this analysis were updated as of November 2012.
Results: Among 38 patients enrolled for SMILE phase II study, data of all 20 survived patients were renewed. No patients were lost during follow-up. The median follow-up time of the survivor was 51 months (range: 37 to 60 months). No patients received additional therapy other than SMILE or stem cell transplantation until relapse, as stipulated in the protocol. The overall survival (OS) and progression-free survival (PFS) at 3 years were 50% (95% CI: 33–65%) and 45% (95% CI: 29 LYFO 60%), respectively. Three patients experienced relapse after the first analysis, but only one patient died of disease. Two patients relapsed after auto transplant are alive, one after allo transplant and the other with indolent course (table).
Conclusions: These findings suggest that durable long-term effect can be obtained for ENKL patients by SMILE. The significance of autologous transplant should be determined with further studies with a large number of patients.

Abstract 235 Table. The 3-year OS and PFS

Post-SMILE therapy	*N*	3-year OS	(95% CI)	3-year PFS	(95% CI)
Allo	17	59%	(33–78)	59%	(33–78)
Auto	4	75%	(13–96)	25%	(1–67)
SMILE only (total 3–6 cycles)	17	35%	(14–57)	35%	(14–57)

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THE EFFICACY AND SAFETY OF THE L-ASPARAGINASE-BASED REGIMEN (CHOP-L) IN COMBINATION WITH RADIOTHERAPY IN NEWLY DIAGNOSED EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE: A PROSPECTIVE PHASE II STUDY

N. Lin, J. Zhu, W. Zheng, Y. Song, M. Tu, Y. Xie, X. Wang, L. Ping, Z. Ying, C. Zhang, W. Liu, L. Deng.
Department of Lymphoma, Peking University Cancer Hospital & Institute, Beijing, China.

Introduction: L-asparaginase-based regimens were found to be highly effective in patients with relapsed or refractory extranodal NK/T-cell lymphoma, nasal type (ENKTL). To explore the efficacy and safety of L-asparaginase in newly diagnosed extranodal nature killer NK/T-cell lymphoma, we conducted a phase II study of the L-asparaginase, cyclophosphamide, vincristine, doxorubicin and dexamethasone (CHOP-L) regimen in combination with radiotherapy.
Patients and Methods: Patients with newly diagnosed ENKTL and a performance status of 0 to 2 were eligible for enrollment. Treatment comprised six to eight cycles of CHOP-L chemotherapy (cyclophosphamide, 750 mg/m^2 day 1; vincristine, 1.4 mg/m^2 day 1 (up to a maximal dose of 2 mg); doxorubicin, 50 mg/m^2 day 1; dexamethasone, 10 mg days 1–8; L-asparaginase 6000 u/m^2 days 2–8). Radiotherapy was scheduled after four to six cycles of CHOP-L regimen according to stage and primary anatomic site. The primary endpoint was the complete response (CR) rate after the protocol treatment.
Results: A total of 38 eligible patients enrolled (median, 40.5 years; range, 15 to 71 years) showed the following clinical characteristics: male to female ratio, 24:14; Ann Arbor stage I, 20; II, 11; III, 3; and IV, 4; upper aerodigestive tract ENKTL, 36; B symptoms present, 22; elevated serum lactate dehydrogenase, 7; and leukocytopenia, 9. Eleven patients (28.9%) developed allergic reactions to L-asparaginase and then used polyethylene glycosylated-asparaginase instead of L-asparaginase. After completing the scheduled chemoradiotherapy, the CR rate and overall response rate were 81.6% (95% CI, 69.3% to 93.9%) and 84.2%, respectively. After a median follow-up of 25 months, the 2-year overall survival, progression-free survival and disease-free survival rates were 80.1% (95% CI, 73.3% to 86.9%), 81% (95% CI, 74.5% to 87.5%) and 93.6% (95% CI, 89.3% to 97.9%), respectively. The main toxicities were myelosuppression, liver dysfunction and digestive tract effects. Grade 3 to 4 leukopenia and neutropenia were frequent (76.3% and 84.2%, respectively). No treatment-related deaths were observed.
Conclusion: CHOP-L chemotherapy in combination with radiotherapy is a safe and highly effective treatment for newly diagnosed ENKTL.

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ALLOGENEIC-STEM CELL TRANSPLANTATION UPFRONT IN THE TREATMENT OF PATIENTS WITH ADVANCED T-CELL LYMPHOMA: AN INTENTION-TO-TREAT ANALYSIS FROM A SINGLE INSTITUTION

S. Le Gouill,[1] M.Lloirat,[1] C. Leux,[2] A. Moreau,[3] T. Guillaune,[1] T. Gastinne,[1] J. Delaunay,[1] N. Blin,[1] B. Mahe,[1] V. Dubrille,[1] K. Augeul-Meunier,[1] P. Peterlin,[1] H. Maisonneuve,[4] P. Moreau,[5] H. Jardel,[6] N. Juge-Morineau,[7] P. Moreau,[1] M. Mohty,[8] C. Patrice.[1]
[1]Hematology, Nantes Medical University, Nantes, France; [2]Epidemiologie, Universite de Nantes, Nantes, France; [3]Anatomo-Pathologie, Nantes Medical University, Nantes, France; [4]Hematology, CHD La Roche sur Yon, La Roche sur Yon, France; [5]Hematology, Centre Hospitalier de Lorient, Lorient, France; [6]Hematology, Hopital de Vannes, Vannes, France; [7]Hematology, Centre Catherine de Sienne, Nantes, France; [8]Hematology, Saint-Antoine, Paris, France.

A previous large study suggested that allo-SCT can cure non-cutaneous T-cell lymphomas (TCL) relapsed/refractory patients (Le Gouill, JCO 2008). Therefore, we initiated a single-centre pre-defined treatment algorithm where all patients <70 years (except alk ALTC) with stage >I disease referred to our institution were systematically planned to receive induction chemotherapy followed by allo-SCT upfront. From November 2004 to December 2012, 49 patients met these criteria (33 PTCL-NOS, 4 AITL, 7 Alk neg ALTC; 2 T/gd and three other entities). Median age at diagnosis was 50 years (range, 29–67). An HLA-matched donor (15 siblings, 17 unrelated and 7 suitable CBU units) could be found for 39 patients. Fifteen patients reached CR, and 25 patients reached PR after indution. Eighteen patients received salvage chemotherapy, and 7 could proceed to allo-SCT. In all, 29 patients (60%) proceeded to allo-SCT (mainly using a reduced-intensity conditioning regimen). Twenty patients did not proceed to allo-SCT because of progressive disease ($n = 13$), absence of matched donor ($n = 3$), unfit patients with comorbidities ($n = 3$) or patient refusal ($n = 1$). Disease status prior to allo-SCT was CR1 in 12 cases and PR1 in 17 cases. Three patients underwent Auto-SCT prior allo-SCT because graft was not available on time.
Results: In the whole series, median follow-up (mFU) was 16.3 months. One and 2-year PFS rates from diagnosis were 56% (CI95%; 43.6–71.5) and 51.3% (CI95%; 39–67.5), respectively. One and 2-year OS were 60% (CI95%; 47.7–75.2) and 50.3% (CI95%; 40.6–69.2), respectively. For allo-SCT patients ($n = 29$), 1- and 2-year PFS calculated from time of allo-SCT (mFU = 32.2 months) were 64.7% (CI 95%; 49.2–85.0) and 60.4% (CI95%; 44.5–81.9); 1- and 2-year OS were 71.6% (CI95%; 56.7–90.4) and 71.6 (CI95%; 56.7–90.4), respectively. Nine transplanted patients (including 2 patients in CR at time of allo-SCT) died. Causes of death were disease progression ($n = 6$) or toxicity ($n = 3$). TRM at 1 year was 8.2% (CI95%; 0–18.5).
Conclusion: Allo-SCT is feasible with low toxicity rate when performed upfront in responder patients and can provide long-term disease control. However, only 60% of patients that were planned to undergo allo-SCT at diagnosis could be actually transplanted. Absence of response after induction chemotherapy was the major reason for patients not undergoing allo-SCT, underlining the urgent need for new therapeutic options to increase response rates.

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LATE RELAPSES IN PERIPHERAL T-CELL LYMPHOMA PATIENTS TREATED IN THE NORDIC NLG-T-01 TRIAL

F. D'Amore,[1] G. F. Lauritzsen,[2] T. Relander,[3] E. Jantunen,[4] H. Hagberg,[5] H. Anderson,[6] H. Holte,[2] A. Osterborg,[7] M. Merup,[7] P. Brown,[8] O. Kuittinen,[9] M. Erlanson,[10] B. Ostenstad,[11] U. Fagerli,[12] O. Gadeberg,[13] C. Sundstrom,[14] J. Delabie,[15] E. Ralfkiaer,[16] M. Vornanen,[17] H. Toldbod.[1]
[1]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Oncology, Oslo University Hospital, Oslo, Norway; [3]Department of Oncology, Skane University Hospital, Lund, Sweden; [4]Department of Medicine, Kuopio University Hospital, Kuopio, Finland; [5]Department of Oncology, Uppsala University Hospital, Uppsala, Sweden; [6]Department of Cancer Epidemiology, Lund University, Lund, Sweden; [7]Department of Hematology, Karolinska University Hospital, Stockholm, Sweden; [8]Department of Hematology, Copenhagen University Hospital, Copenhagen, Denmark; [9]Department of Oncology, Oulu University Hospital, Oulu, Finland; [10]Department of Oncology, Umea University Hospital, Umea, Sweden; [11]Department of Oncology, Ulleval University Hospital, Oslo, Norway; [12]Department of Oncology, St. Olav University Hospital, Trondheim, Norway; [13]Department of Internal Medicine, Vejle Hospital, Vejle, Denmark; [14]Department of Pathology, Uppsala University Hospital, Uppsala, Sweden; [15]Department of Pathology, Oslo University Hospital, Oslo, Norway; [16]Department of Pathology, Copenhagen University Hospital, Copenhagen, Denmark; [17]Department of Pathology, Tampere University Hospital, Tampere, Finland.

Introduction: The Nordic Lymphoma Group conducted a large phase II study (NLG-T-01) in previously untreated systemic peripheral T-cell lymphoma (PTCL) to evaluate the efficacy of a dose-dense induction (CHOEP-14) consolidated by upfront autologous transplant (Tx). Twenty-six per cent of the patients experienced treatment failure before Tx. Another fraction of patients progressed/relapsed within the first 2 years after Tx. Because of its long median follow-up, the NLG-T-01 study was able to reveal late relapses occurring more than 2 years post-Tx. The aim of the present study was to analyze the frequency of these late relapses and characterize their clinico-pathological features.
Methods: Patients with previously untreated systemic PTCL aged 18–67 years were included. ALK-positive ALCL cases were excluded. Relapses were histologically verified, and late relapses were defined as patients who achieved a CR/CRu at first post-Tx assessment and subsequently recurred, at least 2 years after the date of Tx.
Results: Of the 160 patients, representing the trial's intention-to-treat population, a total of 114 (71%) underwent HDT/ASCT with 90 in CR/CRu at 3 months post-transplant. At a median follow-up of 60 months, 83 patients were alive. The 5-year overall and progression-free survival values for the entire cohort were 51% and 44%, respectively. Early failures occurred in 26% of the patients. Another 18% relapsed within the first 2 years after Tx, whereas 6% ($n = 9$) relapsed more than 2 years (range: 2.3–6.5 years) after Tx (late relapses). Patients with late relapses had a median age of 58 years (range: 29–63 years) and a preponderance of female cases (M/F ratio 1:2). The large majority (8 out of 9) of these patients had advanced disease, B-symptoms and IPI ≥ 2 at diagnosis. Histologically, the nine late relapse cases included five PTCL-NOS (relapse at 2.3, 3.5, 3.8, 4 and 5.3 years), two ALK-neg ALCLs (relapse at 2.8 and 6.5 yrs), one AILT (relapse at 4.2 years) and one EATL (relapse at 2.3 years). A paired clonality analysis, that is, performed on both the diagnostic and the relapse biopsy, in the patient relapsing after 6.5 years revealed the presence of the same clone in both biopsies.
Conclusions: Late relapses in transplant-eligible PTCL patients treated with a dose-dense schedule followed by upfront autologous transplant are not frequent

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but provide a rationale for maintenance therapy, which may contribute to further improve the overall outcome of these patients.

EXTRANODAL LYMPHOMA

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MANAGEMENT AND PROGNOSIS OF PATHOLOGICAL FRACTURES IN A SERIES OF 373 PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA OF THE BONE (THE INTERNATIONAL EXTRANODAL LYMPHOMA STUDY GROUP #14 STUDY)

S. Govi,[1] D. Christie,[2] C. Messina,[1] M. Bruno Ventre,[1] E. Gracia,[3] M. Gospodarowicz,[4] G. Martinelli,[5] E. Zucca,[6] F. Cavalli,[6] A. J. Ferreri.[1]
[1]Department of Onco-Hematology, San Raffaele Scientific Institute, Milan, Italy; [2]Radiation Oncology, John Flynn Private Hospital, Southport, Australia; [3]Oncology, National Institute of Oncology and Radiobiology, Cuba, Cuba; [4]Radiation Oncology, Princess Margaret Hospital, Toronto, Canada; [5]Hematology, European Institute of Oncology, Milan, Italy; [6]Medical Oncology, IOSI, Bellinzona, Switzerland.

Background: Ten to 20% of patients (pts) with diffuse large B-cell lymphoma (DLBCL) of the bone (B-DLBCL) present with pathological fractures (PF). The effects of PF on management and prognosis have not been analyzed in a large series.
Methods: The clinical features and the effects of PF on treatment and prognosis were analyzed in a retrospective series of 373 B-DLBCLs, comparing 78 pts with PF at presentation (cases) and 295 pts without PF (controls).
Results: Poor ECOG-PS, higher LDH levels and involvement of limbs were significantly more common among pts with PF. There were no differences in treatment between subgroups: 68 (87%) cases and 270 (92%) controls received chemotherapy (CHT); anthracycline-based regimen in 96% and 98% of pts, respectively. Sixty-nine cases and 233 controls received radiotherapy (RT) as part of first-line treatment; it was the exclusive treatment in 10 cases and 24 controls. RT dose and volume were similar in both subgroups. At a median f-up of 53 months (3–246), pts with PF exhibited lower response [overall response rate (ORR): 78% vs 85%; $p = 0.17$], progression-free survival (PFS) (5 years: 53% vs 61%; $p = 0.01$) and overall survival (OS) (5 years: 54% vs 68%, $p = 0.008$) rates than controls. Upfront surgical treatment of the PF did not change outcome, with a 5-year PFS of 48% and 56% ($p = 0.24$), respectively, for the 30 pts referred to upfront stabilization and the other 48 pts, with a 5-year OS of 39% and 58% ($p = 0.20$), respectively. Anticipating fracture irradiation was associated with significantly poorer outcome in pts treated with combined strategy: the 23 pts irradiated upfront (RT ≥ CHT) showed significantly poorer ORR (52% vs 92%; $p = 0.0005$), PFS (5 years: 33% vs 55%; $p = 0.005$) and OS (5 years: 47% vs 62%; $p = 0.008$) with respect to the 36 pts managed with CHT ≥ RT. Cox analysis adjusted for age and other IPI variables confirmed the independent association between PF and worse OS and the negative effect of initial PF irradiation.
Conclusions: PF seems to be an adverse event in B-DLBCL. Upfront PF irradiation is associated with poorer outcome; anthracycline-based CHT followed by RT is the recommended approach. Initial PF stabilization does not seem to improve outcome; however, it can be used to improve patient's quality of life provided that CHT is not delayed.

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CONSTITUTIVELY ACTIVE STAT6 REPRESSES BCL6 IN PRIMARY MEDIASTINAL B-CELL LYMPHOMA

J. K. Lennerz,[1] K. Rommel,[2] K. Dorsch,[1] E. Kelsch,[1] J. Melzner,[1] M. Buck,[1] K. Leroy,[3] S. Bruederlein,[1] P. Moller,[1] O. Ritz.[1]
[1]Institute of Pathology, University Ulm, Ulm, Germany; [2]Institute for Research in Biomedicine, Institute for Research in Biomedicine, Bellinzona, Switzerland; [3]INSERM, Universite Paris Est Creteil, Creteil, France.

Background: Primary mediastinal b-cell lymphoma (PMBL) is characterized by constitutive activation of JAK/STAT signalling resulting in pSTAT6 positivity. Notably, pSTAT6 positivity is heterogeneous and resembles the micro-heterogeneity seen with the key lymphomagenesis factor BCL6 in PMBL. The specific role of the intratumoural heterogeneity of both factors in PMBL has not been addressed.
Design: Here, we used co-labelling of BCL6 and pSTAT6 as a starting point for a combined immunohistochemical, molecular-biological and small-molecule inhibitor study.
Results: Double-fluorescence labelling in 10 primary PMBL cases and all three available PMBL models (MedB-1, K1106 and U2940) showed only a minute fraction of double-positive nuclei (0.6%) with large fractions of single-labeled pSTAT6 or BCL6 positive nuclei. This coexistence of two single-labeled pSTAT6+ and BCL6+ subpopulations suggested a negative interaction between these factors. We screened the STAT6/BCL6 promoters for their respective consensus binding sites and found five STAT-sites in the BCL6 promoter and confirmed STAT6 binding *in vitro* and *in vivo*. Luciferase assays, RNA interference and ectopic overexpression confirmed that pSTAT6 is sufficient to transcriptionally repress BCL6. To explore the relevance of these coexistent populations, we employed recently developed small molecule inhibitors that drastically reduce BCL6 target gene expression (79-6) or pSTAT6 levels (TG1 01348), respectively. Cell viability assays demonstrate significant reduction with each inhibitor (~30%), and combined exposure resulted in additive efficacy (~60%).
Conclusion: The delineated pSTAT6-mediated molecular repression mechanism link JAK/STAT to BCL6 signalling in PMBL and may carry therapeutic potential.

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HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL SUPPORT FOR PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A RETROSPECTIVE ANALYSIS FROM THE ADULT LYMPHOMA WORKING GROUP OF THE JAPAN SOCIETY FOR HEMATOPOIETIC CELL TRANSPLANTATION

E. Kondo,[1] S. Mizuta,[2] T. Ito,[3] H. Ogawa,[4] H. Sakamaki,[5] K. Kato,[6] Y. Morishima,[7] R. Suzuki,[8] J. Suzumiya.[9]
[1]Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan; [2]Department of Hematology, Fujita Health University Hospital, Toyoake, Japan; [3]Division of Hematology, Second Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan; [4]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [5]Department of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [6]Division of Hematology Oncology, Childrens Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [7]Division of Epidemiology and Prevention, Aichi Cancer Research Center, Nagoya, Japan; [8]Department Of HSCT Data Management/Biostatistics, Nagoya University Graduate School Of Medicine, Nagoya, Japan; [9]Department of Oncology/Haematology, Shimane University Hospital Cancer Centre, Izumo, Japan.

Background: Given that the prognosis of primary mediastinal large B-cell lymphoma (PMLBCL) is improved with rituximab-based chemotherapy, the role of high dose therapy (HDT) with autologous stem cell transplantation (ASCT) for PMLBCL is controversial. We report the results of HDT/ASCT for PMLBCL from the JSHCT Registry.
Methods: Detailed records on 114 patients with PMLBCL received HDT/ASCT between 1995 and 2010 were evaluated. Overall survival (OS) and progression-free survival (PFS) were calculated using Kaplan–Meier method.
Results: Median age at diagnosis was 29 years old (range 17–63). Median time from diagnosis to transplant was 9.5 months (range 3.2 to 59.7). Disease status at HDT/ASCT: first CR (or CRu) 48 patients (pts), firs PR 23 pts, second CR(or CRu) 13 pts, second PR 7 pts and refractory 21 pts. The median $CD34^+$ cell number collected for ASCT was 3.0×10^6/kg body weight (range, 1.5 to 19.5). HDT regimens were varied. Five patients (4.4%) died of toxicity related to the procedure within 100 days after ASCT. With a median follow-up period of 3.1 years (range 0.1 through 14.7), OS and PFS from transplant at 3 years were, respectivery, 88% and 84% for first CR pts, 87% and 55% for first PR pts, 83% and 72% for second CR, 38% and 21% for second PR pts, and 62% and 47% for refractory pts. In patients, achieved CR after transplant (42% of first PR; 33% of second PR and 53% of refratory pts), disease-free survival at 3 years were 53%, 100% and 63% in first PR, second PR and refratory pts, respectively. Disease progression was the main cause of death (62%). In univariate analysis, LDH, clinical stage, age and performans status did not exert significant effects on survival. Disease status at HDT/ASCT in all groups, and CR after ASCT in non-CR groups showed a strong relationship with the outcome.
Conclusions: The results are encouraging, even for PR and refractory patients. Further prospective studies for PMLBCL patients in salvage setting are warranted to confirm the efficacy of HDT/ASCT.

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INTENSIVE THERAPY OF THE PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

J. Mangasarova, A. Magomedova, E. Baryakh, V. Vorobiev, D. Maryin, E. Gemdjan, S. Kravchenco
Chemotherapy and Intensive Care, Hematology Research Center, Moscow, Russia.

Primary mediastinal large B-cell lymphoma (PMBCL) is an aggressive diffuse large B-cell lymphoma entity. It represents less than 3% of all non-Hodgkin

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lymphoma cases but is over-represented in the young adult population. Authors' goal was to evaluate the efficacy and toxicity of the modified programme NHL-BFM-90 (mNHL-BFM-90) for adult patients with PMBCL. We studied 64 patients (21 men, 43 women) from 18 to 70 years (mean age was 30 years). PMBCL staging criteria developed by Ann Arbor were used to stage the patients. All patients were diagnosed with II stage. Bulky mediastinal disease was found in 54 (90%) patients with PMBCL. Age-adjusted international prognostic index 1 was established in 14 patient (22%), 2 in 50 (78%), and 0 and 3 in 0. Serum lactate dehydrogenase level was increased in 58 (91%) patients with PMBCL. Between November 2004 and December 2012, they have started the NHL-BFM-90 protocol was modified by us (a dose of methotrexate was reduced to 1500 mg/m^2 (12 h) in course A, B and C; doxorubicine (50 mg/m^2) was included in course A) treatment of 63 newly diagnosed, previously untreated PMBCL patients. Four to six courses were performed, their quantity being determined depending on achieving the remission time. The patients with residual tumour in mediastinum underwent radiotherapy as consolidating treatment in total dose of 36 Gray. During an average follow-up of 36 months, the 5-year disease-free survival (DFS) and overall survival (OS) were 83% and 89% with PMBCL. One of the patients has died from chemotherapy complications. Eight patients turned out to be primary resistant. The modified mNHL-BFM-90 is a highly effective protocol. The 5-year DFS and OS were 83% and 89% with PMBCL.

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CLINICAL FEATURES AND TREATMENT OUTCOMES OF PATIENTS WITH EXTRANODAL NK/T-CELL LYMPHOMA IN GASTROINTESTINAL TRACT: RESULTS OF MULTICENTRE RETROSPECTIVE ANALYSIS

W. Kim,[1] S. Kim,[1] H. Kang,[2] J. Won,[3] C. Suh,[4] Y. Kwong,[5] K. Tay,[6] Y. Song,[7] J. Cao,[8] W. Chng,[9] R. Suzuki,[10] S. Chuang,[11] T. Lin.[12]

[1]Medicine, Samsung Medical Center, Seoul, SouthKorea; [2]Internal Medicine, Korea Cancer Center Hospital, Seoul, South Korea; [3]Internal Medicine, Soon Chun Hyang University Hospital, Seoul, South Korea; [4]Oncology, Asan Medical Center, Seoul, South Korea; [5]Medical Oncology, Queen Mary Hospital, Hong Kong, Hong Kong; [6]Lymphoma, National Cancer Center, Singapore, Singapore; [7]Internal Medicine, Peking University Cancer Hospital, Beijing, China; [8]Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China; [9]Hematology, Singapore, Singapore; [10]HSCT Data Management & Biostatistics, Nagoya University, Nagoya, Japan; [11]Pathology, Chi-Mei Medical Center, Tainan and Taipei Medical University, Tainan, Taiwan; [12]Medical Oncology, Sun Yat-Sen University, Guangzhou, China.

Extranodal NK/T-cell lymphoma (ENKTL) in gastrointestinal tract is less frequent than nasal tract ENKTL, and gastrointestinal involvement is known as an unfavourable sign. However, owing to its rarity, there are few data regarding clinical features and outcomes of gastrointestinal ENKTL. Thus, we performed a multicentre retrospective analysis focusing on ENKTL involving gastrointestinal tract. The clinical information of 81 patients with ENKTL in gastrointestinal tract was gathered from 12 institutes of Asian countries. The median age was 45 years (range: 17–79 years), and male was predominant (68.5%). The most common site of involvement was small intestine including jejunum and ileum ($n = 57$, 70.3%). The pattern of gastrointestinal involvement was mainly diffuse invasion with lymph node enlargements. The involvement of nasal cavity or nasopharynx was also found in 18 patients (22.2%). Thus, more than a half of patients presented as Lugano stage IV ($n = 45$, 55.6%). B symptoms and elevation of serum LDH were commonly observed (50% and 63%, respectively), however, the invasion of bone marrow was only found in seven patients (8.6%). As a primary treatment, 42% of patients received systemic chemotherapy, whereas 41% underwent surgery followed by chemotherapy. However, the majority of patients relapsed or progressed, resulting in death. Furthermore, treatment-related mortality such as sepsis was also commonly observed. As a result, the median overall survival was 7.8 months (95% CI: 3.9–11.7), and progression-free survival was 5.4 months (95% CI: 2.5–8.3). The common prognostic parameters for lymphoma such as age, serum LDH and stage failed to show a statistical significance for survival. As a result, the international prognostic index and NK prognostic index did not show a significant association with prognosis, either. The type of chemotherapy regimen also failed to prove survival benefit. However, surgery followed by chemotherapy showed a trend of better survival than chemotherapy or surgery alone. In conclusion, ENKTL involving gastrointestinal tract showed dismal prognosis despite active treatment including chemotherapy and surgery. Thus, more effective treatment strategy is required for this disease entity.

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MICRORNAS IN CEREBROSPINAL FLUID AS BIOMARKER FOR DISEASE COURSE MONITORING IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

A. Baraniskin,[1] E. Zaslawska,[1] U. Schlegel,[2] W. Schmiegel,[1] S. Hahn,[3] R. Schroers.[1]

[1]Department of Medicine, Hematology and Oncology, Knappschaftskrankenhaus, Ruhr-University of Bochum, Bochum, Germany; [2]Department of Neurology, Knappschaftskrankenhaus, Ruhr-University of Bochum, Bochum, Germany; [3]Department of Molecular GI-Oncology, Ruhr-University of Bochum, Bochum, Germany.

The diagnosis of primary central nervous system lymphoma (PCNSL) depends on histopathology of brain biopsies, because disease markers in the cerebrospinal fluid (CSF) with sufficient diagnostic accuracy are not available yet. In our previous study, combined expression analyses of miR-21, miR-19b, and miR-92 in CSF revealed that CSF levels of miRNA could differentiate, with high specificity (96.7%) and sensitivity (95.7%), patients with PCNSL from other neurologic disorders. In the current study, we expanded the original cohort ($n = 23$) with 41 patients to further substantiate the results of our prior study. In the enlarged PCNSL cohort, the high sensitivity of 96.9% was demonstrated, viz. 62 of 64 patients with PCNSL were classified correctly. These data confirm our previous findings and further support the potential of miRNA quantification in the CSF as a non-invasive test for PCNSL. In a different study, we found fragments of U2 snRNA (RNU2-1f) circulating in serum to be a novel encouraging diagnostic biomarker for pancreatic ductal adenocarcinoma and colorectal cancer. U2 snRNA composes with several proteins the U2 small nuclear ribonucleoprotein, which forms together with four other snRNPs the spliceosome. Subsequently, we focused on the detection of RNU2-1f in cerebrospinal fluid. Primarily, we found out that RNU2-1f was detectable in CSF as well. Moreover, our results demonstrated that the levels of RNU2-1f, as measured in qRT-PCR assays, were significantly increased in CSF samples from patients with PCNSL. RNU2-1f CSF levels could distinguish, with considerable sensitivity (92%) and specificity (81%), patients with PCNSL from other neurologic disorders, most importantly inflammatory CNS diseases. RNU2-1f had a significant diagnostic value for PCNSL and yielded an AUC of 0.94 in receiver operating characteristic analyses. In this study, we show for the first time that U2 snRNA fragments can be used as mono-marker for the discrimination of patients with PCNSL from controls with high sensitivity and specificity. Furthermore, the diagnostic value of U2 snRNA fragments as single biomarker exceeds the diagnostic value of the previously shown markers miR-21, miR-19b and miR-92 used as single biomarker, respectively.

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ONCOGENIC MICRORNAS IN CERBEROSPINAL FLUID AND SERUM: SENSITIVE TOOL FOR DETECTION OF CENTRAL NERVOUS SYSTEM LYMPHOMA IN RESPONSE TO THERAPY

V. Pospisil,[1] H. Mocikova,[2] M. Palickova,[2] Z. Vernerova,[3] T. Kozak,[2] M. Trneny,[4] T. Stopka.[1]

[1]Pathophysiology, First Faculty of Medicine Charles University, Prague, Czech Republic; [2]Department of Clinical Hematology, Third Faculty of Medicine, Charles University Hospital Kralovske Vinohrady, Prague, Czech Republic; [3]Pathology, Third Faculty of Medicine, Charles University Hospital Kralovske Vinohrady, Prague, Prague, Czech Republic; [4]Clinical Hematology, First Faculty of Medicine and General Faculty Hospital, Charles University, Prague, Czech Republic.

Introduction: Lymphomas overexpress oncogenic microRNAs (such as miR-17–92 and miR-155) that influence tumour growth and spreading. Unlike longer species of RNA, miRs are preserved and detectable in body fluids including serum/plasma and cerebrospinal fluid (CSF); however, their kinetics in body fluids upon course therapy has not been explored. We investigated and compared abundance of miRs in the CSF and sera of lymphoma patients with ($N = 22$) or without ($N = 21$) central nervous system (CNS) involvement and tested whether their levels are elevated at diagnosis or changed upon the treatment.

Methods: CSF and sera were collected at multiple (5–20) time points before, during and after application of therapy (DLBCL 29, MCL 12 and Burkitt 2). Twenty candidate miRs were quantitated by TaqMan RT-PCR and adjusted to levels of control miRs and normal donors.

Results: Our data demonstrate that prominently changed miRs (members of miR-17–92 cluster and miR-21) were elevated to similar extent in CSF in PCNSL ($N = 11$) and systemic lymphomas with CNS involvement (SCNSL, $N = 11$). Additionally, all SCNSL patients and ~60% of systemic lymphomas without CNS involvement expressed high levels of these oncomiRs in the sera. Surprisingly, also a subset of PCNSL patients has elevated these miRs in the sera, and in turn, a subset of systemic

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lymphomas without CNS involvement showed partially elevated oncomiRs in the CSF. These data indicate that (i) lymphoma patients with CNS involvement are characterized by presence of oncogenic miRs in CSF and (ii) blood brain barrier is permissive for miRs in both directions. Next, we investigated the dynamics of these microRNAs during treatment in 10 patients with PCNSL or SCNSL. In therapy responders ($N=4$), the oncomiRs gradually decreased in CSF. However, their levels were not completely normalized. In contrast, refractory lymphoma ($N=3$) displayed gradual increase of these miRs in CSF upon treatment. Interestingly, clinical CNS relapse of two lymphoma patients was preceded by gradually elevated miRs in CSF by 3 months.
Conclusions: The measurement of oncomiRs in CSF and sera represents a likely sensitive tool for CNS lymphoma detection, for estimation of therapy efficacy and prediction of the relapse. Role of oncomiRs in promoting tumourogenesis in distant tissues is being further studied.
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SIMILAR CHEMOKINE RECEPTOR PROFILES IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA AND SECONDARY CENTRAL NERVOUS SYSTEM INVOLVEMENT OF SYSTEMIC DIFFUSE LARGE B-CELL LYMPHOMA

A. Pasanen,[1] S. Lemma,[1] K. Haapasaari,[2] A. Sippola,[1] R. Sormunen,[3] Y. Soini,[4] E. Jantunen,[5] P. Koivunen,[6] N. Salokorpi,[7] R. Bloigu,[8] T. Turpeenniemi-Hujanen,[1] O. Kuittinen.[1]
[1]Department of Oncology and Radiotherapy, University of Oulu and Oulu University Hospital, Oulu, Finland; [2]Department of Pathology, University of Oulu and Oulu University Hospital, Oulu, Finland; [3]Biocenter Oulu, University of Oulu, Oulu, Finland; [4]Department of Clinical Pathology and Forensic Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; [5]Department of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; [6]Department of Otorhinolaryngology, University of Oulu and Oulu University Hospital, Oulu, Finland; [7]Department of Neurosurgery, University of Oulu and Oulu University Hospital, Oulu, Finland; [8]Medical Informatics Group, University of Oulu, Oulu, Finland.

Introduction: Central nervous system (CNS) relapse of diffuse large B-cell lymphoma (DLBCL) is a devastating complication of a disease that is otherwise often curable with modern treatment. The mechanisms causing CNS tropism in DLBCL are not known. Chemokines and their receptors control the normal migration and homing of lymphatic cells and also guide the organ-specific metastasis of solid tumours. In this study, we evaluated the expression and microlocalisation of the chemokine receptors CXCR4, CXCR5 and CCR7 in samples from patients with primary CNS lymphoma (PCNSL) or a systemic DLBCL, with or without CNS relapse.
Methods: The patient material consists of 89 DLBCL patients, including 35 cases of PCNSL, 21 secondary CNS lymphomas (sCNSL) and 33 systemic DLBCL cases without CNS involvement. Immunohistochemical stainings for CXCR4, CXCR5 and CCR7 were performed on diagnostic tissue samples. Immuno-electron microscopy (IEM) was performed on samples from TWO additional PCNSL cases, ONE sCNSL, ONE nodular systemic lymphoma and ONE reactive lymph node sample, after staining with antibodies for CXCR4 and CXCR5, and their ligands CXCL12 and CXCL13.
Results: High nuclear CXCR4 positivity (80–100% of malignant cells positive) was associated with systemic DLBCL, whereas high cytoplasmic CXCR5 correlated with CNS involvement ($p=0.003$ and $p=0.039$, respectively). IEM revealed a nuclear CXCR4 staining pattern in the reactive lymph node and overexpression of CXCR5 in PCNSL and sCNSL compared with the nodular DLBCL and reactive lymph node samples. The proportion of CXCR5 receptors forming complexes with CXCL13 was largest in the reactive lymph node and smallest in PCNSL.
Conclusions: In this study, we found that DLBCL patients with CNS involvement presented with a different chemokine receptor profile compared with patients without CNS involvement. High cytoplasmic CXCR5 expression of malignant B-cells may be associated with increased CNS tropism of DLBCL. High nuclear CXCR4 seems to correlate with nodular disease and act as a CNS protective factor. Our findings give new information on the CNS tropism of DLBCL, and if verified, in the future, they may also contribute to more effective targeting of CNS prophylactic treatment among DLBCL patients.

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RITUXIMAB IN COMBINATION WITH METHOTREXATE-BASED CHEMOTHERAPY WITH BLOOD–BRAIN BARRIER DISRUPTION IN NEWLY DIAGNOSED PRIMARY CNS LYMPHOMA

N. D. Doolittle,[1] R. Fu,[2] L. L. Muldoon,[1] R. M. Tyson,[1] C. A. Lacy,[1] E. A. Neuwelt.[1]
[1]Neurology, Oregon Health & Science University, Portland, Oregon, USA; [2]Public Health and Preventive Medicine, Oregon Health & Science University, Portland, Oregon, USA.

Introduction: Primary central nervous system lymphoma (PCNSL) is an aggressive primarily large B-cell lymphoma confined to the CNS and/or eyes at presentation. High-dose methotrexate (MTX)-based chemotherapy is standard of care in PCNSL. Rituximab has been added to MTX-based PCNSL regimens because of positive outcomes in systemic B-cell lymphomas. On the basis of efficacy in a rat model of human B-cell CNS lymphoma, and safety and efficacy of enhanced delivery of rituximab with blood–brain barrier disruption (BBBD) in recurrent PCNSL, we treated patients with newly diagnosed B-cell PCNSL with rituximab in combination with MTX-based chemotherapy and BBBD. We previously reported outcomes in 12 patients (Blood 116:21,2010; abstract 2792); here, we report updated outcomes in 24 patients.
Methods: We obtained IRB permission to retrospectively evaluate newly diagnosed PCNSL patients who were treated with rituximab in combination with MTX-based chemotherapy and BBBD. Treatment was MTX [2500 mg/day, intra-arterial (i.a.)] and carboplatin (200 mg/m^2/day, i.a.) with BBBD, for two consecutive days every 4 weeks for 1 year. Rituximab (375 mg/m^2, i.v.) was every 4 weeks, 12 hbefore BBBD. Response rate, progression-free survival (PFS), overall survival (OS), and toxicities were evaluated.
Results: Patients ($n=24$) were treated between April 2003 and July 2012. At diagnosis, the median age was 64 years; 16 patients were 60 years or older. The median Karnofsky Performance Score (KPS) was 65. At diagnosis, all patients had brain parenchyma involvement; cerebrospinal fluid cytology was positive in three patients, and three had ocular involvement. The overall response rate was 92% (75% CR), median PFS was 2.1 years, and median OS was 5.1 years. Twelve patients who attained CR were alive at data cut-off;five patients remain in CR 2 years or more after diagnosis.The most frequent toxicities were hematologic; 16 (67%) patients developed grade 3 or 4 hematologic toxicity. Three (13%) patients developed grade 3 infection.
Conclusions: We previously reported a 58% CR rate and median OS of 3.1 years in 149 newly diagnosed PCNSL patients treated with MTX-based BBBD without rituximab. The addition of rituximab shows improved CR rate (75%) and OS (5.1 years), with 16 patients over 60 years old and 12 patients with KPS less than 70 at diagnosis. A phase II prospective study is underway.

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METHOTREXATE, RITUXIMAB AND TEMOZOLOMIDE IN CNS LYMPHOMA: THE MAYO CLINIC EXPERIENCE

V. Gupta, R. C. Wolf, P. B. Johnston.
Hematology, Mayo Clinic, Rochester, USA.

Background: We present a tertiary centre experience with high-dose methotrexate, rituximab and temozolomide (MRT) chemotherapy in both primary and secondary central nervous system (CNS) lymphoma.
Methods: A retrospective analysis was performed for a total of 27 patients (20 male, 7 female) who underwent treatment at the Mayo Clinic, Rochester between November 2010 and October 2012. The median age was 63 years (range 23–73). Of these, 12 patients were diagnosed with primary CNS lymphoma, whereas 15 patients had secondary CNS lymphoma. All patients received at least one cycle of MRT. The most common histological subtype was diffuse large cell lymphoma (23 of 27 patients). Other histological subtypes were high grade lymphoma (two patients), Burkitt's lymphoma (one patient) and Mantle cell lymphoma (one patient).
Results: Of 26 patients, who underwent imaging studies for response assessment after MRT, 14 patients achieved complete response (CR), 4 patients achieved partial response (PR), 2 patients had stable disease, and 6 patients had evidence of disease progression. Overall, the median number of chemotherapy cycles with MRT was 3 (range 1–6). For patients, who achieved CR, the median number of MRT cycles was 4 (range 2–6). MRT was well tolerated. Of 27 patients, who underwent their entire treatment at Mayo Clinic, grade 3 or higher treatment related toxicity was noted to be as follows: neutropenia (9/27), thrombocytopenia (4/27), anaemia (7/29), and transaminitis (10/27). Sixteen of 27 patients proceeded to high dose chemotherapy and autologous stem cell transplant. Out of 16 patients, 14 received BCNU/Thiotepa as conditioning regimen, whereas two patients underwent BEAM conditioning. All 16 patients who underwent transplantation remain in CR at this time. Of the remaining 11 patients, 4 are being planned for autologous transplantation, 2 received and responded to salvage chemotherapy, and 5 patients died of progressive disease.
Conclusions: Overall, the MRT regimen was well tolerated. Overall response rate was noted to be 67% (18 of 27 patients) and achieved CR in 54% patients (14 of 26 patients). Patients who underwent autologous stem cell transplantation as consolidation therapy continue to do well at a median follow-up of 12.5 months (range 2–20 months).

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PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: A RETROSPECTIVE ANALYSIS OF DATA FROM THE DANISH LYMPHOMA REGISTRY LYFO

D. Stoltenberg,[1] P. D. Brown,[1] B. Oestergaard,[2] T. Eberlein,[3] S. Pulczynski,[4] J. Madsen,[5] C. R. Tanem,[6] L. Munksgaard,[2] G. Thorsell,[5] T. Klausen,[6] J. Soenderskov,[1] E. J. Pulczynski.[3]

[1]*Hematology, Rigshospitalet, Copenhagen, Denmark;* [2]*Hematology, Odense Universitetssygehus, Odense, Denmark,* [3]*Hematology, Aarhus Universitetshospital, Aarhus, Denmark;* [4]*Hematology, Holstebro Hospital, Holstebro, Denmark;* [5]*Hematology, Aalborg Universitetssygehus, Aalborg, Denmark;* [6]*Hematology, Herlev Universitetshospital, Herlev, Denmark.*

Introduction: Primary central nervous system lymphoma (PCNSL) remains a challenge because of poor prognosis, lack of uniform strategy and increasing incidence. We analyzed data from LYFO database of the period 2000–2010 to identify factors of prognostic importance.

Method: Patients (pts) with biopsy verified PCNSL were extracted from the LYFO database. Incidence rates were calculated and compared with incidence of all NHL. Clinical and laboratory data at time of diagnosis were entered in a Cox proportional hazards multivariate regression analysis (MVA).

Results: Nineteen pts with PCNSL were identified with a median age 68 (31–88) years; 106 were males. Histology was diffuse large B-cell lymphoma in 93.2% and other NHL in 6.8%. The incidence in females was unchanged in the study period; however, in men, the incidence increased from 1.9×106 to 8.8×106 ($p = 0.003$) and was not explained by an increase in incidence of all NHL. Median follow-up was 74 months. Five-year overall survival (OS) was 25%, median OS 12 months. In patients aged 15–69 years, 5-year OS was 34%, median OS 25 months, and in patients aged 70–88 years, 5-year OS was 13%, median OS 4 months. In an MVA of OS for all patients, the hazard ratio (HR) for age group was 1.78 (1.23–2.58), performance status (PS) 2.00 (1.37–2.90) and comorbidity 1.48 (1.02–2.16). MVA including only treated patients showed age HR 1.78 (1.21–2.62), PS HR 2.23 (1.50–3.30) and monotherapy with HD-MTX HR 4.24 (2.41–7.44) to be of prognostic value. In contrast to monotherapy with HD-MTX HR 9.68 (4.08–22.99), whole brain radiotherapy HR 0.28 (0.08–0.93) and treatment containing rituximab HR 0.29 (0.10–0.81) were associated with a favourable outcome in a subanalysis of patients aged 70–88years.

Conclusion: In the period 2000–2010, a significant increase in incidence of PCNSL was observed in males but not females. High age, performance status and comorbidity were identified as factors associated with poor outcome. Furthermore, in elderly patients, treatment containing rituximab or WBRT only resulted in superior outcome, whereas patients treated with MTX as monotherapy had a very poor outcome.

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IELSG 34—FINAL RESULTS OF A MULTICENTRE PHASE II STUDY TO EVALUATE THE CLINICAL ACTIVITY AND THE SAFETY OF EVEROLIMUS IN MARGINAL ZONE B-CELL LYMPHOMAS

A. Conconi,[1] M. Raderer,[2] S. Franceschetti,[1] L. Devizzi,[3] A. Ferreri,[4] M. Magagnoli,[5] L. Arcaini,[6] P. L. Zinzani,[7] G. Martinelli,[8] U. Vitolo,[9] E. Porro,[10] A. Stathis,[11] F. Cavalli,[11] G. Gaidano,[1] E. Zucca.[11]

[1]*Hematology, AOU Maggiore della Carita', Novara, Italy;* [2]*Department of Internal Medicine I, University of Wien, Wien, Austria;* [3]*Medical Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy;* [4]*Department of Oncology, Unit of Lymphoid Malignancies, San Raffaele Scientific Institute, Milan, Italy;* [5]*Hematology, Humanitas Cancer Center, Milano Rozzano, Italy;* [6]*Hematology, IRCCS Policlinico San Matteo, Pavia, Italy;* [7]*Institute of Hematology and Medical Oncology, Policlinico S.Orsola-Malpighi, Bologna, Italy;* [8]*Division of Clinical Haemato-Oncology, European Institute of Oncology, Milan, Italy;* [9]*Hematology, A.O. Citta' della Salute e della Scienza di Torino San Giovanni Battista, Torino, Italy;* [10]*IELSG Operation Office, Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland;* [11]*Lymphoma Unit, Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland.*

Introduction: The antitumour activity of the mTOR inhibitor everolimus has been observed in different subtypes of non-Hodgkin's lymphomas.

Aim: The IELSG coordinated a phase II study aimed to evaluate the activity and safety of everolimus in marginal zone B-cell lymphomas (MZLs).

Methods: Thirty patients (pts) with relapsed/refractory MZLs received everolimus, 10 mg daily, from day 1 to day 28 for up to six cycles or until progression. The planned sample size allowed the detection of minimum activity of interest corresponding to overall response rate (ORR) of 20% [with at least six objective responses (OR)].

Results: Median age was 71 years (range, 51–88 years). Twenty pts had extranodal MZL, six had splenic MZL, and four had nodal MZL. Median number of prior therapies was 2 (range, 1–5). Six pts had Ann Arbor stage I, two pts had stage II, and 22 pts had stage IV. Seventeen pts had intermediated-high/high risk according to IPI criteria. Among the 24 pts assessable for response, the ORR was 25% (95% CI: 10%-47%), with one complete and five partial responses. Eleven pts experienced stable disease, and seven had disease progression (PD) during therapy. Grade 3–4 adverse events were neutropenia and thrombocytopenia (17% of pts, each), mucositis and odontogenic infections (13%), lung toxicity (10%), and other infectious events (7%). Seventeen pts early interrupted the study treatment, in most cases because of toxicity. Median number of cycles delivered was 4.5 (range, 1–16). After a median follow-up of 14 months, two pts had PD, 2 and 3 months after a prior PR; the other OR lasts +3, +4, +7, and +11 months, respectively. Five deaths were reported, in two pts due to PD, in one pt to toxicity, and in two pts to unknown cause. In an intent to treat analysis, progression-free survival was 14 months.

Conclusions: Everolimus is active in relapsed/refractory MZLs, but detection of significant toxicity suggests the need of strategies, perhaps combination therapies, to potentiate activity and overcome the otherwise limited applicability of the drug in these indolent entities.

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A PHASE II TRIAL OF RITUXIMAB PLUS LENALIDOMIDE IN PATIENTS WITH LYMPHOMA OF THE MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT-LYMPHOMA): AGMT (ARBEITS-GEMEINSCHAFT MEDIKAMENTOESE TUMORTHERAPIE) MALT 2 STUDY

B. Kiesewetter,[1] M. Fridrik,[2] W. Willenbacher,[3] E. Wllenbacher,[3] R. Greil,[4] M. Raderer.[1]

[1]*Department of Internal Medicine I, Division of Oncology, Medical University Wien, Wien, Austria;* [2]*Department of Internal Medicine, Division of Oncology, AKH Linz, Linz, Austria;* [3]*Department of Internal Medicine V, Division of Hematology and Oncology, Medical University Innsbruck, Innsbruck, Austria;* [4]*Department of Internal Medicine III, Paracelsus Medical University Salzburg, Salzburg, Austria.*

Introduction: Recently, the ability of lenalidomide to induce objective responses in lymphoma of the mucosa associated lymphoid tissue (MALT-lymphoma) patients has been demonstrated in a pilot study performed at our institution. In view of this, and the fact that rituximab (R) combination regimens are active in a variety of B-cell NHL malignancies, we have initiated a phase II trial of rituximab plus lenalidomide in patients with MALT-lymphoma.

Patients and Methods: Patients with histologically verified advanced MALT-lymphoma (in case of gastric MALT-lymphoma with demonstrated refractoriness to HP-eradication) were included in the study. Treatment consisted of 375 mg/m^2 rituximab on day 1 and lenalidomide 20 mg p.o. days 1–21, with a 7-day break after each cycle. Patients with documented complete remission (CR) after six courses stopped therapy, whereas patients with partial remission (PR) or stable disease (SD) were given another two cycles. Restaging was scheduled after three, six and eight courses, respectively.

Results: To date, a total of 21 patients have been included in the trial, and as of February 2013, 10 are evaluable for response. Currently, three patients have completed six cycles, resulting in a CR, whereas seven additional patients had CR after cycle 3 with treatment currently ongoing. All patients who underwent evaluation so far responded to R-lenalidomide. Haematologic adverse events were mild with neutropenia grade II in three patients, thrombopenia grade I in three patients and anaemia grade I in one patient. Other side effects consisted mainly of mild fatigue ($n = 5$), pruritus ($n = 6$, one patient grade III), mild constipation ($n = 4$), vertigo ($n = 4$, grade III in one patient) and mild exanthema ($n = 3$). Toxicity required dose reduction to 15 mg in three and 10 mg in one patient (due to nausea/emesis, headache/exanthema and infection, respectively).

Discussion: These preliminary data suggest high activity and good tolerability of R-lenalidomide in patients with MALT-lymphoma. More mature results will be available and presented at the meeting.

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HODGKIN LYMPHOMAS

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SILENCER OF CYTOKINE SIGNALLING 1 GENE MUTATIONS IN CLASSICAL HODGKIN'S LYMPHOMA

J. K. Lennerz, K. Hoffmann, N. Ruether, A. Viardot, P. Moller.
Institute of Pathology, University Ulm, Ulm, Germany.

Background: Gene mutations in the suppressor of cytokine signalling 1 (SOCS1) are frequent in classical Hodgkin's lymphoma; however, the prognostic relevance of these mutations is unexplored.
Methods: We performed laser-capture microdissection of Hodgkin/Reed–Sternberg (HRS) cells out of fresh-frozen tumour samples followed by full-length SOCS1 gene sequencing in a cohort of 84 well-characterized patients with follow-up.
Results: SOCS1 mutations in HRS cells are present in 62% of tumours ($n = 52/84$). Affected DNA motifs as well as patterns of intratumoural accumulation imply that these mutations are the result of aberrant somatic hypermutation in HRS cells. By predicted mutational consequence, the 52 mutated tumours can be separated into those with HRS cells that harbour non-foreshortening point mutations ('minor'; $n = 40/52 = 80\%$) and tumours with HRS cells that harbour at least one foreshortening mutation ('major'; $n = 12/52 = 23\%$). Clinical characteristics did not differ between tumours with wild-type versus SOCS1 mutated, minor, or major HRS cells, respectively. However, when outcome measures were compared to patients with tumours composed of wild-type HRS cells, SOCS1 mutations were associated with favourable outcome. Importantly, subgroup analysis revealed that the prognostic difference was due to a significantly reduced relapse rate in classical Hodgkin's lymphomas with SOCS1 minor mutations in HRS cells, whereas patients with HRS cells that contained SOCS1 major mutations suffered from early relapse and demonstrated significantly shorter overall survival. Thus, for nearly two thirds of the classical Hodgkin lymphoma patients, the SOCS1 mutation status and, in particular, the mutation subtype predict treatment course and overall survival.
Conclusions: SOCS1 mutation status is a single-gene prognostic biomarker for over 60% of classical Hodgkin lymphoma patients.

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RISK FACTORS AND STAGING SYSTEMS IN EARLY STAGE HODGKIN LYMPHOMA PATIENTS HAVE SIGNIFICANT IMPACT ON TREATMENT OUTCOME AFTER MODERN COMBINED MODALITY TREATMENT

P. Borchmann,[1] B. Klimm,[2] H. Goergen,[2] M. Fuchs,[2] B. von Tresckow,[1] V. Diehl,[2] A. Engert.[1]
[1]*1st Department of Internal Medicine, University Hospital of Cologne, Cologne, Germany;* [2]*1st Department of Internal Medicine, German Hodgkin Study Group, Cologne, Germany.*

In early-stage Hodgkin lymphoma (HL), treatment according to the early favourable or unfavourable subgroup is mostly guided by a staging system including the presence of certain risk factors. However, risk factors have grown historically and differ between the various study groups worldwide. We analyzed the impact of risk factors used in different international staging systems on outcome in early-stage HL. In 1173 early-stage patients treated with four cycles of ABVD followed by involved-field radiotherapy within the German Hodgkin Lymphoma Group (GHSG) trials HD10 and HD11, the impact of three international staging systems (GHSG, EORTC, and NCCN) in discriminating early favourable and unfavourable treatment groups for progression-free survival (PFS) and overall survival (OS) was assessed. Univariate analyses of the respective single risk factors as well as multivariate analyses were carried out using logistic regression, in order to evaluate the relevance and interaction of single factors. All three staging systems selected an unfavourable 'high risk' group out of early-stage patients of comparable size (55%, 54%, and 56% of patients with the GHSG, EORTC, and NCCN system, respectively) with significant impact on PFS and OS. For example, with the GHSG system, PFS was 95.8% and 86.4% for the early favourable and unfavourable group at 5 years, with a significant difference of −9.4%. Sensitivity for HL-related failure was high for all systems (84%, 79%, and 83%, respectively); however, there were high rates of false-positive results (1-specifity 54%, 53%, and 55%, respectively). In general, models of high sensitivity included risk factors indicating large tumour burden and high tumour activity. For the first time, the relevance of selecting an unfavourable risk group out of early-stage patients was proven in a large cohort of HL patients without treatment bias. We found a significant impact on PFS and OS. Discriminating early favourable and unfavourable patients with HL by the identified risk factors remains important in the modern combined modality treatment era.

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DEEP INSPIRATION BREATH HOLD RADIATION THERAPY REDUCES RADIATION DOSE TO HEART IN PATIENTS TREATED FOR MEDIASTINAL HODGKIN LYMPHOMA

P. M. Petersen,[1] M. C. Aznar,[2] A. K. Berthelsen,[2] A. L. Jacobsen,[3] D. A. Schut,[2] M. V. Maraldo,[2] T. L. Klausen,[3] F. Andersen,[3] L. Specht.[1]
[1]*Department of Haematology, The Finsen Centre, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark;* [2]*Department of Radiation Oncology, The Finsen Centre, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark;* [3]*Department of Clinical Physiology, Nuclear Medicine and PET, The Finsen Centre, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.*

Introduction: In this study, we investigated whether a precise target definition using PET-CT combined with accurate radiation delivery using deep inspiration breath hold (DIBH) technique can reduce the dose to cardiac structures without compromising the dose to the target in patients with early Hodgkin lymphoma (HL).
Patients and Methods: Nineteen patients with mediastinal HL had chemotherapy followed by involved node radiation therapy. All had pre-chemo-FDG-PET/CT in free breathing (FB) and in DIBH. Involved nodes were contoured independently on both scans. After chemotherapy, all patients had a planning CT in both FB and DIBH. Two radiotherapy treatment plans were made in all patients, using fused images from pre-chemo and post-chemo DIBH and FB scans, respectively. The optimal plan using opposing fields (APPA) or intensity modulated treatment (IMRT) was chosen. Planning target volume (PTV) coverage (% of PTV receiving 95% dose level) ($V_{95\%}$), heart, left anterior descending coronary artery (LAD) and left main coronary artery (LMA) mean doses were calculated for each patient in both DIBH and FB as well as heart volume receiving ≥30 Gy or ≥20 Gy (V_{30}, V_{20}). Wilcoxon's signed rank test for paired data was used for statistical analysis. The patients were treated with the plan determined to be the best in terms of clinical target volume/PTV coverage and sparing organs at risk.
Results: FB plan was chosen in three patients, and 16 had treatment with DIBH. Nine had APPA treatment and 10 IMRT. PTV coverage was equal. Dose characteristics and cardiac structures are shown in the table.
Volumes and dose characteristics for deep inspiration breath hold and free breathing.
Conclusion: Substantial reduction of radiation dose to cardiac structures is feasible without compromising the dose to the target in some patients with mediastinal HL.

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TAILORING THERAPY IN HODGKIN LYMPHOMA BASED ON PREDEFINED RISK FACTORS AND EARLY INTERIM PET/CT: ISRAELI H2 STUDY—PRELIMINARY REPORT ON 306 PATIENTS

E. J. Dann,[1] O. Bairey,[2] R. Bar-Shalom,[3] M. Izak,[4] A. Korenberg,[4] L. Akria,[5] D. Attias,[6] K. Filanovsky,[7] U. Abadi,[8] R. Ruchlemer,[9] R. Abdah-Bortnyak,[10] N. Goldschmidt,[11]

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Parameter	FB (median, range)	DIBH (median, range)	*p* value (Wilcoxon's signed rank test)
PTV $V_{95\%}$	94 (61–97)%	93 (78–96)%	0.43
Heart V_{30}	2.0 (0.0–35)%	0.0 (0.0–27)%	0.02
Heart V_{20}	20 (0.0–76) %	4.5 (0.0–66)%	<0.01
Mean heart dose	7.3 (0.12–23) Gy	4.4 (0.10–17) Gy	<0.01
Mean LAD dose	8.4 (0.10–29) Gy	4.9 (0.09–27) Gy	<0.01
Mean LMA dose	24 (0.25–32) Gy	17 (0.2–32) Gy	0.01

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R. Epelbaum,[10] I. Avivi,[1] D. Lavie,[11] J. M. Rowe,[9] O. Shpilberg,[2] O. Paltiel.[11]
[1]Hematology and BMT, Rambam Medical Center, Haifa, Israel; [2]Hematology and BMT, Rabin Medical Center, Petach Tikva2, Israel; [3]Nuclear Medicine, Rambam Medical Center, Haifa, Israel; [4]Hematology, Assaf Harofeh Medical Center, Zerifin, Israel; [5]Hematology, Western Galillee Medical Center, Nahariya, Israel; [6]Hematology, Bnai Zion Medical Center, Haifa, Israel; [7]Hematology, Kaplan Medical Center, Rehovot, Israel; [8]Hematology, Meir Medical Center, Kfar Saba, Israel; [9]Hematology, Shaare Zedek Medical Center, Jerusalem, Israel; [10]Oncology, Rambam Medical Center, Haifa, Israel; [11]Hematology, Hadassah Medical Center, Jerusalem, Israel.

Introduction: The aim of Hodgkin lymphoma (HL) therapy is to maximize response and minimize long-term toxicity.
Methods: Our multicentre ongoing study, initiated in 2006, prospectively evaluates outcome of HL patients (pts) whose therapy is chosen according to baseline prognostic factors and is tailored based on PET/CT results performed upon two cycles of chemotherapy. Pts with classic HL aged 18–60 years, stages I–IV, are eligible. Early disease is categorized as favourable (EFD) or unfavourable (EUD). After two ABVD cycles, EFD pts with negative (neg) PET have involved nodal radiation therapy (INRT); EUD pts have two more ABVD cycles (total of four) followed by INRT. If interim PET is neg, RT may be replaced with 2XABVD. Pts with positive (pos) interim PET receive two more ABVD cycles (total 4/6) followed by RT. Pts with advanced HL (B symptoms or stages III/IV) are treated based on IPS. Pts with IPS 0–2 receive two ABVD cycles, and pts with IPS ≥ 3 have two cycles of escalated BEACOPP (EB). If interim PET is neg or shows minimal residual uptake in a single site, further therapy with four ABVD cycles is given with no RT. If interim PET is pos with no evidence of HL progression, therapy is escalated to EB with RT given to involved site.
Results: Three hundred twenty-four pts have been enrolled. Table 1 shows data on pts with interim PET. Therapy was escalated in 13% of early standard risk (31/241),and reduced in 85% of pts with advanced high risk HL (56/65). Two pts died: At a median f/u of 24 months (4–74), this study had PFS of 86% for the whole group, 94% for ED and 82% for advanced disease (AD) pts.
Conclusions: Tailoring therapy based on interim PET is feasible both in ED and AD. Longer follow-up and a larger cohort are needed to conclude about long-term toxicity of this approach.

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PATTERNS OF FAILURE IN A RANDOMIZED PHASE III TRIAL OF ABVD VERSUS STANFORD V +/− RADIATION THERAPY IN LOCALLY EXTENSIVE AND ADVANCED STAGE HODGKIN'S LYMPHOMA: AN INTERGROUP STUDY COORDINATED BY THE EASTERN COOPERATIVE ONCOLOGY GROUP (E2496)

H. Wagner,[1] F. Hong,[2] L. I. Gordon,[3] R. I. Fisher,[4] N. L. Bartlett,[5] P. Desjardins,[6] R. D. Gascoyne,[7] P. Stiff,[8] B. D. Cheson,[9] S. Caplan,[10] R. Advani,[11] B. Kahl,[12] J. Friedberg,[13] K. A. Blum,[14] T. Habermann,[15] J. Tuscano,[16] R. T. Hoppe,[17] T. J. Fitzgerald,[18] F. Laurie,[18] S. J. Horning.[19]
[1]Radiation Oncology, Penn State Hershey Cancer Institute, Hershey, USA; [2]Harvard University, Dana Farber Cancer Institute, Boston, USA; [3]Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, USA; [4]Hematology, Fox Chase Cancer Center, Philadelphia, USA; [5]Siteman Cancer Center, Washington University, St. Louis, USA; [6]Bureau des Hematologistes, Greenfield Park, Greenfield Park, Canada; [7]Pathology, British Columbia Cancer Research Agency, Vancouver, Canada; [8]Hematology, Loyola University Cancer Center, Maywood, USA; [9]Hematology, Georgetown University Hospital, Washington, USA; [10]Hematology, Jewish General Hospital, Montreal, Canada; [11]Hematology, Stanford University, Palo Alto, USA; [12]Hematology, University of Wisconsin Cancer Center, Madison, USA; [13]Hematology, University of Rochester Cancer Center, Rochester, USA; [14]Hematology, Ohio State University, Columbus, USA; [15]Hematology, Mayo Clinic, Rochester, USA; [16]Hematology, University of California Davis, Sacramento, USA; [17]Radiation Oncology, Stanford University, Palo Alto, USA; [18]Radiation Oncology, Quality Assurance Review Center, Providence, USA; [19]Hematology, Stanford University, Palo Alto, USA.

Introduction: E2496 compared treatment with ABVD to Stanford V in patients with stage III/IV and bulky stage II Hodgkin's lymphoma (HL). Radiation therapy (RT) (36 Gy) was given in ABVD only to the mediastinum for patients with bulky disease and in Stanford V to all sites initially ≥5 cm. We report a retrospective comparison of patterns of failure (POF) between these regimens.
Methods: Eight hundred fifty-four patients were registered on E2496; 794 were eligible. With a median follow-up of 6.4 years, 203 failed treatment (progression/relapse, death of any reason) with 99/395 (25%) on ABVD and 104 out of 399 (26%) on Stanford V. POF were ascertained retrospectively and were evaluable in 156 patients (69 ABVD, 87 Stanford V). Proportions were compared with Fisher's exact test.
Results: Failure in the RT field (isolated in-field failure with/without outside-field failure) was 2.3% (9/395) and 8.0% (32/399) ($p = 0.0003$) of the 794 eligible patients for ABVD and Stanford V, respectively. Among the 156 cases with POF data, the corresponding figures are 12.9% and 37.2% ($p = 0.0009$).

Abstract 256 Table POF matched with the volumes included in the RT portals (if used). Results are expressed as counts and as percentage of failing patients with POF data.

Location of relapse ABVD ($n = 69$)	Stanford V ($n = 87$)	No. (%)
Inside treated field only	7 (10.0)	15 (17.4)
Outside treated field only	38 (54.3)	35 (40.7)
Both inside and outside treated fields	2 (2.9)	17 (19.8)
Inside and both (total inside)	9 (12.9)	32 (37.2)
Outside and both (total outside)	40 (57.1)	52 (60.5)
NED/DOT/PD during chemo	23 (32.8)	19 (20.9)

NED, died without evidence of HL; DOT, died of toxicity; PD, progression during chemotherapy or off-study for toxicity.

Conclusion: Overall failure rates were identical for the two treatment arms. More in-field failures were reported for Stanford V reflecting the fact that, by design, more sites of initial involvement were included in the treatment fields. Assuming cases with missing data follow a similar pattern, total in-field failure rates were <10% of all entered patients. These conclusions are limited by variable restaging at treatment failure and non-requirement for PET/CR in this trial.

CLL

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PHARMACOGENETIC ANALYSIS OF CYP2B6 IN THE LRF CLL4 TRIAL: THE *6 ALLELIC VARIANT IS ASSOCIATED WITH INFERIOR EFFICACY FOLLOWING FLUDARABINE PLUS CYCLOPHOSPHAMIDE

G. G. Johnson,[2] K. Lin,[2] T. Cox,[1] M. Oates,[1] D. R. Sibson,[1] R. Eccles,[1] B. Lloyd,[1] L. Gardiner,[1] D. F. Carr,[3] M. Pirmohamed,[3] J. C. Strefford,[4] D. G. Oscier,[5] D. Gonzalez-de-Castro,[6] M. Else,[6] D. Catovsky,[6] A. R. Pettitt.[1]

Abstract 255 Table

	Pts with interim PET	Pos/Neg interim PET	NPV	PPV of a pos study	HL progression pat (%)
Total	306	266/40	%90	%20	(11) 34
ED	148	128/20	%93	%25	(9.5) 14
EFD	21	16/5	%94	%20	(9.5) 2
EUD	127	112/15	%93	%27	(9.4) 12
AD	158	138/20	%88	%15	(12.7) 20
IPS 0–2	93	82/11	%88	%18	(12.9) 12
IPS ≥3	65	56/9	%87	%11	(12.5) 8

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[1]Department of Molecular & Clincal Cancer Medicine, University of Liverpool, Liverpool, UK; [2]Department of Haematology, Royal Liverpool University Hospital, Liverpool, UK; [3]Department of Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK; [4]Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton, UK; [5]Department of Molecular Pathology, Royal Bournemouth Hospital, Bournemouth, UK; [6]Haemato-Oncology Research Unit, Division of Molecular Pathology, The Institute of Cancer Research, Sutton, UK.

Introduction: Fludarabine plus cyclophosphamide (FC) is the chemotherapy backbone of modern chronic lymphocytic leukaemia (CLL) therapy. However, the efficacy and toxicity of FC-based therapy varies between individual patients. CYP2B6 is a polymorphically expressed cytochrome P450 isoform that is involved in the metabolism of cyclophosphamide. We therefore speculated that variant CYP2B6 alleles might affect the efficacy and toxicity of FC chemotherapy in CLL. To address this question, CYP2B6 allelotyping was performed on stored DNA samples from 489 patients treated in the LRF CLL4 trial that compared chorambucil (Chl), fludarabine (F) and FC.
Methods: The CYP2B6 c.516G>T and c.785A>G SNPs were genotyped using Taqman real time PCR SNP genotyping assays and the allelotype related to treatment outcome.
Results: Informative CYP2B6 data were obtained on 455 samples, 428 of which expressed the most common *1 and/or *6 alleles. All further analyses were confined to this latter cohort. There were no differences in baseline characteristics or differential effectiveness of Chl, F and FC (FC associated with superior PFS compared with F or Chl; no difference in OS) in the study cohort compared with the overall CLL4 trial cohort. Two hundred sixty-five patients (61.9%) were classified as *1/*1, 134 (31.3%) as *1/*6 and 29 (6.8%) as *6/*6. The 163 patients (38.1%) with at least one *6 allele were compared with *1 homozygotes for all further analyses. No differences were observed in baseline characteristics or response to Chl or F. However, *6 patients were significantly less likely than *1/*1 patients to achieve a CR following FC (odds ratio 0.27; $p = 0.003$) with a trend towards shorter PFS ($p = 0.059$). FC-treated *6 patients also had more residual lymphocytosis ($p = 0.002$) and lymphadenopathy ($p = 0.036$), had higher post-treatment platelet counts ($p = 0.037$) and spent fewer days in hospital ($p = 0.018$) compared with *1 homozygotes. Furthermore, multivariate analysis established that the CYP2B6 *6 variant is an independent predictor of response following treatment with FC.
Conclusion: In conclusion, our study elucidates CYP2B6 status as an important determinant of response and toxicity following FC chemotherapy in CLL.

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THE PROGNOSTIC IMPACT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA REQUIRING FIRST-LINE THERAPY

J. Delgado, R. Santacruz, M. Aymerich, A. Martinez-Trillos, C. Lopez, A. Navarro, M. Rozman, S. Bea, L. Conde, C. Royo, M. Cazorla, D. Colomer, E. Gine, E. Campo, A. Lopez-Guillermo, N. Villamor.
Hematology, Hospital Clinic, Barcelona, Spain.

Background: A proportion of patients with chronic lymphocytic leukaemia (CLL) achieve a minimal residual disease (MRD) negative status after therapy. We retrospectively evaluated the impact of MRD on the outcome of 255 consecutive patients receiving any frontline therapy in the context of a detailed prognostic evaluation, including age, Binet clinical stage, FISH aberrations, beta$_2$-microglobulin (B2M) concentration, CD38 and ZAP70 expression, and IGHV, TP53, NOTCH1, SF3B1 and MYD88 mutations.
Patients and Methods: All patients from this study signed informed consent and were recruited into the International Cancer Genome Consortium Chronic Lymphocytic Leukemia project, which was reviewed by the Institutional Review Board. IGHV mutation status was assessed using leader or consensus primers. For TP53 mutation analysis, we performed direct Sanger sequencing of genomic DNA in four different PCR reactions encompassing exons 4–9. TP53 mutations were analyzed using multiple sequence alignment tools and wild-type sequences from the IARC Database. Exon 34 of NOTCH1 was amplified in two fragments that cover 97% of NOTCH1 mutations. Exons 14, 15, 16 and 18 of SF3B1 and exon 5 of MYD88 were also sequenced. MRD was assessed on bone marrow samples drawn 3 months after frontline therapy using sensitive flow cytometry (MRD flow). Up until 2001, MRD was studied by using triple monoclonal antibody (MoAb) combinations. After 2001, quadruple MoAb combinations were used following the methodology recommended by Rawstron et al. (2001). Since 2006, MRD was quantified according to the standardized methodology proposed by the ERIC group.
Results: Treatment schemes included chlorambucil (82 patients), CHOP or CHOP-like (24), purine analogues in monotherapy (41), fludarabine plus cyclophosphamide (± mitoxantrone) (46), rituximab, fludarabine plus cyclophosphamide (± mitoxantrone) (51) and others (11). Median follow-up was 53 (range 1–242) months from therapy. Median treatment-free survival (TFS) for patients achieving a MRD negative complete response (CR), MRD positive CR, partial response and no response was 72, 37, 22 and 6 months, respectively ($p < 0.001$). Multivariate analysis revealed that four variables had an adverse impact on TFS: MRD positivity ($p < 0.001$), unmutated IGHV status ($p < 0.001$), presence of NOTCH1 mutations ($p = 0.037$) and elevated B2M ($p < 0.001$). Regarding overall survival (OS), multivariate analysis revealed that MRD positivity was predictive of an unfavourable outcome ($p = 0.015$), together with advanced age ($p < 0.001$), unmutated IGHV status ($p = 0.002$) and elevated B2M ($p = 0.013$).
Conclusions: For patients requiring frontline therapy, achievement of a MRD negative status is associated with a significantly prolonged TFS and OS irrespective of other prognostic markers or treatment administered.

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A HIGH NUMBER OF LOSSES IN 11Q CHROMOSOME IS ASSOCIATED WITH SHORT TIME TO FIRST TREATMENT AND OVERALL SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA. FINAL RESULTS OF DATABASE OF CLL OF SPANISH GROUP OF CYTOGENETICS (GCECGH) AND SPANISH GROUP OF CLL (GELLC)

J. Hernandez,[1] M. Hernandez,[2] A. Rodriguez,[2] R. Santacruz,[3] C. Heras,[1] C. Munoz,[1] R. Collado,[4] A. Puiggros,[5] A. Martin,[2] J. Rodriguez,[6] T. Gonzalez,[7] M. Talavera,[8] A. Batlle,[9] J. Delgado,[3] M. Marco,[10] M. Calasanz,[11] B. Espinet,[5] F. Bosch,[12] M. Gonzalez,[2] J. Hernandez-Rivas.[2]
[1]Hematology, Hospital Universitario Infanta Leonor, Madrid, Spain; [2]Hematology, IBSAL, IBMCC, CIC Universidad de Salamanca-CSIC, Salamanca, Spain; [3]Hematology, Hospital Clinic, Barcelona, Spain; [4]Hematology, Hospital General, Valencia, Spain; [5]Hematology, Hospital del Mar, Barcelona, Spain; [6]Hematology, Hospital Juan Ramon Jimenez, Huelva, Spain; [7]Hematology, Fundacion, Santiago de Compostela, Spain; [8]Hematology, Hospital Ramon y Cajal, Madrid, Spain; [9]Hematology, Hospital Marques de Valdecilla, Santander, Spain; [10]Hematology, Hospital General, Castellon, Spain; [11]Hematology, Universitario de Navarra, Pamplona (Navarra), Spain; [12]Hematology, Hospital Valle Hebron, Barcelona, Spain.

Genetic abnormalities in chronic lymphocytic leukaemia (CLL) define subgroups of patients with different survival. CLL patients with 11q- have a bad prognosis although with a variable outcome.
Objective: To analyze if the number of losses in 11q in patients with CLL has an influence in overall survival (OS) and time to first therapy (TFT).
Methods: A total of 2493 patients were registered in database of CLL of GCECGH and GELLC. Clinical data, FISH information and molecular studies were recorded. Genome-wide expression analysis of clonal B-cell lymphocytes of 11q- patients was also performed using Human Gene 2.0 microarrays (Affymetrix).
Results: A total of 242 patients (10.3%) had 11q-. The final analysis was limited to 197 cases (151 male, median age 65 years) after excluding cases with monoclonal B-cell lymphocytosis, lack of clinical data or inappropriate follow-up. Most of patients (61%) were in Binet's stage A. In 82 out of 197 patients (42%), 11q- was the sole cytogenetic aberration at diagnosis. Median OS of patients with 11q- was 106 months (CI95%, 97–128), and TFT was 25 months (CI95%, 31–44). Interestingly, in patients with loss of 11q > 40% of cells (146 cases, 74%), the OS was 90 months (CI95%, 57–123), whereas in the group with <40%, the OS has not been reached (CI95%, 114–157) ($p = 0.008$). In the univariate analysis, clinical stage, B symptoms, hepatomegaly, splenomegaly, lymphocyte count $>20 \times 10^9$/L, a high serum LDH, β_2M levels and high number of cells 11q- (>40) were significant associated with a short OS. In the Cox analysis for OS, variables included were high LDH (HR 2.07, CI95% 1.06–4.06, $p = 0.035$), high β_2M (HR 2.44, CI95% 1.26–4.75, $p = 0.005$) and del11q ≥40 (HR 3.15, CI95% 1.47–6.47, $p = 0.004$). Regarding TFT, in patients with ≥40% of losses in 11q, the median TFT was 18 months (CI95%, 12–24) vs 44 months (CI95%, 33–55) ($p < 0.0001$). In the univariate analysis, significant variables were clinical stage, LDH, β2M, CD38, ZAP70, unmutated IGVH and del11q ≥40 ($p < 0.0001$). In the multivariate analysis, only unmutated IGVH status resulted significant in predicting TFT (HR 4.98, CI95% 1.38–17.9, $p = 0.014$). Regarding gene expression analysis, patients with 11q- showed a distinctive gene expression profile characterized by an activation of NFkB signalling, because of the overexpression of genes such as BTRC and TLR. In addition, an overexpression of URB4, ILK, BSG and SHH, and downregulation of STC1 and CASR, leading to a decreased apoptosis ($p = 0.006$), as well as upregulation of ILK, BTRC, RPL7A and FOXM1, involved in deregulation of the cell cycle ($p = 0.03$), were observed in 11q- patients. Of note, overexpression of JUNB, described as a proto-oncogen, was also observed in 11q-patients, which could lead to a higher cell proliferation ($p = 0.009$).

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Conclusions: In patients with CLL, a high number of losses in 11q is associated with a shorter TFT and OS. This group of patients is characterized by an activation of NFkB signalling, leading to a decreased apoptosis and high proliferation.

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FLUDARABINE, RITUXIMAB, AND LENALIDOMIDE IN PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

I. W. Flinn,[1] R. S. Cooper,[1] D. S. Thompson,[1] J. K. Waselenko,[2] J. Reeves Jr.,[3] R. L. Wise,[4] J. D. Hainsworth.[5]
[1]Sarah Cannon Research Institute/Tennessee Oncology PLLC, Oncology, Nashville, USA; [2]Oncology, Cincinnati Hematology-Oncology, Inc., Cincinnati, USA; [3]Oncology, Sarah Cannon Research Institute/Florida Cancer Specialists, Ft. Myers, USA; [4]Oncology, Sarah Cannon Research Institute/South Carolina Oncology Associates, Columbia, USA; [5]Oncology, Sarah Cannon Research Institute, Nashville, USA.

Introduction: Lenalidomide has been shown to be clinically active in patients (pts) with chronic lymphocytic leukaemia (CLL) and is a potential alternative to cyclophosphamide as an addition to a fludarabine, rituximab backbone. This is a nonrandomized, open label phase I/II dose-finding study of lenalidomide combined with fixed doses of fludarabine and rituximab in a minimally or untreated population of patients with CLL.
Methods: The phase I portion of this trial used the following dosing schedule: rituximab [375 mg/m^2 cycle (C) 1, over days (D) 1 and 2), 500 mg/m^2 D1 of C 2–6] and fludarabine (25 mg/m^2 on D1, 2, and 3) with one of two dose levels (DL) of lenalidomide, DL1—2.5 mg PO on D8–28 of C1–6 ($n = 6$), and DL2—2.5 mg PO D8–28 of C1 and 5.0 mg on D8–28 of C2–6 ($n = 54$). Patients were restaged post-C3, post-C6, and every 6 months until disease progression. Study endpoints included response rate, progression-free survival (PFS) and overall survival (OS).
Results: Treatment discontinuation due to toxicity occurred in 10% of pts. Grade 3/4 hematologic adverse events (AEs) observed regardless of cause were anaemia (G3 13% and G4 2%), neutropenia (G3 32% and G4 27%), febrile neutropenia (G3 2% and G4 2%), and thrombocytopenia (G3 2% and G4 5%). The most common Grade 3/4 treatment related non-haematologic AEs were rash (G3 17% and G4 2%) and fatigue (G3 13%). Responses were reviewed by FISH risk profile and IWCLL 2008 criteria. Pts were grouped as poor risk or good risk/all others (Table 1). Kaplan–Meier estimates of PFS and OS at 24 months for all pts were 77% and 89%, respectively. The estimated PFS and OS rates at 24 months were 92% and 98%, respectively, for the good risk/all other group and 47% and 66%, respectively ,for the poor risk group.
Conclusion: Lenalidomide in combination with fludarabine and rituximab is a potential alternative to FCR. While response rates appear lower than studies with FCR, so does toxicity. A unique side effect is rash that can occur at even the lowest doses of lenalidomide.

Abstract 260 Table 1

Response assessment	Poor risk 11q and or 17p deletion pts ($N = 15$)	Good risk/all other pts ($N = 45$)	Total ($N = 60$)
CR	27%	24%	25%
PR	33%	56%	50%
SD	20%	11%	13%
PD	0	0	0
ORR	60%	80%	75%

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PLATINUM AND HIGH-DOSE CYTARABINE-BASED REGIMENS ARE EFFICIENT IN ULTRA HIGH-RISK CHRONIC LYMPHOCYTIC LEUKAEMIA: RESULTS OF A RETROSPECTIVE MULTICENTRE STUDY

E. Durot,[1] V. Leblond,[2] S. Lepretre,[3] A. Michallet,[4] Q. Le,[1] A. Delmer.[1]
[1]Service d'Hematologie Clinique, Hopital Robert Debre, CHU de Reims, Reims Cedex, France; [2] Assistance Publique-Hôpitaux de Paris, Hematologie, Pitié Salpêtrière Hospital, Paris, France; [3]Hematologie, Centre Henri Becquerel, Rouen, France, [4]Hematologie, Centre Hospitalier Lyon Sud, Pierre Benite, France.

Introduction: Ultra high-risk chronic lymphocytic leukaemia (CLL) and Richter's syndrome (RS) usually display a poor prognosis. Although they are widely used as salvage therapy in many types of lymphomas, platinum and cytarabine (Pl + AraC)-based regimens have not been evaluated in large cohorts of patients (pts) with CLL or RS. We conducted a retrospective study aimed to assess the efficacy of these Pl + AraC regimens in this particular setting.
Methods: We have analyzed the characteristics and outcome of 75 patients (pts) who received at least one course of Pl + AraC-based regimen for relapsed/refractory CLL or RS in four French centres during the period 2000–2012. Cytogenetic data and beta2 microglobulin were available for almost all cases. The regimens were either DHAP ± rituximab, ESHAP ± rituximab or OFAR (each administered as previously reported) but were analyzed together.
Results: Forty-seven pts had CLL (of whom 36 met the criteria of ultra high-risk CLL) and 28 pts had RS. Median age was 62 years (range, 18–79). The median number of previous therapies was 3 (range, 1–7) including fludarabine-based regimens (75%) and alemtuzumab (32%), and 61% of pts were refractory to the last treatment. Deletions of chromosomes 17p and 11q were found in 40% and 39% of cases, respectively. The overall response rate was 60% with 24% complete response (CR) in CLL and 43% with 25% CR in RS. The median progression-free survival and overall survival were 11 and 14.6 months, respectively. Fludarabine refractoriness and 17p deletion were not associated with a poorer outcome. In multivariate analysis, the only factors associated with a shorter survival were PS ECOG ≥ 2 ($p = 0.04$) and albumin level <35 g/L ($p = 0.004$). The main toxicities were myelosuppression and infectious complications (toxic death 15%). Twenty-one patients underwent thereafter autologous or allogeneic stem cell transplantation.
Conclusions: Pl + AraC-based regimens provide high response rate in high-risk CLL and RS. In ultra-high-risk CLL (17p deletion or fludarabine refractoriness), these regimens should be considered as an option for tumour control before allogeneic transplantation.

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CYTOKINE QUANTIFICATION IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS TREATED WITH IBRUTINIB AND RITUXIMAB

I. de Weerdt,[1] J. Hoellenriegel,[1] S. O'Brien,[1] M. J. Keating,[1] W. G. Wierda,[1] J. J. Buggy,[2] J. A. Burger.[1]
[1]Department of Leukemia, MD Anderson Cancer Center, Houston, TX, USA; [2]Pharmacyclics, Inc., Sunnyvale, CA, USA.

Introduction: The B-cell receptor (BCR) pathway plays an important role in chronic lymphocytic leukaemia (CLL), affecting proliferation and survival. BTK is a critical kinase in BCR signal transduction. Ibrutinib, an oral BTK inhibitor, disrupts BCR signalling as well as other signalling pathways, such as the CXCL12–CXCR4 axis and shows encouraging early clinical results. The aim of the current study was to evaluate *in vivo* effects of ibrutinib on plasma cytokine and chemokine levels in CLL patients.
Methods: High-risk CLL patients (deletion of 17p or tp53 mutation; remission duration <3 years after firstline chemoimmunotherapy or previously treated 11q del) were treated with ibrutinib and rituximab. Using multiplex bead assays (Millipore), the levels of 32 cytokines and chemokines were measured in plasma samples. Blood samples before treatment, after 1 month and 3 months of treatment were available from 26 patients. In addition, plasma levels of soluble BAFF, APRIL and CD40L were measured with ELISA.
Results: Plasma levels of TNF-α were significantly reduced from 76.12 pg/mL (± 9.794) to 24.39 pg/mL (± 2.868) and 21.3 pg/mL (± 3.599) after 1 and 3 months, respectively. Furthermore, levels of IFN-α2 and IL-8 decreased significantly after 1 month of treatment. Ibrutinib treatment results in a shift of CLL cells from the lymph nodes to the peripheral blood. In line with this effect, we found marked reductions in chemokine levels of CCL19, CCL21 and CXCL13, chemokines that stimulate lymphocytes homing in lymphatic tissues. Interestingly, levels of CXCL12 were unaffected by ibrutinib treatment. TNF members APRIL and CD40 ligand levels remained stable after treatment, but there was a robust upregulation of BAFF, especially after 3 months. Mean levels increased from 0.9913 ng/mL (± 0.069) to 1.068 ng/mL (± 0.071) after 1 month and 1.419 ng/mL (± 0.154) after 3 months.
Conclusion: Our results demonstrate that ibrutinib has multiple effects *in vivo* besides inhibition of BCR signalling. Levels of several cytokines, which may provide *in vivo* stimuli to CLL cells, decrease. Furthermore, we saw a reduction of tissue homing chemokines.

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BRAF INHIBITION IN HAIRY CELL LEUKAEMIA

S. Dietrich,[1] F. Peyrade,[2] G. Follows,[3] H. Glimm,[4] D. Re,[2] M. Andrulis,[5] M. Hundemer,[1] C. von Kalle,[4] A. Ho,[1] T. Zenz.[1]

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[1]Medizin V, University of Heidelberg, Heidelberg, Germany; [2]Department of Medical Oncology, Centre Antoine Lacassagne, Nice, France; [3]Department of Haematology, Addenbrookes Hospital, Cambridge University, Cambridge, UK; [4]Department of Translational Oncology, National Center for Tumor Diseases and German Cancer Research Center, Heidelberg, Germany; [5]Institute of Pathology, University of Heidelberg, Heidelberg, Germany.

Activating mutations of the BRAF kinase (BRAF V600E) are found in virtually all cases of classical hairy cell leukaemia (HCL), suggesting disease-specific oncogene dependence.
Methods: We report on the combined experience of three centres (Heidelberg, Cambridge and Nice) with six HCL patients who were treated with a short course of low dose vemurafenib, a specific BRAF inhibitor.
Results: Presence of the BRAFV600E mutation was confirmed in all patients. Before vemurafenib treatment, all patients had massive HCL bone marrow infiltration and splenomegaly (two patients had prior splenectomy), leading to severe cytopenias. Four patients had at least four prior lines of therapy and have been refractory to purine analogues. One patient was treated upfront and another patient after two lines of therapy because of poor performance status. Treatment was started with 2×240 mg and slowly escalated to $2 \times 960 \times$ mg in one and 2×480 mg in another patient. Because a major response was already seen at 2×240 mg, the following patients were treated with 2×240 mg. Two patients achieved a CR (duration of treatment: 56 days) and two patients a PR (duration of treatment: 86 and 58 days), and two patients are currently on treatment. Blood counts rapidly recovered in all patients: platelets >100.000/μL after a median of 27 days (range: 11–35), neutrophils >1000/μL after 24 days (range: 11–42) and haemoglobin after 68 days (range: 42–98). Of note, neutrophil counts decreased in three patients before recovery. The spleen sized normalized within days ($n = 4/4$) as did sCD25 ($n = 3/3$), which is considered a reliable marker of HCL load. Side effects were arthritis ($n = 2$), which could be managed with low dose steroids and NSAR, elevated bilirubin ($n = 3$), wart-like lesions and other skin changes ($n = 3$), and grade 3 hematologic toxicity (neutropenia, $n = 3$). A heavily pre-treated and refractory patient showed disease recurrence at last follow-up and was successfully re-started on vemurafenib. Detailed immunohistological assessments are provided. After vemurafenib treatment, p-ERK signalling was almost completely abolished in HCL cells *in vivo*, followed by apoptosis of HCL cells as shown by Tunnel staining.
Conclusion: Targeting of a single mutated oncogene can provide disease control in this leukaemia. Further studies with careful assessment of vemurafenib dosing in HCL are urgently needed.

TRANSPLANTATION

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EFFECT OF MOLECULAR SUBCLASS ON OUTCOMES AFTER RADIOIMMUNOTHERAPY-BASED AUTOLOGOUS STEM CELL TRANSPLANTATION IN DIFFUSE LARGE B-CELL LYMPHOMA

T. Siddiqi,[1] N. Tsai,[2] Q. Huang,[3] J. Palmer,[2] S. J. Forman,[1] A. Krishnan.[1]
[1]Hematology/Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, USA; [2]Biostatistics, City of Hope National Medical Center, Duarte, USA; [3]Pathology Department, City of Hope, National Medical Center, Duarte, USA.

Introduction: Classification of molecular subclass by cell of origin (COO) for diffuse large B-cell lymphoma (DLBCL) has improved risk stratification in the first-line setting as shown by DNA microarray analysis and immunohistochemistry (IHC). Five-year overall survival (OS) for DLBCL patients with germinal centre B-cell-like (GCB) COO is significantly better than that for non-GCB in newly diagnosed patients. In the relapsed/refractory setting, following autologous stem cell transplant (ASCT), there are conflicting data on the effects of COO on survival. The ibritumomab tiuxetan (Z)-BEAM conditioning regimen for ASCT in DLBCL has produced promising response rates and progression-free survivals (PFS), and we seek to assess the role of COO in predicting outcome with this regimen.
Methods: We evaluated COO (GCB, non-GCB) using the Hans IHC algorithm in 50 patients undergoing ASCT with Z-BEAM conditioning from 2002 to 2012. The median age at transplant was 52.6 years. Ten patients were in first CR/PR (20%), 16 failed induction therapy (32%), and 24 were in first or higher relapse (48%). Forty-one patients had chemosensitive disease (82%), median prior treatments was 2, and all had prior rituximab exposure. IPI at transplant was low risk (76%) primarily. Thirty-three patients had GCB (66%), and 17 (34%) had non-GCB COO. We applied Cox regression analysis to examine variables affecting survival including disease status at transplant, number of prior regimens, chemosensitivity, salvage therapy, IPI at transplant and COO; variables significant to the level of $p = 0.1$ in univariate were included in the multivariate analysis.
Results: Twenty-one patients relapsed and 14 died. The median follow-up for surviving patients was 38.6 months. The major cause of death was relapse/disease progression. OS and PFS at 2 years were 80% (95% CI: 69–88) and 57% (95% CI: 48–64), respectively. Patients with GCB COO had significantly better OS (88 vs 65% at 2 years, $p = 0.046$) and PFS (63% vs 42% at 2 years, $p = 0.028$) compared with patients with non-GCB COO. Only GCB versus non-GCB significantly affected PFS in multivariate analysis.
Conclusion: Z-BEAM ASCT for DLBCL in this cohort resulted in excellent 2-year survivals for both GCB and non-GCB patients, with significantly better outcomes in patients with GCB COO.

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TREATMENT OF PERIPHERAL T-CELL LYMPHOMA WITH AGGRESSIVE INDUCTION CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANT USING DENILEUKIN DIFTITOX FOR *IN VIVO* PURGING AND POST-TRANSPLAT THERAPY: PRELIMINARY RESULTS OF A PHASE II CLINICAL TRIAL

N. K. Gupta,[1] N. Bartlett,[2] W. Z. Ai,[1] J. Hwang,[3] C. Andreadis,[1] W. Stock,[4] L. E. Damon,[1] C. A. Linker,[1] L. D. Kaplan.[1]
[1]Medicine/Hematology-Oncology, University of California, San Francisco, CA, USA; [2]Hematology-Oncology, Washington University, St Louis, MO, USA; [3]Biostatistics, University of California, San Francisco, CA, USA; [4]Medicine, University of Chicago, Chicago, IL, USA.

Introduction: Peripheral T-cell lymphomas (PTCL) are associated with high rates of early relapse and inferior survival compared with aggressive B-cell lymphomas. Both gemcitabine (G) and denileukin diftitox (DD) are active agents in PTCL. We evaluated incorporation of G-based induction, aggressive stem cell mobilization with DD *in vivo* purging, and ASCT followed by DD maintenance to improve rate of ASCT and duration of progression-free survival (PFS).
Methods: Patients with untreated PTCL with IPI >1 and excluding ALK-1 + ALCL received two cycles of G + vinorelbine + liposomal doxorubicin [Doxil] (GVD) induction followed by two cycles of augmented CHOP (cyclophosphamide 2 gm/m^2) + methotrexate 3000 mg/m^2 (M-CHOP). Patients were restaged after GVD × 2 and after augmented M-CHOP. Those in PR/CR received stem cell mobilization with high-dose cytarabine, etoposide (EA) and DD, 9 μg/kg/day days 6–10. This was followed by ASCT with CBV prep. Two cycles of DD, 18 μg/kg/day × 5d q 21d were administered beginning day +90. The primary objectives were improvement in 3-year PFS from 30% to 50% compared with historical controls and evaluation of safety.
Results: Between July 2008 and June 2011, 21 of a planned 45 patients (median age = 58, international prognostic index 2 = 6, 3 = 12, and 4/5 = 3) were enrolled. Histology: AILT (8), PTCLU (9), ALCL (1), enteropathy-associated (1), subcutaneous (1) and hepatosplenic (1). Overall response rate (ORR) following GVD was 72% (CR 28%, PR 44%); ORR following M-CHOP was 90% (CR 65%, PR 25%); and 16 (76%) patients went on to ASCT with a mean CD34+ dose of 23.5×10^6/kg. At the median f/u of 22.5 months, 62% were alive and progression-free. Toxicities included grade 4 cytopenias (all patients), febrile neutropenia 11 (52%), documented sepsis/bacteremia 6 (29%), reversible grade 3/4 LFT abnormalities associated with DD (3) and severe capillary leak syndrome (1). Premature study closure was due to manufacturing shortages of both DD and Doxil.
Conclusions: The combination of GVD and augmented M-CHOP produced high initial response rates, with a favourable safety profile. EA-DD mobilization provided robust stem cell collection in patients with PTCL proceeding to ASCT in first CR/PR. Preliminary survival data compare favourably with historical controls. MRD data will be presented.

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AUTOLOGOUS AND ALLOGENEIC STEM-CELL TRANSPLANTATION FOR TRANSFORMED NON-FOLLICULAR INDOLENT LYMPHOMA: A REPORT OF THE CANADIAN BLOOD AND MARROW TRANSPLANT GROUP

D. Villa,[1] M. Crump,[2] T. Panzarella,[3] K. Savage,[1] C. Toze,[4] D. Stewart,[5] D. MacDonald,[6] R. Buckstein,[7] M. Rubinger,[8] R. Foley,[9] A. Xenocostas,[10] M. Sabloff,[11] N. Chua,[12] F. Couture,[13] J. F. Larouche,[14] S. Cohen,[15] J. Connors,[1] K. Ambler,[4] J. Kuruvilla.[2]
[1]Medical Oncology, British Columbia Cancer Agency, Vancouver, Canada; [2]Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, Canada; [3]Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; [4]Leukemia/BMT Program of BC, Vancouver Hospital and Health Sciences Centre, Vancouver, Canada; [5]Hematology, Tom Baker Cancer Centre, Calgary, Canada; [6]Hematology,

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Queen Elizabeth II Health Sciences Centre, Halifax, Canada; [7]Hematology, Sunnybrook Health Sciences Centre, Toronto, Canada; [8]Hematology, CancerCare Manitoba, Winnipeg, Canada; [9]Hematology, Hamilton Health Sciences Corporation, Hamilton, Canada; [10]Hematology, London Health Sciences Centre, London, Canada; [11]Hematology, The Ottawa Hospital, Ottawa, Canada; [12]Hematology, Cross Cancer Institute, Edmonton, Canada; [13]Hematology, Hotel Dieu de Quebec, Quebec City, Canada; [14]Hematology, Hopital de L'Enfant Jesus, Quebec City, Canada; [15]Hematology, Maisonneuve Rosemont Hopital, Montreal, Canada.

Background: The role of autologous (AUTO) and allogeneic (ALLO) stem cell transplantation in the management of patients with transformed non-follicular non-Hodgkin lymphoma is undefined. The aim of this study was to evaluate outcomes after AUTO or ALLO in this group of patients.
Methods: This is a multicentre, retrospective cohort study of patients with biopsy-proven indolent B-cell non-follicular non-Hodgkin lymphoma, and simultaneous or subsequent biopsy-proven aggressive histology transformation who were treated with AUTO or ALLO during 1994–2010. All patients received myeloablative conditioning regimens. Patient, disease, treatment, and outcome data were collected from each participating transplant centre and combined for analysis. Outcomes were compared to a cohort of 257 patients with transformed follicular lymphoma who also underwent AUTO ($n = 199$) or ALLO ($n = 58$).
Results: Twenty-nine patients were identified with the following underlying indolent histologies: 15 (52%) marginal zone lymphoma, 6 (21%) small lymphocytic lymphoma, 5 (17%) chronic lymphocytic leukaemia, and 3 (10%) lymphoplasmacytic lymphoma. The median age at transformation was 55 years (range 25–65). Patients received anthracycline or platinum-containing chemotherapy regimens for transformation, including rituximab in 20 (69%). Eighteen (62%) subsequently underwent AUTO, while 11 (38%) underwent ALLO. Thirteen (45%) received stem-cell transplants as part of their upfront therapy (after one line of chemotherapy), while the other 16 (55%) received it in the relapsed setting (after 2–3 lines of chemotherapy). Following stem-cell transplantation, the 5-year overall survival (OS) was 52% (AUTO 62%, ALLO 36%), and 5-year progression-free survival (PFS) was 51% (AUTO 62%, ALLO 34%). Transplant-related mortality was 6% AUTO and 36% ALLO. Adjusted for type of stem-cell transplantation, 5-year OS, and PFS were similar to those of patients with transformed follicular lymphoma receiving AUTO and ALLO ($p = 0.26$ and $p = 0.29$, respectively).
Conclusions: AUTO and ALLO may be reasonable treatments for selected patients with transformed indolent non-follicular lymphoma, although outcomes and toxicity appear to be more favourable with AUTO.

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POST-TRANSPLANTATION CYCLOPHOSPHAMIDE IS SAFE AND EFFECTIVE TO PREVENT IMMUNOLOGICAL REACTION AFTER UNMANIPULATED HAPLOIDENTICAL TRANSPLANTATION FOLLOWING NON-MYELOABLATIVE CONDITIONING FOR ADVANCED LYMPHOMAS

L. Castagna,[1] S. Bramanti,[1] S. Furst,[2] L. Giordano,[1] R. Crocchiolo,[2] E. Mauro,[1] L. Morabito,[1] R. Bouabdallah,[3] M. Balzarotti,[1] A. Anastasia,[1] E. Todisco,[1] C. Carlo-Stella,[1] E. Brusamolino,[1] D. Blaise,[2] A. Santoro.[1]
[1]Hematology, Istituto Clinico Humanitas, Rozzano, Italy; [2]Transplantation Unit, Institut Paoli Calmettes, Marseille, France; [3]Lymphoma Unit, Institut Paoli Calmettes, Marseille, France.

Introduction: Several studies have demonstrated the feasibility of allogeneic transplantation in advanced lymphomas. Only few patients had an HLA-identical sibling or matched unrelated donor (mud), and alternative non-identical donors such as cord blood or haploidentical (haplo)-related donors could expand the accessibility to transplantation for all patients.
The aims of this study were to confirm the reproducibility of haplo transplantation in terms of toxicity and efficacy in a cohort of lymphoma patients treated in two institutions.
Methods: From April 2009, 47 lymphoma patients were included in two institutions because of relapse after high dose chemotherapy (ct) or refractoriness to conventional salvage ct in an auto-allo programme. All patients received conditioning regimen consisting of cyclophosphamide (Cy), fludarabine, and low dose TBI (2 Gy). Graft versus host disease (GVHD) prophylaxis was post-infusion Cy (days +3 and 4) and tacrolimus/cyclosporine and MMF. T cell replete bone marrow or peripheral blood was infused at day 0. Prophylaxis against moulds, bacteria, and CMV was used.
Results: The median follow-up was 10 months (3–42). Two patients developed donor-specific antibody-related graft failure. The median time (mt) to ANC >0.5 and platelets $>20\,000$ was 20 (15–32) and 27 days (20–46). CMV reactivation was 26%, with non-fatal two CMV-related diseases. Epstein–Barr virus (EBV) reactivation was 23% without EBV-related disease. BK virus-related cystitis was observed in 22% of patients. Invasive fungal infection incidence was 6%.
The incidence of grade 2–4 aGVHD was 26%, and only two patients had grade IV. The mtto aGVHD was 56 days (39–133). The incidence of moderate cGVHD was 4%, and the median time to cGVHD was 167 days. The 1-year overall survival, progression-free survival, and NRM were 70%, 62%, and 15%. Infections were the main cause of death. The relapse incidence was 13%, and the mt to relapse was 4.4 months (1.1–8.3). The mt to discharge from hospital was 28 days (19–84). In univariate analysis, the disease status before transplantation affected the outcome.
Conclusions: This study confirms the feasibility of T cell replete haplo transplantation with post-infusion Cy. Furthermore, the efficacy was strongly suggested in a cohort of advanced lymphomas patients. Infectious complications were frequently and were the first cause of mortality.

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AUTOGRAFTING (ASCT) FOLLOWED BY REDUCED INTENSITY CONDITIONING REGIMEN (RICT) AS TREATMENT OF RESISTANT HODGKIN LYMPHOMA. FOURTEEN YEARS OF EXPERIENCE

A. Carella,[1] F. Guolo,[1] C. Ghiggi,[1] E. Todisco,[2] L. Castagna,[2] A. Santoro.[2]
[1]UOC Ematologia 1, IRCCS AOU San Martino-IST, Genova, Italy; [2]Ematologia, Humanitas Cancer Center, Milano, Italy.

Introduction: The outlook of patients with HL who do not enter a CR after first-second line therapy is poor. Autografting has become the standard therapy for these patients, but, unfortunately, the relapses remain the most important cause of treatment failure. The main objective of this study was to update the results achieved in these categories of patients with a dual transplant approach started in Genoa in 1997 (Carella et al. JCO 2000;18:3918–24).
Methods: Fifty-four patients were treated with ASCT/RICT procedure, but 34 patients only are here evaluated since they received the RICT procedure at a median time of 2 months from autologous stem cell transplantation (ASCT). The median age was 27 years (range, 15–47 years). None of these patients achieved CR after ABVD ± RT and second to third line therapies. Before ASCT, 9 patients had partial remission, 1 patient stable disease and 24 patients progressive disease. The high-dose therapies utilized were CVB, BEAM or melphalan. The conditioning regimen for RICT consisted of fludarabine + cyclophosphamide (24 patients) or fludarabine + melphalan ± TBI (10 patients). The donor type was matched sibling in 29 (85%) patients and matched unrelated donor in 5 (15%) patients. Donor mobilized hematopoietic stem cells collections were prepared for fresh infusion and were not T-cell depleted. Methotrexate and cyclosporine were used to prevent graft-versus-Hodgkin disease (GVHD) prophylaxis.
Results: All patients had full donor cell engraftment. Eleven (32%) patients achieved CR after ASCT and 18 (53%) patients after ASCT/RICT. Eight patients developed >grade 2 acute GVHD and 26 chronic GVHD (10: limited; 6: extensive). Seven patients, who did not respond, received donor lymphocyte infusion and developed grade 3 aGVHD (two patients) and extensive cGVHD (one patient). Three of them achieved CR. Twenty-four patients are currently alive at a median of 71 months (range, 25–170 months), and 16 patients are in continuous CR. Ten patients died: seven of progressive disease and three of progressive disease combined with cGVHD and infection.
Conclusions: The use of ASCT to debulk lymphoma and less toxic nonmyeloablative preparative regimens enabled engraftment and generation of a GVHD effect that was responsible for much of the benefit in mediating these dramatic lymphoma regressions.

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BUSULFAN, MELPHALAN AND ETOPOSIDE FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION ON PATIENTS WITH NON-HODGKIN's LYMPHOMA: MULTICENTRE STUDY FROM CONSORTIUM FOR IMPROVING SURVIVAL OF LYMPHOMA IN KOREA

J. Won,[1] H. Park,[1] K. Kim,[1] W. Kim,[2] S. Park,[3] M. Lee,[4] S. Sohn,[5] C. Suh,[6] H. Lee,[7] H. Kang,[8] C. Choi,[9] S. Bae,[10] J. Park,[11] E. Park,[12] J. Kwak.[13]
[1]Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul, South Korea; [2]Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; [3]Department of Internal Medicine, Soonchunhyang University College of Medicine, Bucheon Hospital, Seoul, South Korea; [4]Department of Internal Medicine, Konkuk University Medical Center and School of Medicine, Seoul, South Korea; [5]Departments of Hematology and Oncology, Kyungpook National University Hospital, Daegu, Daegu, South Korea; [6]Department of Oncology, Asan Medical Center, University of Ulsan

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College of Medicine, Seoul, South Korea; [7]Department of Internal Medicine, Kosin University Gospel Hospital, Busan, South Korea; [8]Department of Internal Medicine, Korea Cancer Center Hospital, Korea Institute of Radiological and Medical Science, Seoul, South Korea; [9]Department of Internal Medicine, Korea University Guro Hospital, Korea University College of Medicine, Seoul, South Korea; [10]Department of Internal Medicine, Catholic University of Daegu School of Medicine, Daegu, South Korea; [11]Deparment of Internal Medicine, Gachon University Gil Hospital, Incheon, South Korea; [12]Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, South Korea; [13]Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, South Korea.

Background: Several different high dose therapy (HDT) conditioning regimens have been used for non-Hodgkin's lymphoma (NHL), such as BEAM, BEAC and CBV. Carmustine is an active drug in the HDT of NHL, but the supply of carmustine is limited in some countries including Korea. Intravenous busulfan containing regimens as conditioning regimen have been used for both allogeneic and autologous stem cell transplantation in patients with haematologic malignancies. The purpose of this prospective multicentre phase II study was to evaluate the efficacy and safety of iv busulfan/melphalan/etoposide regimen as a conditioning regimen for high-dose chemotherapy in the patients with relapsed or high-risk NHL.
Methods: Patients with relapsed or primary refractory NHL or chemosensitive high-risk NHL underwent high-dose chemotherapy followed by ASCT at 12 centres in Korea. The conditioning regimen consisted of iv busulfan 3.2 mg/kg/day iv on days −8, −7 and −6, etoposide 400 mg/m^2/day iv on days −5 and −4, and melphalan 50 mg/m^2/day iv on days −3 and −2.
Results: Fifty-one patients were enrolled. All patients had successful stem cell engraftment with a median time to neutrophil recovery of more than 500/mm^3 of 10 days (range, 2 to 30 days). Platelet recovery of more than 20 000/mm^3 was seen after a median of 10 days (range, 2 to 51 days).
Treatment-related toxicities included nausea/vomiting in 28 patients (55%), diarrhoea in 28 patients (55%) and mucositis in 33 patients (65%), which were grade I or II in the majority of cases. There were no VOD and treatment-related death. The median duration of hospitalization for ASCT was 30 days (range, 12 to 80 days).
Forty-one patients (80%) achieved a complete response 1 month after ASCT, while three patients showed progressive disease. At a median follow-up of 27 months, 31 (60%) patients exhibited a relapse or progression, while 19 patients had died of disease and one patient had died of heart failure.
The estimated 2-year overall and progression-free survival for all patients was 58% and 39%, respectively.
Conclusion: This analysis suggests that conditioning regimen of iv busulfan/melphalan/etoposide would be well tolerated and effective in patients with relapsed or high-risk NHL. Accordingly, this regimen may be regarded as an important treatment option to substitute for BEAM regimen.

IMAGING

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BASELINE METABOLIC TUMOUR VOLUME PREDICTS PATIENT'S OUTCOME IN HODGKIN LYMPHOMA

C. Rossi,[1] S. Kanoun,[2] A. Berriolo-Riedinger,[2] O. Humbert,[2] I. Dygai-Cochet,[2] M. Chretien,[1] C. Legouge,[1] L. Berthet,[2] J. Bastie,[1] R. Casasnovas.[1]
[1]Hematology Clinic, CHU Le Bocage, Dijon, France; [2]Medicine Nucleaire, Dijon, France.

Introduction: The presence of a tumour bulk at staging of Hodgkin lymphoma (HL) is a negative predictive factor of patient's outcome. The metabolic tumour volume at baseline (MTV0) computed on PET may improve the tumour burden evaluation and better identify subsets of patients (pts) with different outcomes. In this study, we investigated the respective prognosis value of the MTV0, tumour bulk (>10 cm on CT) and interim PET response in a retrospective single centre study.
Methods: From 2007 to 2010, 59 pts with a first diagnosis of HL were treated in our institution. All pts received four to eight cycles of chemotherapy either ABVD (85%) or BEACOPP. Radiotherapy was performed in 14 responding pts with localized disease. PET was performed at baseline (PET0) and after two cycles of chemotherapy (PET2), and therapeutic strategy was not modified according to PET2 result. MTV0 was measured with a semi-automatic method using various volume shapes and systematic 41% SUVmax thresholding. On the basis of a receiver operating characteristic approach, pts with a MTV0 >225 cc were considered to have a hypermetabolic bulky disease. Interim PET was interpreted using SUVmax reduction between PET0 and PET2 (ΔSUV). Pts with a ΔSUV >71% were good responders. Median follow-up was 47 months (6–71).
Results: Median MTV0 was 120 cc (10–1610) and 17 pts (29%) had an MTV0 >225 cc. MTV0 (≤225 vs >225) and tumour bulk were predictive of 4-year progression-free survival (PFS): 85% vs 42%; $p = 0.001$ and 44% vs 79%, $p < 0.03$, respectively). ΔSUV (>71 vs ≤71%) had also an impact on patient's outcome ($p < 0.0001$). In multivariate analysis, using the international prognosis score, ΔSUV, MTV0 and bulky tumour as covariates, only ΔSUV and MTV0 remained independent predictors for PFS ($p = 0.0005$; RR = 6.3, and $p < 0.006$; RR = 4.4, for ΔSUV and MTV0, respectively). Then, three prognosis groups were identified: pts with either ΔSUV >71 and MTV0 ≤225 ($n = 37$; 63%), or ΔSUV <71 or MTV0 >225 ($n = 17$; 29%), or ΔSUV <71 and MTV0 >225 ($n = 5$; 8%), had a 92%, 49%, and 20% 4-year PFS ($p < 0.0001$).
Conclusions MTV0 is more relevant that tumour bulk to predict outcome of HL pts and adds significant prognosis insights to interim PET response assessment. The combination of MTV0 with ΔSUV allows identifying three subsets of HL pts with different outcomes that may help clinicians to guide therapeutic strategy.

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FDG PET-CT AFTER FIRST-LINE TREATMENT PREDICTS PROGRESSION-FREE AND OVERALL SURVIVAL IN PATIENTS WITH FOLLICULAR LYMPHOMA

E. Gainza,[1] G. Gutierrez Garcia,[1] X. Setoain,[2] B. Sanchez-Gonzalez,[3] S. Rodriguez,[4] A. Rodriguez-Trillos,[1] C. Trempat,[5] M. Villamor,[1] A. Gaya,[1] L. Colomo,[1] A. Martinez-Pozo,[6] E. Gine,[1] J. Maiques,[7] M. Garcia,[8] S. Fuentes,[2] E. Campo,[6] A. Salar,[3] A. Lopez-Guillermo.[1]
[1]Hematology, Hospital Clinic, Barcelona, Spain; [2]Nuclear Medicine, Hospital Clinic, Barcelona, Spain; [3]Hematology, Hospital Del Mar, Barcelona, Spain; [4]Radiology, Hospital Clinic, Barcelona, Spain; [5]Nuclear Medicine, Hospital Del Mar, Barcelona, Spain; [6]Pathology, Hospital Clinic, Barcelona, Spain; [7]Radiology, Hospital Del Mar, Barcelona, Spain; [8]Pathology, Hospital Del Mar, Barcelona, Spain.

Background: Follicular lymphoma (FL) is characterized by a relative long survival, with a pattern of continuous relapses. Achievement of complete response is an important issue for the outcome of the patients. The role of PET-CT, well established in aggressive lymphomas, is not completely elucidated in FL. The purpose of this study was to assess the usefulness of PET-CT in the evaluation of response of patients with FL.
Patients and Methods: One hundred sixteen patients (62M/54F; median age 59 years) with histologically proven FL (grades 1, 2, 3a 91; grade 3b, 9; not determined 16) underwent ^{18}F-FDG PET-CT at diagnosis in all cases and after treatment (immunochemotherapy in the vast majority) in 90. FDG uptake was determined by standardized uptake value (SUV).
Results: Initial PET-CT showed FDG uptake in 100% of assessable patients, with a median SUV of 6.9 (range, 2–24). PET detected more lesions than CT scan in 27 cases. Only 30% of proven bone marrow involvement was detected by PET-CT. Patients with higher histological grade and those with elevated serum LDH showed higher FDG avidity. After induction, PET-CT showed no residual mass with no FDG uptake in 45 (50%), residual mass with no FDG uptake in 21 (23%) and residual mass with FDG uptake in 24 (27%). After a median follow-up of 4.2 years, 4-year progression-free survival (PFS) was 76%, 85% and 57% for these groups, respectively. In multivariate analysis, PET positivity and intermediate or high-risk FLIPI were the most important variables to predict PFS. Post-induction PET-CT also predicted overall survival (OS), with a 4-year OS of 95%, 89% and 77% for the aforementioned groups, respectively. Indeed, in multivariate analysis, PET positivity after induction and intermediate or high-risk FLIPI predicted poor OS.
Conclusions: PET-CT has demonstrated its usefulness in both initial staging and in assessment of response in FL. PET-CT status after first-line treatment was along with FLIPI the most important factor to predict PFS and OS.

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PROGNOSTIC VALUE OF 18FDG BASELINE FUNCTIONAL PET PARAMETERS IN PRIMARY MEDIASTINAL DIFFUSE LARGE B-CELL LYMPHOMA

L. Ceriani,[1] E. Zucca,[2] P. L. Zinzani,[3] A. Ferreri,[4] U. Vitolo,[5] C. Stelitano,[6] E. Brusamolino,[7] G. Cabras,[8] L. Rigacci,[9] F. Salvi,[10] L. Gargantini,[11] M. Balzarotti,[12] F. Merli,[13] G. Pinotti,[14] S. Montoto,[15] A. Lopez-Guillermo,[16] F. Cavalli,[2] L. Giovanella,[1] P. W. Johnson,[17] M. Martelli.[18]
[1]Department of Nuclear Medicine and PET-CT Centre, Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland; [2]Lymphoma Unit, Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Switzerland; [3]Institute of Hematology and Medical Oncology, Policlinico S.Orsola-Malpighi, Bologna, Italy; [4]Department of Oncology, Unit of Lymphoid Malignancies, San Raffaele

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Scientific Institute, Milan, Italy; [5]Hematology, A.O. Citta' della Salute e della Scienza di Torino San Giovanni Battista, Torino, Italy; [6]Hematology, A.O "Bianchi-Melacrino-Morelli', Reggio Calabria, Italy, [7]Hematology, IRCCS Policlinico San Matteo, Pavia, Italy; [8]Hematology, Ospedale Businco, Cagliari, Italy; [9]Hematology, Policlinico Careggi, Firenze, Italy; [10]Hematology, A.O. SS. Antonio e Biagio e C. Arrigo, Alessandria, Italy; [11]Hematology, Ospedale Niguarda Ca' Granda, Milan, Italy; [12]Hematology, Humanitas Cancer Center, Milano Rozzano, Italy; [13]Hematology, Arcispedale Santa Maria Nuova-IRCCS, Reggio Emilia, Italy; [14]Medical Oncology, Ospedale di Circolo e Fondazione Macchi, Varese, Italy; [15]Centre for Haemato-Oncology, Barts Cancer Institute, London, UK; [16]Hematology, Hospital Clinic, Barcelona, Spain; [17]Cancer Research UK Clinical Centre, Southampton General Hospital, Southampton, UK; [18]Hematology, La Sapienza University, Rome, Italy.

Introduction: The IELSG 26 study was defined to evaluate the role of PET in the treatment of primary mediastinal diffuse large B-cell lymphoma (PMLBCL), in particular determining the metabolic response rate at the end of immunochemotherapy. We have previously shown (Ceriani et al. ASH 2012) that liver FDG uptake was the best cut-off to predict outcome for patients (pts), providing the first validation of utility for the Deauville criteria in end-of-therapy PET evaluation of PMLBCL. We report here the analysis of the prognostic impact of functional PET parameters at baseline in PMLBCL. The aim of the study was to assess the prognostic value of (i) the metabolic activity as measured by maximum standard uptake value (SUVmax), (ii) the metabolic tumour burden as measured by metabolic tumour volume (MTV) and (iii) total lesion glycolysis (TLG) estimated on 18FDG PET-CT performed at baseline, in pts enrolled in the IELSG-26 trial.

Patients and Methods: SUVmax, MTV and TLG were measured following a standard protocol in 103/125 pts (baseline PET was not available in 20 pts having been omitted because of the urgency of treatment, and in two for technical problems). All pts received R-CHOP(-like) or R-MACOP-B(-like) regimens; 93 pts had consolidation radiotherapy to the mediastinum. Cut-offs were calculated for each parameter using the receiver operating characteristic curve. The impact on progression-free (PFS) and overall survival (OS) of the PET functional parameters and of the main clinical features (LDH, stage, B-symptoms, bulk, performance status and international prognostic index) was analyzed.

Results: At a median follow-up of 36 months, the PFS and OS rates were 87% and 94%, respectively. By univariate analysis (logrank test), SUVmax, MTV and TLG, as well as bulk >10 cm and stage >2, were significantly associated with worse PFS and OS. In Cox models including SUVmax, MTV, TLG, bulk >10 cm and stage >2, only TLG retained statistical significance for both OS ($p = 0.038$) and PFS ($p = 0.002$). At 5 years, OS was 100% for pts with low TLG vs 80% for those with high TLG ($p = 0.0001$), while PFS was 97% vs 64%, respectively ($p < 0.0001$).

Conclusions: TLG on baseline PET is a powerful predictor of PMLBCL outcome, and its utility to risk stratify patients may warrant further studies.

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18F-FLUORODEOXYGLUCOSE OUTPERFORMS 18F-FLUORO-THYMIDINE IN IDENTIFYING TRANSFORMATION OF FOLLICULAR LYMPHOMA, IN PARTICULAR THROUGH HETEROGENEITY IN UPTAKE

M. Wondergem,[1] N. Rizvi,[2] O. Hoekstra,[2] R. Boellaard,[2] S. Cillessen,[3] J. Regelink,[1] M. Chamuleau,[1] N. Hoetjes,[2] P. van de Ven,[4] S. Zweegman,[1] J. Zijlstra.[1]
[1]Hematology, VU University Medical Center, Amsterdam, Netherlands; [2]Nuclear Medicine, VU University Medical Center, Amsterdam, Netherlands; [3]Pathology, VU University Medical Center, Amsterdam, Netherlands; [4]Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, Netherlands.

Introduction: Diagnosing transformation of follicular lymphoma (FL) to diffuse large B-cell lymphoma is important, because therapy regimens for FL are not effective in transformed lymphoma (TF NHL). Currently, transformation is probably underdiagnosed as it is known to occur focally, whereas in general, a biopsy is performed randomly. FDG-PET, imaging glucose utilization, is known to correlate with proliferation rate. However, FLT might more specifically image proliferation through visualization of thymidine uptake. Therefore, we performed a prospective study to identify which PET-tracer, FDG or FLT, can be used to distinguish between FL and TF NHL.

Methods: FDG-PET and FLT-PET scans were performed in 17 patients with FL and 9 patients with biopsy-proven TF NHL. We measured standardized uptake value (SUVmax) of the lymph node with the highest uptake per patient and the range of uptake in involved nodes per patient. To reduce partial volume effects, only lymph nodes larger than 3cc (measured as the A50 isocontour on the PET scan) were incorporated in the analysis. Scans were made on the Philips Gemini TF PET-CT camera, 1 h after injection of 185 MBq of FDG or FLT.

Results: The SUVmax was significantly higher in TF NHL as compared with FL for both FDG (median 22.0, range 14.6–42.4 in TF NHL and 10.9 (5.2–20.4) in FL, $p < 0.0001$) and FLT (median 11.5, range 5.5–16.3 in TF NHL and 8.0 (3.6–16.6) in FL, $p = 0.03$), however, with considerable overlap. Additionally, we determined the range of FDG and FLT uptake in each individual patient. The FDG range was significantly higher in patients with TF NHL versus FL (6.0–37.5 versus 0.03–7.9, $p < 0.001$), allowing discrimination between TF NHL and FL. In contrast, FLT did not discriminate ($p = 0.07$, receiver operating characteristic (ROC) curve: AUC for FDG 0.967 vs FLT 0.716). Using ROC curve analysis, cut-off values could be determined. Using a cut-off of 14.5 for FDG SUVmax in the lymph node with the highest uptake, TF NHL was diagnosed with a sensitivity of 100% and a specificity of 82%. With a cut-off of 6 for FDG range, sensitivity was 100% and specificity 71%. For FLT, no cut-off values could be determined because of overlap of values. For validation, we analyzed FDG PET scans in four additional patients with TF NHL and five patients with FL using these cut-off values. Moreover, we analyzed 9 TF NHL and 11 FL from literature (Bodet Millin Haematologica 2008). In these validation sets, the 100% sensitivity could only be retained using the cut-off of 6 for FDG range. The lower specificity found in the validation set (55–80%) is acceptable because it only leads to excess biopsies (when a FL is misclassified as a TF NHL) and not to mistreatment of a patient (when a transformation is missed).

Conclusions: FDG-PET distinguishes better than FLT-PET between FL and TF NHL. An intraindividual FDGrange of 6 or higher is highly suspicious of transformation and should guide diagnostic procedures.

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INTERIM PET SUVMAX REDUCTION IS SUPERIOR TO VISUAL ANALYSIS TO PREDICT PATIENT'S OUTCOME IN HODGKIN LYMPHOMA

C. Rossi,[1] S. Kanoun,[2] A. Berriolo-Riedinger,[2] O. Humbert,[2] I. Dygai-Cochet,[2] C. Legouge,[1] M. L. Chretien,[1] L. Berthet,[2] J. Bastie,[1] R. Casasnovas.[1]
[1]Hematology Clinic, CHU Le Bocage, Dijon, France; [2]Medicine Nucleaire, Centre G. F. Leclerc, Dijon, France.

Introduction: PET performed after two cycles of chemotherapy could predict outcome of Hodgkin lymphoma (HL) patients (pts) (Gallamini 2007), but suitable criteria to interpret interim PET (iPET) remain to be established. A visual analysis (VA) using a 5-point scale (5PS) was proposed to assess iPET response. However, SUV may improve iPET accuracy, and SUV reduction between baseline and iPET was shown to be superior to VA in patients (pts) with diffuse large B-cell lymphoma (Casasnovas 2011). To compare the clinical usefulness of both methods in HL pts, we analyzed iPET according to visual and SUV criteria in a retrospective single centre study.

Methods: From 2007 to 2010, 59 pts with a first diagnosis of HL were treated in our institution with four to eight cycles of either ABVD (85%) or BEACOPP. Radiotherapy was performed in 14 responding pts with localized disease. PET was carried out at baseline (PET0) and after two cycles of chemotherapy (PET2), and treatment was not modified according to PET2 result. PET scans were interpreted using the 5PS (PET2 positivity for 5PS score 4 or 5). Standard uptake value (SUVmax) reduction values between PET0 and PET2 (ΔSUV) were available for all pts, and pts with a ΔSUV >71% (receiver operating characteristic curve) were considered as good responders. Median follow-up was 47 months (6–71).

Results: Using VA, 46 (78%) pts achieved a negative PET2, and 7 of them failed to treatment [negative predictive value (NPV) = 85%]. Forty-nine (83%) pts had a ΔSUV >71%, and 6 of them failed to treatment (NPV = 88%). PET2 positive predictive value was significantly better for ΔSUV (70%) compared with VA (46%). Using ΔSUV analysis, 6 (46%) of the 13 PET2 positive pts could be reclassified as good responder. While visual PET2 positivity was related to a lower 4-year progression-free survival (PFS) (45%) compared with PET2 negativity (81%, $p < 0.002$), ΔSUV (>71 vs ≤71%) was more accurate to identify pts with different 4-year PFS (82% vs 30%; $p < 0.0001$). In multivariate analysis, using the international prognosis score and ΔSUV as covariates, ΔSUV remains the unique independent predictor for PFS ($p = 0.0001$; RR: 8.1).

Conclusions: ΔSUV was more accurate than VA based on the 5PS to predict outcome of HL pts. ΔSUV reduces the excess of positive results related to the VA and appears to be the best method so far to assess iPET response in HL.

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WHOLE-BODY DIFFUSION WEIGHTED MRI IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AND HODGKIN LYMPHOMA AT STAGING AND DURING TREATMENT

V. Safar,[1] E. Itti,[2] A. Luciani,[3] S. Katsahian,[4] S. Toledano,[3] B. Bresson,[2] C. Lin,[5] A. Charles-Nelson,[6] K. Belhadj,[7] J. Dupuis,[7] P. Zerbib,[3] B. Zegai,[3] J. Moroch,[8] Y. Huang,[9] M. Meignan,[2] C. Haioun,[7] A. Rahmouni.[3]

[1]Hematology, CHU Lyon Sud, Pierre-Benite, France; [2]Nuclear Medicine, CHU Mondor, Creteil, France; [3]Imagerie Medicale, CHU Mondor, Creteil, France; [4]Equipe 22 Inserm, Centre de Recherche des Cordeliers, Creteil, France; [5]Nuclear Medicine and Molecular Imaging, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taoyuan, Taiwan, China; [6]Unite de Recherche Clinique, CHU Mondor, Creteil, France; [7]Hemopathies Lymphoides, CHU Mondor, Creteil, France; [8]Anatomic Pathology, CHU Mondor, Creteil, France; [9]Anatomic Pathology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taoyuan, Taiwan, China.

Introduction: Evaluation of whole body diffusion weighted MRI (WB-DW-MRI) using apparent diffusion coefficient (ADC) parametric images for staging and follow-up in diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL) by comparison with PET/CT as the reference method.

Methods: Twenty-seven consecutive patients presenting with newly diagnosed DLBCL ($n=15$) and HL ($n=12$) prospectively underwent both WB-DW-MRI and 18-F FDG-PET/CT at staging and after two cycles (interim) of inductive chemotherapy. WB-DW-MRI analysis included size and visual ADC measurements for the 23 defined nodal sites and the six defined organs allowing Ann Arbor staging at baseline and for response assessment. Data of FDG-PET/CT were analyzed using Deauville criteria. WB-DW-MRI and PET/CT images were both analyzed by a junior and a senior reader independently. The baseline staging and the interim response based on WB-DW-MRI and PET/CT were compared. Agreement between junior and senior readings was compared on a per-site basis (Kappa).

Results: At baseline, Ann Arbor stages were concordant between PET/CT and WB-DW-MRI in 22 patients: four patients were understaged on WB-DW-MRI because of lung ($n=2$), iliac nodes ($n=1$), and bowel involvement ($n=1$); one was overstaged (bone marrow involvement). By using size measurements at interim, MRI and PET showed concordant responses in 12 patients; 14 patients were considered in partial response by MRI and complete metabolic response by PET, and 1 patient had a complete response by MRI and a partial response by PET. When including ADC information, MRI was concordant with PET in 22 patients: only four patients had persistent low ADC with no abnormal uptake on PET, and one patient had abnormal FDG uptake not detected on MRI (mediastinal mass). Interestingly, interobserver agreement for PET reading ranged 0.63–0.70 (good), whereas for MRI reading, the range was 0.86–0.96 (excellent).

Conclusion: WB-DW-MRI using ADC mapping is a potentially valuable technique for initial staging and interim response assessement, with excellent interobserver agreement.

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COMPARATIVE ASSESSMENT OF BONE MARROW INVOLVEMENT BY BONE MARROW BIOPSY OR POSITRON EMISSION TOMOGRAPHY IN HODGKIN LYMPHOMA

T. Vassilakopoulos,[1] M. Angelopoulou,[1] V. Prassopoulos,[2] S. Chatziioannou,[3] M. Moschogiannis,[4] P. Tsirkinidis,[5] C. Poziopoulos,[6] P. Repoussis,[7] C. Matsouka,[8] F. Kontopidou,[9] E. Variami,[10] N. Viniou,[10] K. Petevi,[1] G. Boutsikas,[1] A. Kanellopoulos,[1] P. Panayiotidis,[11] G. Pangalis,[4] I. Datseris,[12] J. Meletis,[1] P. Rondogianni.[12]

[1]Haematology Department, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece; [2]Department of Nuclear Medicine, Ygeia Hospital, Athens, Greece; [3]Department of Nuclear Medicine, Academy of Athens Foundation, Athens, Greece; [4]Haematology Department, Athens Medical Center, Athens, Greece; [5]Department of Internal Medicine, 401 Military General Hospital, Athens, Greece; [6]Haematology Department, Metropolitan Hospital, Athens, Greece; [7]Haematology Department, Metaxa General Hospital, Athens, Greece; [8]Haematology Unit, Alexandra General Hospital, Athens, Greece; [9]2nd Department of Internal Medicine, Hippokration General Hospital, National and Kapodistrian University of Athens, Athens, Greece; [10]1st Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece; [11]1st Propedeutic Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece; [12]Department of Nuclear Medicine, Evangelismos General Hospital, Athens, Greece.

Introduction: Bone marrow involvement (BMI) is observed in ~6% of Hodgkin lymphoma (HL) patients (pts). Recent data suggest that few pts have positive bone marrow biopsy (BMB) in the absence of positron emission tomography (PET) evidence, questioning the need of BMB.

Aims: (i) To correlate BMB and BM PET findings in 148 pts; (ii) to assess the impact of our published clinical prediction rule (Vassilakopoulos et al, Blood 2005) on the frequency of BMI detected by either method; and (iii) to assess the ability to omit BMB in selected pts.

Methods: After reviewing all baseline PET reports from three centres, data regarding BMB, clinical and laboratory characteristics were retrieved from medical records. PET data were reviewed for BM uptake and were visually graded as follows: (i) no uptake; (ii) diffuse uptake ≤liver; (iii) diffuse uptake >liver; (iv) solitary osseous/BM focus without CT correlate; and (v) multiple osseous/BM foci. Pts were classified according to our prediction rule for BMI in low-risk, standard-risk and high-risk groups.

Results: Combined PET and BMB data were available for 148 pts: PET was negative for BMI in 122 pts (82%; 71, 24 and 27 pts with scores 1, 2 and 3) and positive in 26 pts: 3 had a single focus and 23 had multiple foci. Only 10 pts had BMI by BMB (6.8%). None of the pts of PET categories 1, 2, 3 or 4 had positive BMB; 10/23 pts graded as '5' had positive BMB (44%). Our clinical prediction rule was well validated: the frequency of BMI by BMB was 0% in low-risk and standard-risk groups vs 20% in the high-risk group, while the frequency of BMI by PET was 0% in low-risk, 6.5% in standard-risk and 40% in the high-risk groups. The outcome of the 10 pts with BMI by BMB was marginally inferior (3-year FFS 43% vs 85%, $p=0.055$). The difference was more pronounced for the 23 pts with BMI by PET (3-year FFS 43% vs 85%, $p=0.0002$). Pts with BMI by PET and negative or positive BMB had similar outcomes (3-year FFS 28% vs 52%, $p=0.33$).

Conclusions: PET revealed ~2.5 times more cases of BMI than BMB. Our clinical prediction rule was adequately validated regarding the prediction of BMI by either BMB or PET, suggesting that the selected prognostic covariates are valid for BMI. There was no case of positive BMB in the absence of BMI by PET. Thus, BMB can be safely omitted in pts staged by PET, because there was no high-risk group who might benefit from the combination of PET and BMB.

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ONO-4059, A NOVEL ORAL BRUTON'S TYROSINE KINASE (BTK) INHIBITOR THAT DEMONSTRATES POTENT PHARMACODYNAMIC ACTIVITY THROUGH PHOSPHORYLATED BTK (P-BTK) INHIBITION, IN ADDITION TO EFFECTIVE ANTI-TUMOUR ACTIVITY IN A DLBCL XENOGRAFT MODEL

T. Yoshizawa[1], J. Birkett,[2] T. Yasuhiro,[1] S. Hotta,[1] Y. Ariza,[1] Y. Ueda,[1] M. Narita,[1] Y. Shichino,[3] K. Kawabata.[4]

[1]Discovery Research Laboratories 3, Ono Pharmaceutical Co., Ltd., Osaka, Japan; [2]Clinical Science, Ono Pharma UK Ltd, London, United Kingdom; [3]Discovery Research Laboratories, Ono Pharmaceutical Co., Ltd., Osaka, Japan; [4]Research Headquarters, Ono Pharmaceutical Co., Ltd, Osaka, Japan.

Introduction: Bruton's tyrosine kinase (Btk) is a key regulator of the BCR signaling pathway and abberant BCR signaling has been implicated in the survival of malignant B-cells. The activated B-cell-like (ABC) sub-type of diffuse large B-cell lymphoma (DLBCL) is associated with poor prognosis and new therapies, preferably chemo-sparing therapies, or as add-on to existing treatment regimens, are required to help treat patients with ABC-DLBCL.

Methods: ABC-DLBCL cell lines (TMD-8 and U-2932) were incubated with ONO-4059 monotherapy (1-1000 nmol/L) and in combination with various agents that target various pathways thought to be involved in DLBCL. Apoptosis was determined by AnnexinV-FITC/7AAD (AnnV+/7AAD-) staining. In vivo studies were performed with mice bearing TMD-8 cells that were subcutaneously injected into female SCID mice. ONO-4059 was administered orally, twice a day (BID) at doses of 1, 3 and 10 mg/kg. After the final dose of ONO-4059, the total RNAs were extracted from frozen re-sected tumour tissue specimens (10 samples at each dose, along with 10 vehicle tissue samples). Samples were analyzed using Agilent microarray technology.

Results: ONO-4059 is highly potent with an IC50 in the sub-nmol/L range. Apoptosis was detected in TMD-8 cells at 10 nmol/L at the 48 hr time point but not in U-2932 cells. In the TMD-8 xenograft studies, the inhibitory level of Phosphorylated-Btk (P-Btk) achieved indicates the profound anti-proliferative activity of ONO-4059. Gene expression results revealed down-regulation of CXCL-10 ($p < 0.001$) after the treatment of ONO-4059. CXCL-10 is reported to be up-regulated in lymphoma patients.

Conclusions: Our data show that ONO-4059 has potent anti-tumour activity in ABC-DLBCL cells. ONO-4059 is currently being developed in a Phase I clinical trial for the treatment of B-cell malignancies. Additional combination studies are underway using the TMD-8 xenograft model, testing ONO-4059 in combination with various agents.

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GENOMIC INSTABILITY AND ACTIVATION OF THE DNA DAMAGE RESPONSE PATHWAY IS AN INDEPENDENT PREDICTOR OF POOR PROGNOSIS AND A PROMISING TARGET FOR THERAPY IN DIFFUSE LARGE B CELL LYMPHOMA

E. Derenzini,[1] C. Agostinelli,[2] E. Imbrogno,[1] I. Iacobucci,[1] A. Ferrari,[1] A. Ghelli,[1] E. Brighenti,[1] B. Casadei,[1] S. Pileri,[2] G. Martinelli,[1] P. Zinzani.[1]
[1]University of Bologna, Institute of Hematology and Medical Oncology L.A. Seragnoli, Bologna, Italy; [2]University of Bologna, Unit of Hemolymphopathology, Bologna, Italy.

Introduction: Genomic instability and constitutive activation of the DNA damage response (DDR) pathway have been recently described in models of aggressive myc-driven lymphoid malignancies. The checkpoint kinases 1 (CHK1) and 2 (CHK2) are serine–threonine kinases involved in the DDR pathway, and DDR activation triggers the phosphorylation of the DNA damage marker histone H2AX.
Methods: Immunohistochemistry (IHC) for phospho (γ) H2AX was performed in 106 consecutive patients treated at our Institution between 2004 and 2011 with R-CHOP/CHOP-like regimens, with available paraffin embedded tissue from initial diagnosis. To asses the therapeutic potential of DDR pathway inhibition in diffuse large b-cell lymphoma (DLBCL), the DLBCL cell lines HBL-1, U2932, TMD8, SUDHL-6, BJAB, SUDHL-4 and primary DLBCL cells were incubated with the CHK inhibitor PF-0477736 (Pfizer).
Results: A significant growth inhibition (WST-1 assay) was evident after 48 h in all cell lines (IC50 140–230 nM). PF-0477736 25–500 nM induced cell death by apoptosis (annexin V–propidium iodide staining) in a time-dependent and dose-dependent manner. Notably, PF-0477736 demonstrated activity also in primary DLBCL cells (IC 50 of 50–500 nM, 24 h). All cell lines showed detectable baseline activation of CHK1/CHK2 and/or H2AX phosphorylation. We observed inhibition of phosphorylation of the downstream targets CDC25c and CDC2, coupled with a marked increase in γH2AX and CHK1 phosphorylation (ser317 and 345) following treatment. In the HIC study, 48.1% of patients ($n = 51$) displayed high levels of basal γH2AX (>50% of positive cells). Five-year survival rate was 61% vs 33% for γH2AX-low and γH2AX-high patients, respectively ($p = 0.01$). Remarkably, these results were independent from the international prognostic index score in multivariate analysis.
Conclusions: A significant fraction of DLBCL shows high levels of inherent genomic instability, and the DDR activation marker γH2AX is a poor prognostic predictor in DLBCL. The CHK inhibitor PF-0477736 shows high single agent activity in DLBCL cell lines and primary cells. These data provide strong rationale for targeting the DDR pathway and for clinical investigation of CHK inhibitors in DLBCL.

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TARGETING SIGNALLING PATHWAYS IN MYCOSIS FUNGOIDES AND SEZARY SYNDROME

W. Ai,[1] T. Rakhshandehroo,[1] L. Pincus,[2] F. McCormick,[3] S. Bandyopadhyay.[3]
[1]Medicine, University of California, San Francisco, San Francisco, USA; [2]Dermatology, University of California, San Francisco, San Francisco, USA; [3]HDF Comprehensive Cancer Center, University of California, San Francisco, San Francisco, USA.

Background: Mycosis fungoides/Sezary syndrome (MF/SS) is a group of heterogeneous diseases. Although generally indolent at early stage, approximately 40–50% patients with advanced stage disease eventually succumb to their illness. Recent studies demonstrated dysregulation of signalling pathways in MF/SS, including PI3K/Akt, jak/stat, RAS and NFkB pathways. Here, we performed a high throughput drug screening to identify potential new therapeutic agents targeting these signalling pathways in MF/SS.
Methods: We compiled a drug library of 94 compounds targeting major signalling pathways as well as HDAC, proteosome, DNA repair and apoptosis.We then evaluated the anti-proliferation effect of these compounds on four MF/SS cell lines in high throughput proliferation assays. In addition, we searched for compounds in the drug library that synergize with BKM120, a PI3K inhibitor, to induce apoptosis.
Results: We identified 14 compounds with strong anti-proliferative activities in MF/SS. They represent inhibitors for PI3K, mTOR, HDAC, proteosome and heat shock proteins.Of these compounds, BKM120 induces apoptosis in MF/SS cell lines.The combination of HDAC inhibitors with BKM120 resulted in greater apoptosis than each agent alone.
Conclusion: BKM120, which targets the PI3K/Akt pathway, is a promising new therapeutic agent for MF/SS. The combination of BKM120 with HDAC inhibitors induces greater appoptosis than each agent alone.

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A NOVEL IRREVERSIBLE BTK INHIBITOR PLS-123 WITH ANTI-TUMOUR ACTIVITY IN B-NHL PRECLINICAL MODELS

N. Ding,[1] X. Zheng,[1] Y. Song,[1] X. Li,[2] Y. Tian,[3] L. Ping,[1] Y. Shi,[4] W. Zheng,[1] N. Lin,[1] X. Wang,[1] M. Tu,[1] Y. Xie,[1] W. Liu,[1] L. Deng,[1] Z. Ying,[1] C. Zhang,[1] Z. Pan,[2] J. Zhu.[1]
[1]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Lymphoma, Peking University Cancer Hospital & Institute, Beijing, China; [2]Key Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen, China; [3]Department of Internal Medicine Oncology, Peking University First Hospital, Beijing, China; [4]Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Pathology, Peking University Cancer Hospital & Institute, Beijing, China.

B-cell non-Hodgkin's lymphoma (B-NHL) is the malignant growth of B-lymphocytes in the lymph system that contains various subtypes with distinct characteristics in pathology, clinical features and prognosis. The BCR signal pathway has gained significant attention as a potential treatment target because of its essential role in pathogenesis of B-NHL. Recently, several BCR signal targeting drugs, especially the Btk inhibitor ibrutinib, have demonstrated promising therapeutic effects in relapsed/refractory chronic lymphocytic leukaemia (CLL) and B-NHL patients. We recently developed a novel Btk inhibitor PLS-123, also a covalent irreversible inhibitor as ibrutinib; but unlike ibrutinib that only inhibits Btk's catalytic activity, PLS-123 exhibits a dual-action mode of inhibition for both the catalytic activity of Btk and its own activation. We examined PLS-123's effects on preventing the proliferation of 15 kinds of B-NHL cell lines (DLBCL: OCI-Ly7, SU-DHL-6, SU-DHL-16, Pfeiffer and OCI-Ly3; FL: DoHH2, RL and WSU-NHL; CLL: JVM-3; MCL: JVM-2, JVM-13, Jeko-1, Mino, Z138 and Granta519). In 14 of them, PLS-123 demonstrated more anti-proliferative effects than ibrutinib in a dose-dependent manner. The DLBCL cell line OCI-Ly7 and FL cell line WSU-DHL showed the highest sensitivity towards PLS-123 treatment with a GI50 in the nanomolar range, whereas ibrutinib has a GI50 in the micromolar range. PLS-123 also inhibited more strongly in proliferation of B-NHL patients' primary tumour cells. Furthermore, the phosphorylation levels of key regulatory enzymes (Btk, PLCγ2 and Erk1/2) of BCR signal pathway within lymphoma cells were more significantly diminished after treatment of PLS-123 than ibrutinib in the presence or absence of anti-IgM treatment. PLS-123 but not ibrutinib also activated apoptosis-related proteins PARP and cleaved-caspase-3 during the anti-proliferative process. Interestingly, chemokine CCL3 and CCL4 productions from B-NHL cells, which were highly regulated by BCR signalling pathway, were also more dramatically reduced after PLS-123 treatment comparing with ibrutinib. Finally, PLS-123 also demonstrated a significant anti-tumour effect in a mice xenograft model. Taken together, the novel irreversible Btk inhibitor PLS-123 has a dual-action mode of inhibition and exerts more profound effects on B-NHL cell lines than ibrutinib. Our results could suggest a new direction of the next generation Btk inhibitors for the treatment of B-NHL patients.

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A NEW THERAPEUTIC APPROACH FOR THE THERAPY OF B-CELL DISORDERS: DRUG-LOADED ANTI-CD20 NANOPARTICLES

G. Pozzato,[1] N. Mezzaroba,[2] S. Zorzet,[2] E. Secco,[2] S. Biffi,[3] C. Tripodo,[4] M. Calvaruso,[4] L. Nunez,[5] R. Spretz,[6] G. Larsen,[7] S. Noriega,[6] C. Garrovo,[2] G. H. Marin,[8] E. Mansilla,[8] P. Macor.[2]
[1]Department of Surgical and Medical Sciences, University of Trieste, Trieste, Italy; [2]Department of Life Sciences, University of Trieste, Trieste, Italy; [3]Area Science Park, Optical Imaging Laboratory, CBM, Trieste, Italy; [4]Department of Human Pathology, Univerisity of Palermo, Palermo, Italy; [5]Department of Life Sciences, University of Chicago, Chicago, United States; [6]Reseach and Development, LNK Chemsolutions, Lincoln, United States; [7]Reseach and Development, Bio-Target, Chicago, United States; [8]Ministry of Health, CUCAIBA, La Plata, Argentina.

The treatment of B-cell indolent diseases like chronic lymphocytic leukemia (CLL) as well as of aggressive disorders like Burkitt lymphoma is based on chemotherapy and immunotherapy via monoclonal antibodies (Ab). These treatments show high toxicity and a fraction of patients does not respond. Nanoparticles designed with specific Ab-coating represent a new strategy to target only tumor cells with high-dose chemotherapy. We characterized the effects of new biodegradable nanoparticles (BNP) coated with anti-CD20 and loaded with Chlorambucil and Hydroxychloroquine (CLB+HCQ) or with Fludarabine (Fl). To investigate the effects of BNP on cell viability, MEC-1 cells and BJAB cells (two CLL and Burkitt cell lines both expressing CD20 and mutated p53) were incubated with BNP. The viable cells were determined with MTT and cell death with PARP-1 cleavage and Annexin V detection. The BNP with anti-CD20 induced a selective penetration in cells

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expressing this antigen, as demonstrated by confocal and electron microscopy studies. After 48 hours all cancer cells were destroyed with CLB-HCQ loaded BNP while those with Fl showed a lower killing rate. The results were confirmed in primary cells isolated from 40 cases of untreated CLL. The "*in vivo*" safety of antiCD20-BNP was evaluated by endovenous (e.v.) or intra-peritoneal (i.p.) injection of BNP in mice. The BNP wiht or without drugs did not induced tissue damages, weight loss or death, while the same amount of free cytotoxic drugs caused death of all mice. BNP elimination was documented in liver and intestine. The i.p. injection of BJAB cells and the e.v. injection of MEC1 cells in SCID mice allowed the development of a human/mouse models of lymphoma/leukemia. Both BJAB and MEC1 determined a rapid diffusion of leukemic cells in all organs and tissues and early death of all mice. In both models, eight injections of antiCD20 BNP containing CLB-HCQ were able to increase survival in 100% of mice and 90% of animals were cured. The same amount of free CLB+HCQ killed all the mice in about a week for toxicity. This study demonstrates that antiCD20-BNP containing HCQ-CLB can be effective in controlling both models of aggressive or indolent B-cell disorders and provides a rationale for adopting this new therapy in clinical trials.

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ALISERTIB (INVESTIGATIONAL AGENT) PLUS RITUXIMAB AND VINCRISTINE IS SYNTHETIC LETHAL IN MYC/BCL2 CO-EXPRESSING AGGRESSIVE B-CELL NON-HODGKIN LYMPHOMA MOUSE MODELS

D. Mahadevan,[1] C. Morales,[1] L. S. Cooke,[1] A. Manziello,[2] D. O. Persky,[3] D. Mount,[2] R. I. Fisher,[4] T. P. Miller,[2] W. Qi.[1]
[1]Medicine–Hematology/Oncology, University of Tennessee/West Clinic, Memphis, USA; [2]Medicine, University of Arizona Cancer Center, Tucson, USA; [3]Medicine, Yale Cancer Center, Newhaven, USA; [4]Medicine, University of Rochester Medical Center, Rochester, USA.

Introduction: Concurrent MYC and BCL2 translocation and/or protein over-expression defines double-hit B-cell lymphomas (DH B-NHL) characterized by poor outcomes with R-CHOP. Aurora (A and B) are oncogenic Ser/Thr kinases that play critical roles in regulating the mitotic phase of the cell cycle. Aberrant Myc expression leads to aurora dysregulation with associated genetic instability, polyploidy and resistance to microtubule targeted agents (MTAs). We **hypothesized** that co-expression of Myc and Bcl2 is functionally equivalent of DH B-NHL targetable by inhibition of aurora in the presence of MTAs and rituximab.
Methods: We evaluated ABC-DLBCL cell lines TMD-8, U-2932, OCI-Ly10 and MCL cell line Granta-519 for Myc and Bcl2 expression in the context of a chronic active BCR pathway. Anti-lymphoma activity of alisertib (MLN8237, M) was evaluated in the presence of vincristine (V) rituximab (R) in cell culture and SCID mouse models of U-2932 and Granta-519. Gene expression profiling, qRT-PCR, IHC and Western blotting of harvested tumours at the end of treatment were interrogated for mechanistic role(s) of aurora inhibition when added to V R.
Results: Myc and Bcl2 are differentially expressed in U-2932, TMD-8, OCI-Ly10 and Granta-519. Treatment with alisertib for 48 h maintained expression of Myc, Bcl2 and BTK, except p53 levels increased significantly in Granta-519. Alisertib (M) was synergistic with V R (MVR) for inhibition of cell proliferation, abrogation of cell cycle checkpoints and enhanced apoptosis compared with single or doublet treatments. A U-2932 mouse model showed tumour regression (TR) with MR and VR but relapsed 10 days after discontinuing therapy. In contrast, MVR showed TR with no relapse >40 days after stopping therapy. A Granta-519 mouse model showed TR with MV but relapsed 20 days after discontinuing therapy, whereas MVR showed TR with no relapse >120 days after stopping therapy. Genes upregulated by MVR are linked to karyokinesis (CENP-E, KIF20A/B, SGOL-2) in metaphase.
Conclusions: MVR represents a novel therapeutic strategy in Myc/Bcl2 co-expressing aggressive B-NHL and is under clinical evaluation sponsored by Millennium Pharmaceuticals (C14011). Correlative study of Myc and Bcl-2 amplification in relation to clinical outcome may warrant further investigation.

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DUAL TARGETING OF HISTONE DEACETYLASE AND NF-KAPPAB in B-CELL AND T-CELL LYMPHOMA

C. Deng, R. Rodriguez, L. Scotto, J. Xavier, O. A. O'Connor.
Center for Lymphoid Malignancies, Department of Medicine, Columbia University Medical Center, New York, USA.

Introduction: The lymphomas represent one select type of cancer where a strong rationale for targeting the acetylation mediated epigenetic apparatus exists. Increased levels of histone deacetylase (HDAC) have been correlated with aggressive behaviours of B-cell and T-cell lymphomas. Conversely, mutations of histone acetyltransferases have been shown to occur frequently in B-cell lymphoma. NF-kappaB (NF-kB) is a family of inducible transcription factors that are critical for cell proliferation and survival and constitutively activated in select types of B-cell and T-cell lymphoma. Simultaneous inhibition of HDAC and NF-kB is a promising strategy for the treatment of lymphoma.
Methods: We first investigated the pharmacologic activity of a novel IKK2 inhibitor, LY2409881, *in vitro*. Sensitivity of lymphoma cells to the compound was determined using the ATP-based assay, Cell Titer-Glo. The effect of LY2409881 on NF-kB was determined by Western blot and immunofluorescence microscopy. We investigated the drug : drug interaction of LY2409881 and HDAC inhibitors in these cells by calculating the relative risk ratio.We confirmed the *in vitro* activity of LY2409881 using mouse xenograft models of human B-cell lymphoma. Blood and tissue samples were obtained from these mice to perform pharmacokinetic and pharmacodynamic studies.
Results: LY2409881 specifically inhibited the activation of NF-kB and enhanced apoptosis, and were toxic to cells addicted to NF-kB. Furthermore, LY2409881 demonstrated marked anti-lymphoma synergy with HDAC inhibitors; the synergism varied significantly in a cell line and HDAC inhibitor-dependent manner. The distinct patterns of synergism among different HDAC inhibitors with LY2409881 were not solely attributable to difference in HDAC inhibitor-mediated acetylation of p65, as these inhibitors all stimulated binding of p65 to its target DNA in a similar manner. Ongoing experiments will determine the activity of LY2409881 *in vivo* in mouse xenograft models of human lymphoma, as a single agent and in combination with HDAC inhibitors.
Conclusion: A novel selective IKK2 inhibitor, LY2409881, and HDAC inhibitors synergistically inhibited the growth of B-cell and T-cell lymphoma.

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DARATUMUMAB, A NOVEL HUMAN CD38 MONOCLONAL ANTIBODY FOR THE TREATMENT OF B-CELL NON-HODGKIN LYMPHOMA

A. Matas-Cespedes,[1] A. Vidal-Crespo,[1] V. Rodriguez,[1] G. Roue,[1] A. Lopez-Guillermo,[2] E. Campo,[2] D. Colomer,[2] J. J. Lammerts Van Bueren,[3] J. Bakker,[3] A. Wiestner,[4] P. W. Parren,[3] P. Perez-Galan.[1]
[1]Hemato-Oncology, IDIBAPS-Hospital Clinic, Barcelona, Spain; [2]Hematopathology Unit, Hospital Clinic, Barcelona, Spain; [3]Genmab BV, Utrecht, Netherlands; [4]Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes Of Health, Bethesda, MD, USA.

Introduction: Daratumumab (DARA) is a human CD38 mAb that induces killing of tumour cells, mainly via complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). DARA is currently evaluated in phase I/II clinical trials in patients with multiple myeloma with manageable adverse events and marked reductions in paraprotein and bone marrow plasma cells. In this study, we have analyzed the activity of DARA against CD38-expressing tumour cells in B-cell lymphomas including mantle cell lymphoma (MCL) and follicular lymphoma (FL) and in chronic lymphocytic leukaemia (CLL).
Methods: ADCC and CDC killing activity were assessed by Calcein release. Migration assays were performed in migration chambers of 5-μM pore size. Molecules per cell (MCP) were analyzed using Qifikit and flow cytometry. *In vivo* activity was assessed in a prophylactic set up in SCID mice subcutaneously injected with 1×10^7 cells. Mice were given antibodies on days 1, 15 and 30 (20, 10 and 10 mg/kg ip, respestively).
Results: MCL and CLL tumour cells show heterogeneous expression of CD38, whereas FL cells showed invariable high CD38 levels. By using healthy donor PBMC effector cells, DARA induced significant levels of ADCC at 1 μg/mL in cell lines and primary cells of MCL ($n = 4$, 45.16%), FL ($n = 5$, 49.85%) and CLL ($n = 11$, 37.7%). However, DARA did not induce significant CDC in any of these models. Likely, low CDC activity was associated with high expression of the complement inhibitors CD46, CD55 and CD59, and insufficient number of CD38 MCP. Noteworthy, DARA-induced ADCC was significantly increased by preincubation of PBMC with lenalidomide (2–4 fold; $p < 0.05$). CD38 is important for CXCR4–CXCL12-induced migration in CLL. We have found that in CD38 CLL cells with high migratory capacity, DARA (10–30 μg/mL) inhibited CXCL12-mediated migration up to 70% ($n = 6$, mean 38.8%). Noteworthy, DARA exhibited *in vivo* activity and completely prevented the outgrowth of MCL and FL xenograft tumours in SCID mice.
Conclusions: DARA is able to induce ADCC in MCL, FL and CLL tumour cells, interferes with CXCR4–CXCL12-mediated B-cell migration and shows activity *in vivo*. Interestingly, ADCC activity of DARA could be further augmented by lenalidomide. These results suggest that DARA may be a promising therapeutic agent for a set of B-cell neoplasms.

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EFFECTS OF RITUXIMAB AND GA-101 ON *IN VITRO* KILLING OF CELL LINES AND PATIENT LYMPHOMA CELLS BY NORMAL DONOR OR PATIENT EFFECTOR CELLS

H. S. Wu,[1] A. Lunter,[2] E. Spillner,[1] M. Zeis,[2] N. Schmitz.[2]
[1]Institute of Biochemistry, University Hamburg, Hamburg, Germany; [2]Department of Haematology, Oncology and Stem Cell Transplantation, Asklepios Hospital St. Georg, Hamburg, Germany.

Introduction: Immunochemotherapy combining rituximab (R) with CHOP or CHOP-like regimens has significantly improved treatment results in aggressive B-cell lymphoma. Next generation anti-CD 20 antibodies have been generated and currently undergo phase I to III studies in many lymphoma subtypes. *In vitro* testing of new antibodies mostly involved effector cells from normal donors, and lymphoma cell lines served as target cells.
Methods: We compared *in vitro* activities of R and GA-101, a second generation anti-CD-20 antibody, provided by Hoffmann-La Roche AG, using the following functional assays: CD 107a degranulation assay, Europium assay, complement-dependent cytotoxicity (CDC) and apoptosis (annexin V assay). Freshly isolated CD 56-positive NK cells from normal donors or patients with follicular lymphoma, mantle cell lymphoma or diffuse large b-cell lymphoma (DLBCL) were used as effector cells and tested against various B-cell lymphoma cell lines: Su-DHL-4, Ramos, Karpas 422; Oci-Ly 3 and -10 representing DLBCL of ABC subtype are under investigation. We also used single cell suspensions of lymph node material obtained from patients with DLBCL, MCL or FL as target cells.
Results: CD 107a degranulation assays ($n = 9$) using healthy donor cells and Su-DHL-4 cell line confirmed that GA 101 results in superior killing of target cells (5% vs 14%, $p = 0.022$) as compared with R. In contrast to previous reports, we did not find significant differences in CDC and apoptosis when healthy donor cells and Su-DHL-4 cells were tested; a trend in favour of R was found when Ramos cells were used in this type of assay. When testing patient-derived NK cells against autologous fresh lymphoma cells isolated from the same patients, CD3-negative CD 107a-positive expression was significantly lower than observed in all cell lines. So far, no significant differences in terms of cytotoxicity were found when GA 101 was compared with R.
Conclusions: *In vitro* testing of new antibodies using normal donor cells and cell lines obviously does not always reflect the *in vivo* situation where patient effector cells need to kill patient lymphoma cells targeted by CD-20 antibodies. We suggest using patient effector and target cells to evaluate the potential of new antibodies whenever possible.

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SINGLE AND MULTIPLE DOSE-RANGING EVALUATION OF SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF GS-9973, A NOVEL PSYK INHIBITOR

S. Ramanathan,[1] J. Di Paolo,[2] T. Doan,[3] D. Burge.[4]
[1]Clinical Pharmacology, Gilead Sciences, Foster City, USA; [2]Biology, Gilead Sciences, Foster City, USA; [3]Clinical Operations, Gilead Sciences, Foster City, USA; [4]Clinical Research, Gilead Sciences, Foster City, USA.

Background: GS-9973 is a selective, reversible, ATP-competitive small molecule inhibitor of spleen tyrosine kinase (Syk) that effectively blocks BCR-mediated activation and proliferation, blocks Fc receptor signalling and function, and inhibits immune complex-mediated inflammatory cytokine production, including TNFa. The safety, pharmacokinetics (PK), and pharmacodynamics (PD) of GS-9973 were evaluated in a double-blind, single/multiple ascending dose study.
Methods: In sequential cohorts ($n = 8$ active, 2 placebo), subjects received GS-9973 (25 to 1200 mg) as a single dose (Part A) or multiple twice-daily doses for 7 days (Part B) in a fasted manner. Blood sampling was done in Parts A and B (after AM dose on Day 7) to assess PK and PD using functional inhibition of *ex vivo* aIgE-stimulated CD63 expression on basophils, and direct target inhibition of *ex vivo* pervanadate evoked-SYK autophosphorylation. Safety and tolerability assessments were carried out throughout dosing and during 7-day (Part A) or 14-day (Part B) follow-up, with dose escalation gated by stopping criteria. PK-PD relationship was evaluated using GS-9973 concentrations versus CD63 inhibition.
Results: Across dose cohorts, subjects were ≥60% white, ≥50% male, with mean age of 28 to 37 years. Adverse events (AEs) were generally mild to moderate, with no AE-driven study drug discontinuations observed. The PK of GS-9973 will be presented. GS-9973 exposures reached a plateau at doses ≥600 mg BID (due to solubility-limited absorption), provided >90% CD63 inhibition at peak concentrations (~2 h post-dose) and maintained >60% inhibition over the dosing interval, facilitated by a long plasma half-life of 10 to 14 h. PK-PD analyses indicated a sigmoidal relationship between GS-9973 concentrations and inhibition of CD63 expression and pSyk autophosphorylation, with EC_{50} of ~400–450 ng/mL and hill slope >1 for CD63 inhibition.
Conclusions: GS-9973 was generally well tolerated over a 48-fold dose range. Plasma GS-9973 exposures reached a plateau ≥600 mg and provided exposure-dependent robust PD activity over the dosing interval. The overall safety, PK, and PD profile of GS-9973 support continued clinical evaluation of doses that provide strong PD activity.

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EXPLORATORY ANALYSIS OF THE RELATIONSHIP BETWEEN TARGET EXPRESSION AND EFFICACY OF ANTI-CD22-MMAE AND ANTI-CD79B-MMAE ANTIBODY DRUG CONJUGATES IN PHASE I STUDIES

K. Mundt,[1] R. McCord,[1] H. Koeppen,[2] L. Fu,[3] C. Wong,[3] A. Do,[3] T. Sumiyoshi,[3] Y. Wang,[1] R. Bourgon,[4] A. Polson,[5] A. Chai,[6] W. Chu,[7] M. C. Palanca-Wessels,[8] R. H. Advani,[9] F. Morschhauser,[10] B. D. Cheson.[11]
[1]Oncology Biomarker Diagnostics, Genentech, South San Francisco, USA; [2]Research Pathology, Genentech, South San Francisco, USA; [3]OBD, Clinical Assays and Technology Group, Genentech, South San Francisco, USA; [4]Research Bioinformatics, Genentech, South San Francisco, USA; [5]Research, Genentech, South San Francisco, USA; [6]Biometrics, Genentech, South San Francisco, USA; [7]Clinical Development, Genentech, South San Francisco, USA; [8]Fred Hutchinson Cancer Center, University of Washington, Seattle, WA, USA; [9]Stanford Cancer Center, Lymphoma Clinic, Stanford University, Palo Alto, CA, USA; [10]Claude Huriez, Centre Hospitalier Regional, Lille, France; [11]Lombardi Comprehensive Cancer Center, Georgetown University Hospital, Washington, DC, USA.

Introduction: Anti-CD22-MMAE and anti-CD79b-MMAE are antibody drug conjugates (ADCs) directed against B-cell surface antigens CD22 and CD79b, respectively. Phase 1 studies are ongoing for both to assess safety and tolerability in relapsed or refractory B-cell non-Hodgkin's lymphoma (NHL) patients. Both ADCs have demonstrated encouraging levels of activity at doses ≥1.8 mg/kg (ASH 2012: # 59 Advani et al; # 56 Palanca Wessels et al).
Methods: Archival tissue was collected from patients to assess levels of CD22 and CD79b by IHC across both trials ($n = 94$). CD22 and CD79b mRNA levels were analyzed by qRT-PCR normalized to housekeeping genes and universal mRNA expressed as log2–(ΔΔCT). Correlation of response of efficacy evaluable patients at doses ≥1.8 mg/kg with the respective target expression level and other biomarkers (e. g. apoptotic regulators, diffuse large B-cell lymphoma subgroup) was explored.
Results: High levels of CD79b expression by IHC were observed in virtually all specimens consistent with its role as signal transducing subunit of the essential B-cell receptor. Interestingly, CD22 expression by IHC using antibody clone FPC-1 was unexpectedly low, with only 10–20% exhibiting expression levels ≥ IHC 2+ and significant numbers of samples being IHC negative for CD22. These results were confirmed with a second anti-CD22 antibody clone SP104. To assess the correlation between protein and mRNA expression, samples were also analyzed by qRT-PCR for CD22 and CD79b, which suggested comparable levels of mRNA for both targets. The diverse patient population and dosing regimens in this phase 1 study do not permit a meaningful statistical assessment of correlation of target expression and response at this stage. However, responses, including CRs, were observed at IHC levels of 1+ and correspondingly low mRNA expression.
Conclusion: The difference between CD22 protein expression by IHC and mRNA may reflect a biological difference in protein translation or sensitivity of the protein to tissue processing. Overall, no striking correlation of target expression with response was observed. There was no treshhold of target expression for response: Anti-tumour responses with ADC treatment were observed in patients whose tumours had low levels of target expression by both IHC and qRT-PCR. Data will be updated for the conference.

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DENDRITIC CELL VACCINE FOR INDOLENT B-CELL NON-HODGKIN LYMPHOMA: PRELIMINARY RESULTS OF TRIAL LS1081

S. F. Cordes,[1] T. D. Atwell,[2] M. L. Maas,[3] A. S. Armstrong,[3] P. A. Bulur,[3] M. P. Gustafson,[3] Z. Zhang,[4] M. R. Callstrom,[2] S. M. Ansell,[4] L. F. Porrata,[4] P. B. Johnston,[4] S. N. Markovic,[4] D. A. Gastineau,[4] A. B. Dietz,[3] T. E. Witzig,[4] Y. Lin.[4]
[1]Internal Medicine, Mayo Clinic, Rochester, USA; [2]Radiology, Mayo Clinic, Rochester, USA; [3]Transfusion Medicine, Mayo Clinic, Rochester, USA; [4]Medicine - Hematology, Mayo Clinic, Rochester, USA.

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Introduction: We present the preliminary results of clinical trial LS1081 in progress testing two antigen delivery methods in a vaccine strategy for patients (pts) with relapsed indolent B-cell non-Hodgkin lymphoma (NHL). The primary objective is safety and feasibility. Secondary objectives are to describe treatment responses and correlate clinical response with immune changes.
Methods: Autologous dendritic cells (DC) are manufactured from leukapheresed cells from NHL pts. Pts with tumour deemed amenable to cryoablation (arm A) received cryoablation on day 1 followed by intratumoural injection of DC on days 1 and 14. Pts without an ablatable tumour (arm B) have node excision to generate tumour lysate *ex vivo*. DC are pulsed with lysate during maturation and injected intradermally q14days × 4. Pts are monitored for 1 year after vaccination for adverse events and systemic tumour response. Correlative studies include immune phenotype and T-cell intracellular cytokine productions. Planned accrual is 10 patients per arm.
Results: To date, four pts have accrued to arm A and 2 pts to arm B. All pts tolerated vaccine treatments without major adverse events. Of the five pts who have completed treatments, there were two partial remission, one stable disease, and two progressions. Tumour regression is steady but can be slow, reaching Cheson criteria for response 6 months after vaccines. The two responding patients continue in response at 12 and 15 months. Interestingly, correlative studies suggest that immune changes are different in responding patients and may predict treatment response. Compared with those of pts with progression, T cells in non-progressing pts had significantly increased IFNg than IL4 production in response to stimulation. In addition, hierarchical clustering analysis of pts' composite blood immune phenotype showed clustering away from phenotype profile of healthy controls. With treatments, the composite phenotype profile of the pts changed with respect to baseline.
Conclusions: Cryoablation and intratumoural DC vaccination is a feasible and safe strategy in NHL. Biosystems analysis can be used to develop novel assays as predictive biomarkers for treatment response. Accrual is ongoing to further define the response rate and biomarkers.

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A DOSE-FINDING STUDY OF BENDAMUSTINE, RITUXIMAB AND LENALIDOMIDE IN RELAPSED AND REFRACTORY LYMPHOMA

B. D. Cheson,[1] J. G. Crawford.[2]
[1]*Hematology-Oncology, Georgetown University Hospital, Washington, D.C., USA;* [2]*Oncology, Lombardi Comprehensive Cancer Center, Washington, D.C., USA.*

Background: Despite progress in treating non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL), many patients (pts) are refractory to initial therapy or relapse and require additional approaches. Bendamustine (B), rituximab (R) and lenalidomide (L) have single-agent activity in this setting. Thus, the purpose of the study was to identify a tolerable phase II dose of the combination. Pts were required to have NHL or HL and failed standard therapies.
Methods: The first four pts were treated with B at 90 mg/m^2 d 1,2 q 28 d, and L at 5 mg qd 28/28 d, which was poorly tolerated because of grade 3 rash (subsequently dropped as a DLT), grade 4 neutropenia and grade 3 anaemia, considered DLTs. The protocol was amended such that L was escalated in a standard 3 × 3 design from 5 mg 21/28 consecutive days to a maximum of 20 mg, up to six monthly cycles, followed by 6 months of L at the same dose. Response was assessed every 3 months by physical exam and CT. At the highest dose of BL, rituximab 375 mg/m^2 d1 of each cycle was added to pts with B-cell NHL (level 4R).
Results: Twenty pts were accrued: 12M/8F, median age 65.5 years, median two prior therapies (1–7), histologies: diffuse large B-cell lymphoma 11, transformed 1, mycosis fungoides 1, Waldenstrom's 1, marginal zone 3, HL 2, mantle cell 1. Median cycles received −5.2 pts not evaluable for toxicity died soon after cycle 1. DLT was based on first cycle toxicities: gr 3/4 neutropenia 3/1, gr 3 anaemia −3, gr 3 rash −1, gr 3 nausea −1, gr 3 fatigue −1. Over all cycles, gr 3–4 toxicities included neutropenia gr 3–8, gr 4–9, anaemia gr 3–3, gr 4–1, thrombocytopenia gr 3–2, rash gr 3–3, nausea gr 3–1. L was discontinued in one pt after cycle 4 for profound fatigue. L doses were reduced in three pts, B in two, both in 1 by 1 dose level. Dose delays were levels 1–3, levels 2–0, levels 3–1, levels 4–3, and levels 4R–0. Treatment is ongoing in five patients. To date, 16 pts were evaluable for response:CR 4, PR 7, and PD 5.
Conclusions: BRL at the dose of 20 mg L, 21/28 d is tolerable and worth pursuing in phase II–III trials.

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PHASE I STUDY OF INOTUZUMAB OZOGAMICIN COMBINED WITH R-GDP FOR RELAPSED CD22+ B-CELL NON-HODGKIN LYMPHOMA

R. Sangha,[1] A. Davies,[2] N. H. Dang,[3] M. Ogura,[4] L. Paccagnella,[5] R. Ananthakrishnan,[6] E. Vandendries,[7] Y. Goh.[8]
[1]*Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada;* [2]*Cancer Sciences Division, Somers Cancer Research Building, University of Southamptom, Southampton, UK;* [3] *University of Florida, Gainesville, FL, USA;* [4]*Nagoya Daini Red Cross Hospital, Nagoya, Japan;* [5]*Pfizer, Groton, CT, USA;* [6]*Pfizer, Cambridge, MA, USA;* [7] *Pfizer, Cambridge, MA, USA;* [8] *Singapore General Hospital, Singapore.*

Introduction: CD22 is expressed on most B-cell non-Hodgkin lymphoma (B-NHL). Inotuzumab ozogamicin (INO), an anti-CD22 antibody linked to calicheamicin, has activity in refractory B-NHL. This study hypothesized that INO plus rituximab, gemcitabine, dexamethasone, and cisplatin (R-GDP) for relapsed CD22+ B-NHL was safe and tolerable.
Methods: Patients (pts) with relapsed CD22+ B-NHL treated with ≥1 prior R-chemo regimen were enrolled using an up-and-down independent dose-escalation schema for G and P. INO (0.8 mg/m^2 day 2) was combined with R-GDP (R 375 mg/m^2, G, and P day 1; oral D 40 mg days 1–4) on a 21-day cycle for up to six cycles. Dose-limiting toxicity (DLT) included febrile neutropenia, grade (Gr) 4 ANC lasting ≥7 days, Gr 4 platelets ≥7 days, Gr ≥3 platelets with bleeding and transfusion support, Gr ≥3 QTc prolongation, Gr 4 AST/ALT, Gr 2 bilirubin ≥7 days, G-CSF during cycle 1, Gr ≥3 clinically significant or drug-related nonhematologic toxicity ≥7 days, and Gr ≥2 drug-related nonhematologic toxicity, causing dose delay of ≥7 days.
Results: Thirty-seven pts were treated: 15 DLBCL, 11 FL, 7 MCL, 1 MZL, 1 SLL, and 2 indolent B-NHL. Characteristics: aged 33 to 81 years (median 65 years); 34 with ECOG PS ≤1; median of two prior chemo regimens (range 1–6); five refractory to prior therapy. No DLTs were observed at the starting dose of G 500 mg/m^2, P 37.5 mg/m^2 (n = 6); two DLTs (febrile neutropenia, Gr 2 platelets) at G 1000 mg/m^2, P 37.5 mg/m^2 (n = 3); no DLTs at G 1000 mg/m^2, P 0 mg/m^2 (n = 6); two DLTs (febrile neutropenia; Gr 4 ANC ≥7 days) at G 500 mg/m^2, P 50 mg/m^2 (n = 8); and two DLTs (Gr 3 hypokalemia, Gr 4 neutropenic sepsis) at G 500 mg/m^2, P 75 mg/m^2 (n = 4). In a maximum tolerated dose (MTD) confirmation cohort of 10 additional pts, three pts had DLTs (two with Gr 4 platelets, one with febrile neutropenia); thus, MTD was determined to be INO 0.8 mg/m^2, R 375 mg/m^2, G 500 mg/m^2, D 40 mg, and P 50 mg/m^2. Gr ≥3 adverse events included thrombocytopenia (68%), neutropenia (54%), lymphopenia (32%), anaemia (27%), leukopenia (24%), hypokalemia (22%), fatigue (11%), and febrile neutropenia (11%). Median treatment cycle was 4 (range 1–6). There were eight complete and nine partial responses.
Conclusions: INO 0.8 mg/m^2 with R-GDP is tolerable at reduced doses of G (500 mg/m^2) and P (50 mg/m^2). Preliminary efficacy is being explored in the ongoing MTD expansion cohort.

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EFFICACY AND SAFETY OF IBRUTINIB IN COMBINATION WITH RITUXIMAB IN HIGH-RISK CLL PATIENTS

J. A. Burger,[1] M. J. Keating,[1] W. G. Wierda,[1] J. Hoellenriegel,[1] A. Ferrajoli,[1] S. Faderl,[1] S. Lerner,[1] G. Zacharian,[1] X. Huang,[2] D. F. James,[3] J. J. Buggy,[3] H. Kantarjian,[1] S. M. O'Brien.[1]
[1]*Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, USA;* [2]*Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, USA;* [3]*Pharmacyclics, Inc., Sunnyvale, USA.*

Background: The Bruton tyrosine kinase (BTK) inhibitor ibrutinib targets BCR signalling and induces remissions in a majority of chronic lymphocytic leukaemia (CLL) patients, even in high-risk CLL. To further improve and accelerate responses to ibrutinib, we combined ibrutinib with rituximab (iR) in a single-centre trial, which accrued 40 patients between February and July 2012.
Methods: Patients received ibrutinib 420 mg PO continuously daily until progressive disease (PD) with rituximab 375 mg/m^2 weekly × 4 (cycle 1), then D1 monthly until cycle 6. Study inclusion required high-risk CLL (del17p or TP53 mutation, or short PFS <36 months after frontline chemo-immunotherapy, or relapsed CLL with del11q).
Results: Patients had a median of two prior therapies, a median Rai stage of 4, 20 patients had del17p or TP53 mutation, and 13 patients had del11q. At a median follow-up of 8 months, 37 of 40 patients continue on therapy without disease progression. Four patients achieved a CR, and 30 patients a PR, accounting for an overall response rate of 85%. In addition, we noted two PRs with persistent lymphocytosis, and three SD. Treatment was well tolerated, with only six cases of grade 3/4 toxicities, which were mainly unrelated. Grade 1/2 toxicities were mostly self-limited, such as diarrhoea, myalgias/bone pains, fatigue, upper respiratory infections, bruising, and hot flashes. Two patients died from unrelated infections, and one patient relapsed after initially responding. Biomarker assessment demonstrated rapid normalization of CCL3 and CCL4 chemokine levels, complete BTK occupancy, and inhibition of BCR and chemokine receptor responsiveness.
Conclusion: iR is a safe, well-tolerated regimen, which induces an excellent response rate in high-risk CLL. The addition of rituximab shortens the initial re-distribution

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lymphocytosis, and consequently remissions are achieved faster. These encouraging data emphasize the need for rapid further development of ibrutinib for patients with high-risk CLL, given that the alternative treatment options are disappointing.

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UPDATED INTERIM RESULTS OF AN INTERNATIONAL, MULTICENTRE, PHASE 2 STUDY OF IBRUTINIB (PCI-32765) IN RELAPSED OR REFRACTORY MANTLE CELL LYMPHOMA

M. L. Wang,[1] S. Rule,[2] P. Martin,[3] R. L. Auer,[4] B. S. Kahl,[5] W. Jurczak,[6] R. H. Advani,[7] J. Romaguera,[1] M. E. Williams,[8] J. Barrientos,[9] E. Chmielowska,[10] J. Radford,[11] S. Stilgenbauer,[12] J. M. McGreivy,[13] F. Clow,[14] D. M. Beaupre,[15] L. A. Kunkel,[16] A. Goy,[17] K. A. Blum.[18]

[1]Department of Lymphoma/Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Department of Haematology, Derriford Hospital, Plymouth, UK; [3]Division of Hematology-Oncology, Weill Cornell Medical College, New York, NY, USA; [4]Department Haemato-Oncology, Barts Health NHS Trust, London, UK; [5]Department of Medicine-Hematology/Oncology, University of Wisconsin, Madison, WI, USA; [6]Department of Haematology, Jagiellonian University, Krakow, Poland; [7]Department of Medicine, Division of Oncology, Stanford University Medical Center, Stanford, CA, USA; [8]Hematology-Oncology, University of Virginia School of Medicine, Charlottesville, VA, USA; [9]CLL Research and Treatment Program, Division of Hematology and Medical Oncology, Department of Medicine, Hofstra North Shore-LIJ, Hyde Park, NY, USA; [10]Centrum Onkologii, Oddzial Kliniczny Onkologii, Bydgoszcz, Poland; [11]Institute of Cancer Sciences, The University of Manchester and the Christie NHS Foundation Trust, Manchester, UK; [12]Klinik fur Innere Medizin II, Universitatsklinikum Ulm, Ulm, Germany; [13]Clinical Science, Pharmacyclics, Inc., Sunnyvale, CA, USA; [14]Biostatistics and Programming, Pharmacyclics, Inc., Sunnyvale, CA, USA; [15]Clinical Science, Pharmacyclics, Inc., Sunnyvale, CA, USA; [16]Chief Medical Officer, Pharmacyclics, Inc., Sunnyvale, CA, USA; [17]John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, NJ, USA; [18]Division of Hematology, The Ohio State University, Columbus, OH, USA.

Introduction: Bruton's tyrosine kinase (BTK) is a central mediator of BCR signalling essential for B-cell development. Ibrutinib is an oral BTK inhibitor that induces apoptosis and inhibits migration and adhesion of malignant B-cells. Interim results demonstrated ibrutinib induced rapid nodal responses in relapsed or refractory (R/R) mantle cell lymphoma (MCL) (Wang, ASH 2012).
Methods: R/R MCL patients (pts) who were either bortezomib-naïve (BN) or bortezomib-exposed (BE) were enrolled. Ibrutinib 560 mg PO QD was administered continuously until PD. Tumour response was assessed every two cycles. Primary endpoint: overall response rate (ORR). Secondary endpoints: duration of response (DOR), progression-free survival, overall survival, and safety. The study enrolled 115 pts (65 BN, 50 BE); 111 were treated; 110 were evaluable for response. Baseline characteristics included the following: median age 68 years, time since diagnosis 42 months, # of prior therapies 3; bulky disease 13%, prior SCT 10%, and high risk MIPI 49%.
Results: Safety data are reported for 111 pts. Treatment-emergent adverse events (TEAEs) in ≥15% of pts included the following: diarrhoea (40%), fatigue (36%), URI (23%), nausea (23%), rash (19%), dyspnea (21%), and peripheral edema (19%). Grade 3 AEs ≥5% were neutropenia (13%), anaemia (8%), thrombocytopenia (7%), and 5% each of abdominal pain, diarrhoea, dyspnea, and pneumonia. Grade 4 treatment-related AEs included neutropenia (5%), hyperuricaemia (2%), and 1% each pancytopenia, thrombocytopenia, and sepsis. One Grade 5 pneumonia was reported as treatment related.
Median time on study was 9.2 months; 47% of pts remain on therapy. Median PFS was 13.9 months; DOR not yet reached.

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Evaluable	BN ($n=63$)	BE ($n=47$)	Total ($n=110$)
ORR (%)	65	72	68
CR (%)	21	23	22
PR (%)	44	49	46

Responses increased with longer treatment. Median time to PR was 1.9 months and to CR was 5.5 months. With longer follow-up on the subset of 51 pts reported at ASH 2011 (median time on study then was 3.7 months, now 14.7 months), the CR rate increased from 16% to 39%, and the ORR increased from 69%to 75%.

Conclusions: Updated results of this phase 2 study of single agent ibrutinib will be presented. Longer follow-up demonstrates the durability of responses and confirms the unprecedented single agent activity of ibrutinib in R/R MCL. The TEAEs were consistent with safety data previously reported. Pivotal studies in R/R MCL have been initiated.

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PRELIMINARY SAFETY AND EFFICACY OF IPI-145, A POTENT INHIBITOR OF PHOSPHOINOSITIDE-3-KINASE-δ,-γ, IN PATIENTS WITH RELAPSED/REFRACTORY T-cell LYMPHOMA

S. Horwitz,[1] A. Younes,[2] I. W. Flinn,[3] M. Patel,[4] B. Kahl,[5] Y. Oki,[6] J. Sweeney,[7] K. Allen,[7] J. Dunbar,[7] P. Kelly,[7] F. M. Foss.[8]

[1]Department of Medicine, Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York, USA; [2]Department of Medicine, Chief, Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York, USA; [3]Director, Hematologic Malignancies Research Program, Sarah Cannon Research Institute, Nashville, USA; [4]Florida Cancer Specialists, Sarah Cannon Research Institute, Sarasota, USA; [5]Department of Medicine, University of Wisconsin, Madison, USA; [6]Department of Lymphoma/Myeloma, MD Anderson Cancer Center, Houston, USA; [7]Clinical, Infinity Pharmaceuticals, Cambridge, USA; [8]Yale Medical Oncology, Yale University Cancer Center, New Haven, USA.

Background: PI3-kinases are pivotal in cell signalling and regulate multiple cellular functions relevant to oncogenesis. IPI-145, a potent oral inhibitor of the PI3K-δ and PI3K-γ isoforms, is being developed in patients (pts) with haematologic malignancies. Early phase 1 results in pts with T-cell lymphoma (TCL) are presented here.
Methods: This dose-escalation (DE) study evaluates the safety, maximum tolerated dose (MTD), clinical activity, and pharmacokinetics (PK)/pharmacodynamics (PD) of IPI-145. PD assessment included chemokine/cytokine profiling. IPI-145 is given orally twice daily (BID) in 28-day cycles. Tumour response is based on standard disease-specific criteria. Expansion cohorts (EC) at ≤ the MTD are enrolling.
Results: Overall, 65 pts have been dosed with IPI-145, including 10 TCL pts enrolled in DE [$n=1$, 25 mg; $n=1$, 50 mg; $n=4$, 60 mg; $n=2$, 75 mg; and $n=2$, 100 mg (all BID)]. The MTD was declared at 75 mg BID after two pts dosed at 100 mg BID experienced a dose-limiting toxicity in cycle 1 (Grade 3 rash, Grade 3 ALT elevation). Available PK data from the DE cohort at the MTD showed a C_{max} of 3225 ng/mL and an AUC_{0-8} of 12 938 ng h/mL, sufficient to inhibit both the PI3K-δ and PI3K-γ enzymes. In TCL pts, the median (range) number of cycles was 1.7 (1–7), and 30% remain on study. Treatment-related adverse events (TRAEs) occurred in five (50%) TCL pts. The most common ≥ Grade 3 TRAEs were increased ALT (30%), rash and pneumonia (20% each). One Grade 4 pneumonitis occurred in a pt dosed at 60 mg BID who later died after declining supportive therapy. Among the eight evaluable pts with TCL, responses included one CR (enteropathy-associated TCL), two PR (one Sézary syndrome and one subcutaneous panniculitis-like TCL), one SD and four PD. All three responses occurred within 3 months of first dose (≥60 mg BID).
Conclusions: IPI-145 appears well tolerated, and at doses ≥60 mg BID, it has shown clinical activity in pts with relapsed/refractory advanced TCL. The MTD has been determined at 75 mg BID, and an EC at this dose level is enrolling both cutaneous and noncutaneous TCL pts. Updated safety, efficacy and PK/PD data from the TCL pts in DE and in the 75 mg BID EC will be presented.

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FINAL REPORT OF A PHASE I STUDY OF IDELALISIB, A SELECTIVE INHIBITOR OF PHOSPHATIDYLINOSITOL 3-KINASE P110δ, IN PATIENTS WITH RELAPSED OR REFRACTORY MANTLE CELL LYMPHOMA

J. P. Leonard,[1] B. S. Kahl,[2] R. R. Furman,[1] I. W. Flinn,[3] N. D. Wagner-Johnston,[4] J. R. Brown,[5] D. M. Benson,[6] J. C. Byrd,[6] S. E. Spurgeon,[7] S. Peterman,[8] D. Johnson,[8] J. Gu,[8] R. D. Dansey,[8] W. R. Godfrey,[8] S. E. Coutre.[9]

[1]Lymphoma/Myeloma Service, Weill Cornell Medical College, New York, USA; [2]Hematology Clinic, University of Wisconsin Carbone Cancer Center, Madison, WI, USA; [3]Hematologic Malignancies Research Program, Sarah Cannon Research Institute, Nashville, TN, USA; [4]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA; [5]Chronic Lymphocytic Leukemia Center, Dana-Farber Cancer Institute, Boston, USA; [6]Division of Hematology, The Ohio State University, Columbus, OH, USA; [7]Center for Hematologic Malignancies,

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Oregon Health & Science University, Portland, OR, USA; [8]Clinical Development, Oncology, Gilead Sciences, Seattle, USA; [9]Hematology Clinic, Stanford Cancer Institute, Stanford, CA, USA.

Background: Phosphatidylinositol 3-kinase p110δ (PI3Kδ) signalling is critical for activation, proliferation and survival of B cells and plays a role in homing and retention in lymphoid tissues. PI3Kδ signalling is hyperactive in many B-cell malignancies. Idelalisib is a first-in-class, selective, oral inhibitor of PI3Kδ. Initial response rate of 42% was previously reported in MCL (Kahl, ICML 2011). Long-term follow-up is now presented.
Methods: This phase 1 study evaluated the activity of continuous (48 weeks) idelalisib monotherapy treatment in patients (pts) with relapsed or refractory haematologic malignancies. Doses ranged from 50 to 350 mg BID in eight cohorts. Response was based on investigator assessments using standard criteria (Cheson et al, 2007). Pts who continued to benefit were able to enrol in an extension study.
Results: Forty pts with recurrent MCL enrolled. Pts were 88% male, median age (range) of 69 (52–83) years, 43% with refractory disease. The median (range) number of prior therapies was 4 (1–14). The median (range) duration of idelalisib treatment was 3.5 (1–26+) months, with six (15%) pts continuing on treatment in the extension protocol. Overall response rate (ORR) was 16/40 (40%), with 2/40 CR (5%). The median duration of response (mDOR) was 2.7 months, and median PFS (mPFS) was 3.7 months. The 1-year PFS was 22%. For pts dosed with ≥100 mg BID, ORR was 12/23 (52%); for pts dosed with ≥150 mg BID, ORR was 11/16 (69%) including both CR (12.5%). Most common adverse events included (total%/≥G3%) diarrhoea (40/18), nausea (33/5), pyrexia (28/0), fatigue (25/3), rash (25/3), decreased appetite (20/15), URI (20/0), and pneumonia (13/13). Abnormal lab values included (total%/≥G3%) ALT/AST elevations (65/20). Six of 40 (15%) pts discontinued therapy due to AEs, potentially treatment related.
Conclusions: The oral PI3Kδ inhibitor idelalisib (GS-1101) is active and well tolerated in heavily pre-treated pts with MCL. A proportion of pts have long-term (>1 year) clinical benefit. These data support further clinical evaluation of idelalisib in MCL. Clinical trials with idelalisib in combination with other agents are in progress.

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FINAL REPORT OF A PHASE I STUDY OF IDELALISIB, A SELECTIVE INHIBITOR OF PI3Kδ, IN PATIENTS WITH RELAPSED OR REFRACTORY INDOLENT NON-HODGKIN LYMPHOMA

B. S. Kahl,[1] R. R. Furman,[2] I. W. Flinn,[3] J. R. Brown,[4] N. D. Wagner-Johnston,[5] S. E. Coutre,[6] J. C. Byrd,[1] D. M. Benson,[7] S. E. Spurgeon,[8] S. Peterman,[9] D. Johnson,[9] Y. Cho,[9] R. D. Dansey,[9] W. R. Godfrey,[9] J. P. Leonard.[2]
[1]Hematology Clinic, University of Wisconsin Carbone Cancer Center, Madison, WI, USA; [2]Lymphoma/Myeloma Service, Weill Cornell Medical College, New York, USA; [3]Hematologic Malignancies Research Program, Sarah Cannon Research Institute, Nashville, TN, USA; [4]Chronic Lymphocytic Leukemia Center, Dana-Farber Cancer Institute, Boston, USA; [5]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA; [6]Hematology Clinic, Stanford Cancer Institute, Stanford, CA, USA; [7]Division of Hematology, The Ohio State University, Columbus, OH, USA; [8]Center for Hematologic Malignancies, Oregon Health & Science University, Portland, OR, USA; [9]Clinical Development, Oncology, Gilead Sciences, Seattle, USA.

Background: PI3K-delta signalling is critical for activation, proliferation and survival of B cells, plays a role in homing and retention in lymphoid tissues, and is hyperactive in many B-cell malignancies. Idelalisib (GS-1101) is a first-in-class, selective, oral inhibitor of PI3Kδ. Initial response rate of 38% was reported previously in indolent non-Hodgkin lymphoma (iNHL) (Kahl, ICML 2011). Long-term follow-up is now presented.
Methods: This phase 1 study evaluated the activity of continuous idelalisib monotherapy treatment in patients (pts) with relapsed haematologic malignancies. Doses ranged from 50 to 350 mg QD or BID in eight cohorts. Response was evaluated on the basis of investigator assessments using standard criteria (Cheson, 2007). Pts who continued to benefit were able to enrol in an ongoing extension study.
Results: Study enrolled 64 pts with indolent iNHL. iNHL subtypes included 38 FL, 11 SLL, 9 LPL/WM, and 6 MZL. Pts were 69% male, median age (range) of 64 (32–91) years, 58% with refractory disease and 53% with bulky disease (LN diameter ≥5 cm). The median (range) number of prior therapies was 4 (1–10). The median (range) duration of treatment was 3.8 (0–41) months, with 19 (30%) pts continuing on treatment extension protocol. Overall response rate (ORR) across all cohorts were 31/64 (48%), with one CR (1.6%). The median duration of response (mDOR) was 18.4 months, and median PFS (mPFS) was 7.6 months. For pts dosed with ≥100 mg BID ($N = 36$), the ORR was 24/36 (67%), the mDOR was 15.4 months, and the mPFS was 16.6 months. The ORR for iNHL subtypes was FL (45%), SLL (64%), LPL/WM (56%), and MZL (33%). Adverse events included (total%/≥G3%) diarrhoea (36/8), fatigue (36/3), rash (27/3), nausea (25/2), pyrexia (20/3), chills (20/0), cough (19/2), pneumonia (17/16), and URI (17/0). Lab abnormalities included (total%/≥G3%) ALT/AST elevations (56/25). Eight of 64 (12.5%) pts discontinued therapy because of potentially treatment-related adverse events.
Conclusions: The oral PI3Kδ inhibitor idelalisib is active in heavily pretreated pts with iNHL, can produce durable responses, and has a favourable safety profile. These data support further clinical development; phase 2 and 3 trials in iNHL are ongoing (NCT01732913, NCT01732926).

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FINAL REPORT OF A PHASE I STUDY OF IDELALISIB (GS-1101) A SELECTIVE INHIBITOR OF PI3Kδ, IN PATIENTS WITH RELAPSED OR REFRACTORY CLL

I. W. Flinn,[1] J. R. Brown,[2] J. C. Byrd,[3] S. E. Coutre,[4] N. D. Wagner-Johnston,[5] B. S. Kahl,[6] S. E. Spurgeon,[7] D. M. Benson,[3] S. Peterman,[8] D. Johnson,[8] D. Li,[8] R. D. Dansey,[8] T. M. Jahn,[8] R. R. Furman.[9]
[1]Hematologic Malignancies Research Program, Sarah Cannon Research Institute, Nashville, TN, USA; [2]Chronic Lymphocytic Leukemia Center, Dana-Farber Cancer Institute, Boston, USA; [3]Division of Hematology, The Ohio State University, Columbus, OH, USA; [4]Hematology Clinic, Stanford Cancer Institute, Stanford, CA, USA; [5]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA; [6]Hematology Clinic, University of Wisconsin Carbone Cancer Center, Madison, WI, USA; [7]Center for Hematologic Malignancies, Oregon Health & Science University, Portland, OR, USA; [8]Clinical Development, Oncology, Gilead Sciences, Seattle, USA; [9]Lymphoma/Myeloma Service, Weill Cornell Medical College, New York, USA.

Background: Signals through PI3K-delta regulate activation, proliferation and survival of B cells, critically influence homing and retention of B cells in lymphoid tissues, and are hyperactive in many B-cell malignancies. Idelalisib (GS-1101) is a first-in-class, selective, oral inhibitor of PI3Kδ that reduces proliferation, enhances apoptosis, and inhibits homing and retention of malignant B cells.
Methods: Patients (pts) with relapsed/refractory CLL were treated continuously with single-agent oral idelalisib from 50–350 mg/dose (QD or BID). Response evaluated by investigators per Hallek (2008) and Cheson (2012).
Results: Fifty-four pts (9F/45M) median (range) age 63 (37–82) years enrolled with bulky lymphadenopathy (80%), refractory disease (70%), extensive prior therapies (median: 5, range: 2–14), unmutated IgHV (91%), del17p and/or TP53 mutation (24%), del11q (28%), and NOTCH1 mutation (17%). The median (range) exposure was 9 (0–41) months. Twenty-five (46%) pts completed the primary study; 23 (43%) enrolled into an extension study. Overall response rate was 30/54 (56%, 2 CR, 28 PR). Of the 28 PR, 22 met Hallek (2008) and six met PR with lymphocytosis (Cheson, 2012). Forty-four of 54 (81%) showed a lymph node response (≥50% reduction in the nodal SPD). Twenty-one of 54 were SD and 3/54 NE. The median (range) time to first response was 1.9 (0.9–12.9) months. Median PFS was 17 months, and median duration of response was 18 months. Idelalisib treatment resulted in resolution of splenomegaly (14/20, 70%) and normalization of cytopenias: anaemia (17/25, 68%), thrombocytopenia (27/34 79%), and neutropenia (15/15, 100%). Most common AEs independent of causality (any Grade/≥Gr 3) included fatigue (31%/2%), diarrhoea (30%/6%), pyrexia (30%/4%), rash (22%/0%), upper respiratory tract infection (22%/0%), and pneumonia (20%/19%). Two per cent of pts had ≥Gr 3 ALT/AST elevation. Fifteen per cent of pts discontinued due to AEs; 7% potentially treatment-related. There were no dose-limiting toxicities.
Conclusions: Idelalisib shows substantial clinical activity and a favourable safety profile in heavily pretreated, refractory and high-risk pts with CLL. Phase 3 trials with idelalisib in combination with rituximab or bendamustine/rituximab are ongoing (NCT01539512, NCT01569295).

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UPDATE ON A PHASE 1 STUDY OF THE SELECTIVE PI3Kδ INHIBITOR, IDELALISIB (GS-1101) IN COMBINATION WITH RITUXIMAB, BENDAMUSTINE, OR BENDAMUSTINE/RITUXIMAB IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA

R. R. Furman,[1] S. E. Coutre,[2] I. W. Flinn,[3] J. C. Barrientos,[10] K. Rai,[10] S. de Vos,[4] M. T. Schreeder,[5] N. D. Wagner-Johnston,[6] T. E. Boyd,[7] N. H. Fowler,[8] J. P. Leonard,[1] L. Holes,[9] D. Johnson,[9] D. Li,[9] R. D. Dansey,[9] T. M. Jahn,[9] J. Sharman.[11]

[1]Lymphoma/Myeloma Service, Weill Cornell Medical College, New York, USA; [2]Hematology Clinic, Stanford Cancer Institute, Stanford, CA, USA; [3]Hematologic Malignancies Research, Sarah Cannon Research Institute, Nashville, TN, USA; [4]Department Hematology/Oncology, University of California, Los Angeles Medical Center, Los Angeles, USA; [5]Department of Medicine, Clearview Cancer Institute, Huntsville, AL, USA; [6]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA; [7]Department of Oncology, Yakima Valley Memorial Hospital/North Star Lodge Cancer Center, Yakima, WA, USA; [8]Department of Lymphoma/Myeloma, M. D. Anderson Cancer Center, Houston, USA; [9]Clinical Development, Oncology, Gilead Sciences, Seattle, USA; [10]Division of Hematology-Oncology, Hofstra North Shore-LIJ School of Medicine, Hyde Park, NY, USA; [11]Department of Medicine, Willamette Valley Cancer Institute/US Oncology Research, Springfield, OR, USA.

Introduction: PI3K-delta signalling is critical for proliferation and survival as well as homing and tissue retention of malignant B cells. Idelalisib is a first-in-class, selective, oral inhibitor of PI3Kδ that has shown considerable monotherapy activity in patients (pts) with heavily pretreated CLL.

Methods: Ph 1 study evaluated idelalisib continuously given at 150 mg BID in combination with rituximab (R) (375 mg/m^2 weekly × 8), bendamustine (B) (70 or 90 mg/m^2 × 2, every 4 weeks × 6), or bendamustine/rituximab (BR) for relapsed/refractory chronic lymphocytic leukaemia (CLL). Clinical response was evaluated according to published criteria.

Results: Fifty-one pts (22F/29M) with a median (range) age of 64 (41–87) years were enrolled. Adverse disease characteristics included bulky lymphadenopathy (63%), refractory disease (51%), multiple prior therapies (median 3, range: 1–9) with 98% receiving prior R and 45% receiving prior B. As of 21 Jan 2013, the median (range) treatment duration was 18 (1–33) months. Thirty-one (61%) pts enrolled into the extension study. Twenty-four pts are continuing idelalisib treatment on the extension study. The overall response rate was 84%, with one CR, and a median (range) time to response of 1.9 (1.5–4.6) months. The 2-year progression-free survival and overall survival were 65% and 85%, respectively. At 2 years of follow-up, 72% of responses were still enduring. There was no difference in outcomes for pts with <3 prior treatments ($n = 21$) vs ≥3 prior treatments ($n = 30$). The most common TEAEs (any Grade%/≥Gr 3%, independent of causality) included pyrexia (45/4), diarrhoea (41/14), cough (31/2), fatigue (29/2), and nausea (28/0). Pneumonia (any Gr%/≥Gr 3%) occurred in 16/12, and rash was seen in 16/0. Eight per cent of patients experienced ≥Gr 3 ALT/AST elevation based on lab values.

Conclusions: A lack of overlapping toxicities allowed idelalisib to be co-administered with R, B, or BR, and all three regimens were highly active, resulting in durable tumour control in pts with heavily pretreated relapsed/refractory CLL. Ph 3 trials evaluating the efficacy of idelalisib in combination with R or BR are ongoing (NCT01539512, NCT01569295).

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PHASE II MULTICENTRE STUDY OF LENALIDOMIDE IN HEAVILY PRETREATED PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA POST-BORTEZOMIB: MCL-001 'EMERGE' STUDY

A. Goy,[1] M. E. Williams,[2] R. Sinha,[3] S. K. Besisik,[4] J. Drach,[5] R. Ramchandren,[6] L. Zhang,[7] S. Cicero,[8] T. Fu,[9] T. Witzig.[10]

[1]Hematology/Oncology, John Theurer Cancer Center at HUMC, Hackensack, USA; [2]Hem/Oncology, University of Virginia Health System, Charlotesville, USA; [3]Hematology/Oncology, Emory University Winship Cancer Institute, Atlanta, USA; [4]Internal Medicine, Istanbul University Faculty of Medicine, Istanbul, Turkey; [5]Hematology, Medical University of Wien, Oncology, Wien, Austria; [6]Hematology, Karmanos Cancer Institute, Detroit, USA; [7]Clinical Development, Celgene Corporation, Summit, USA; [8]Clinical Science, Celgene Corporation, Summit, USA; [9]Biometrics and Data Operations, Celgene Corporation, Summit, USA; [10]Hematology, Mayo Clinic, Rochester, USA.

Introduction: Patients (pts) with relapsed/refractory MCL have limited treatment options, particularly when progressing after bortezomib. The immunomodulatory agent lenalidomide demonstrated clinical activity in previous smaller phase II trials in aggressive NHL including MCL. MCL-001 'EMERGE' was a large global study evaluating the efficacy and safety of lenalidomide in relapsed/refractory MCL post-bortezomib.

Methods: Phase II multicentre study of lenalidomide 25 mg/day PO days 1–21 every 28 days until PD or intolerability. Pts had to have received rituximab, cyclophosphamide, and anthracycline (or mitoxantrone) and be relapsed/progressed (<12 months) or refractory (≥2 cycles) to bortezomib. Primary endpoints were overall response rate (ORR) and duration of response (DOR) by independent central review.

Results: One hundred thirty-four relapsed/refractory MCL pts with heavily pretreated (no limit on prior therapies) and advanced stage disease (93% III–IV) were enrolled. Median age was 67 years, and median number of prior therapies was 4 (range, 2–10); 78% ≥3 prior chemotherapy regimens (55% refractory to last therapy). Average dose was 20 mg/day lenalidomide. Most common grade 3/4 AEs were 43% neutropenia, 28% thrombocytopenia, 11% anaemia, 8% pneumonia and 7% fatigue. By central review, ORR was 28% (7.5% CR/CRu) and median DOR 16.6 months (95% CI, 7.7–26.7). Responses were rapid with median TTR 2.2 months (3.7 months to CR), regardless of tumour load, bulky disease, number and type of prior therapies. Among responders, 17/37 pts (46%) were enrolled within 3 months of last anti-lymphoma therapy (to which they had progressed). In these heavily pretreated pts (median four prior therapies; range, 2–8), median TTR was 1.9 months and median DOR 7.7 months (95% CI, 5.7–NR). Overall median duration of lenalidomide versus patient's last therapy for 17 responders was 267 days (range, 77–995) vs 51 days (range, 1–587).

Conclusions: The MCL-001 study showed rapid and durable response to lenalidomide in heavily pretreated MCL pts (median four prior therapies). For the 17 responders, prolonged duration of lenalidomide treatment over last therapy (>five times longer) suggests clinical benefit in a population often resistant to chemotherapy in that setting.

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BCL-2 EXPRESSION AND CORRELATION WITH ACTIVITY IN A RANDOMIZED PHASE II STUDY OF NAVITOCLAX (ABT-263) IN COMBINATION WITH RITUXIMAB IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

E. Punnoose,[1] B. Zheng,[1] Y. Xiao,[1] H. Savage,[1] A. Do,[1] L. Belmont,[1] E. McKeegan,[2] D. Hurst.[1]

[1]Research and Early Development, Genentech, South San Francisco, USA; [2]Oncology Discovery, AbbVie, North Chicago, USA.

Introduction: Navitoclax or ABT-263 is a BH3-mimetic orally available inhibitor of antiapoptotic Bcl-2 family members with high affinity for Bcl-xL, Bcl-2 and Bcl-w. Bcl-2 is overexpressed in chronic lymphocytic leukaemia (CLL) and is associated with enhanced resistance to apoptosis. We evaluated Bcl-2 family expression in blood and bone marrow aspirates (BMA) of CLL patients enrolled in a phase II trial and correlated with biologic activity of treatment with navitoclax.

Methods: Baseline blood and BMA were collected from patients randomized into the three arms: rituximab (RTX) (Arm A), RTX plus navitoclax daily either for 12 weeks (Arm B) or until disease progression (Arm C). Bcl-2 family expression was measured in patient specimens by gating on CD19/CD5 positive CLL cells in a real-time flow-assisted cell sorting (FACS) assay at central lab or in isolated PBMCs by qRT-PCR analysis at Genentech. Statistical analysis compared baseline expression using median cut-off and best objective response in patients where both Bcl-2 family expression and response assessment were available ($n = 55$; Arm A: 24; Arm B: 15; Arm C: 16). Navitoclax containing Arms B and C were combined for the statistical analysis. The following analytes were evaluated: Bcl-2, Bcl-xL, Mcl-1, A1, Bim, Noxa and ratios of Bcl-2/Mcl-1 and Bcl-xL/Mcl-1.

Results: Bcl-2 was expressed in all patients evaluated with inter-patient dynamic range of fourfold. Both variable expression and intra-patient heterogeneity were observed for Bcl-xL, A1, Bim and Noxa; Mcl-1 was poorly expressed in most patients (<20% of tumour cells positive in 80% of patients). Within the Bcl-2 high group, addition of navitoclax was associated with significantly increased odds of achieving a favourable clinical response (PR or CR by iwCLL response criteria) compared with RTX alone (Bcl-2 high: odds ratio = 12.4, $p = 0.0054$ vs Bcl-2 low: odds ratio = 0.94, $p = 1$). Bcl-2 expression was highly correlated between blood and BMA specimens in the patient (R2: 0.85).

Conclusions: High Bcl-2 expression is a predictive marker of response to navitoclax in combination with RTX. Bcl-2 can be measured effectively by a FACS assay in CLL patient blood.

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WEEKLY MLN9708, AN INVESTIGATIONAL PROTEASOME INHIBITOR, IN PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMA: PHASE 1 DOSE-ESCALATION STUDY

P. Martin,[1] J. Chang,[2] B. D. Cheson,[3] R. Rifkin,[4] S. Hamburg,[5] R. Reyes,[6] A. Hui,[7] J. Yu,[7] N. Gupta,[7] A. Di Bacco,[7] Y. Shou,[7] S. E. Assouline.[8]
[1]Medicine, Weill Cornell Medical College, New York, USA; [2]Hematology, University of Wisconsin Carbone Cancer Center, Madison, USA; [3]Lombardi Comprehensive Cancer Center, Georgetown University Hospital, Washington, DC, USA; [4]Medicine, Rocky Mountain Cancer Center, Denver, USA; [5]Medicine, Tower Cancer Research Foundation, Beverly Hills, USA; [6]Medicine, University of Kansas Medical Center, Kansas City, USA; [7]Millennium: The Takeda Oncology Company, Cambridge, USA; [8]Medicine, Jewish General Hospital, McGill University, Montreal, Canada.

Introduction: MLN9708 (ixazomib citrate), an investigational, reversible proteasome inhibitor, has shown preclinical activity in lymphoma models (Kupperman, Cancer Res 2010; Lee, Clin Cancer Res 2011). Intravenous (IV) and oral formulations are in clinical development. This study is assessing the safety, maximum tolerated dose (MTD), pharmacokinetics, and pharmacodynamics of IV MLN9708 in rel/ref lymphoma.
Methods: Adults with measurable rel/ref lymphoma after ≥2 chemotherapies received IV MLN9708 on days 1, 8, and 15 of 28-day cycles. Dose doubling and dose escalation proceeded based on cycle 1 dose-limiting toxicities (DLT). Blood samples for PK/PD analysis were collected pre-dose and post-dose on days 1 and 15 of cycle 1 and day 1 of cycle 2. Responses were assessed by IWG 2007 criteria. Adverse events (AEs) were graded by NCI-CTCAE v3.0.
Results: At data cut-off (26.10.2012), 30 patients (pts) had received MLN9708 in eight dose levels (0.125–3.11 mg/m^2); median age 57 years (range 23–78), median five prior therapies (range 1–9); 63% male, 43% Ann Arbor stage III–IV disease. Histologies included 10 follicular lymphoma (FL), 5 diffuse large B-cell lymphoma, 4 peripheral T-cell lymphoma (PTCL), and 3 Hodgkin's lymphoma. Pts received a median of two treatment cycles (range 1–34). Four pts treated at 1.76–3.11 mg/m^2 had DLT. Ten pts were treated at the MTD of 2.34 mg/m^2. The MLN9708 safety profile is given below. Four pts had grade 1/2 drug-related peripheral neuropathy (PN); no grade ≥3 PN was seen. One pt died on-study (treatment unrelated). MLN9708 plasma exposure increased dose-proportionally from 0.5 to 3.11 mg/m^2, and terminal half-life was 3.8–10.4 days after multiple dosing. A dose-dependent increase in maximal whole blood 20S proteasome inhibition was seen. Responses included one complete response and two partial responses (PR) in FL, and one PR in PTCL; at data cut-off, duration of therapy in the four responders was 6, 13, 27, and 33 months, respectively.
Conclusions: These data suggest that weekly IV MLN9708 was generally well tolerated and may be clinically active in rel/ref lymphoma, with responses in FL and infrequent PN. Final data will be presented at the meeting.

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Drug-related AE, %	N = 30
Any	90
Grade ≥3	43
Neutropenia	13
Diarrhoea	10
Thrombocytopenia	10
Lymphopenia	7
Renal failure	7
Serious	10
Discontinuation due to AE, %	10

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FINAL RESULTS FROM A PHASE 1 STUDY OF BLINATUMOMAB IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN's LYMPHOMA

M. E. Goebeler,[1] A. Viardot,[2] P. Kufer,[3] M. S. Topp,[1] S. Knop,[1] A. Mackensen,[4] L. Kanz,[5] G. Hess,[6] M. Wilhelm,[7] M. Libicher,[8] D. Nagorsen,[9] G. Zugmaier,[10] H. Einsele,[1] B. Dorsch,[11] P. A. Baeuerle,[12] R. C. Bargou.[1]
[1]Comprehensive Cancer Center Mainfranken (CCCMF), Department of Internal Medicine II, University of Wurzburg, Wurzburg, Germany; [2]Department of Internal Medicine III, University Ulm, Ulm, Germany; [3]Translational Science, Amgen Research (Munich) GmbH, Munich, Germany; [4]Department of Internal Medicine V, University of Erlangen Medical Center, Erlangen, Germany; [5]Department of Medicine II, University of Tubingen Medical Center, Tubingen, Germany; [6]Department of Internal Medicine III, Johannes Gutenberg University Mainz, Mainz, Germany; [7]Department of Internal Medicine V, Klinikum Nurnberg, Nurnberg, Germany; [8]Department of Radiology, Diakoniekrankenhaus Schwabisch-Hall, Schwabisch-Hall, Germany; [9]Hematology and Oncology, Amgen Inc., Thousand Oaks, USA; [10]Hematology and Oncology, Amgen Research (Munich) GmbH, Munich, Germany; [11]Biostatistics, Metronomia Clinical Research GmbH, Munich, Germany; [12]Translational Science, Amgen Research (Munich) GmbH, Munich, Germany.

Introduction: Blinatumomab, an investigational T cell-engagingsingle-chain antibody construct targeting CD19, has shown antitumour activity in relapsed non-Hodgkin's lymphoma (NHL) and acute lymphoblastic leukaemia. We report the final results of the phase 1 study of blinatumomab in patients with relapsed/refractory B-cell NHL.
Methods: This dose escalation study enrolled heavily pretreated patients. Blinatumomab was administered as 4- or 8-week continuous IV infusions at seven different dose levels (0.5 to 90 μg/m^2/day). Endpoints inuded frequency of adverse events (AEs), pharmacokinetics, and overall response rate (ORR) by Cheson criteria.
Results: A total of 76 patients were enrolled and received blinatumomab (primary refractory, $n = 48$). Of those, 42 patients were treated in the study's formal dose escalation phase. The most relevant dose-limiting toxicities were central nervous system (CNS) AEs.The maximum-tolerated dose (MTD) was established at 60 μg/m^2/days. Thirty-four additional patients were treated in the study's extension phase that explored additional efficacy and strategies for the mitigation of CNS AEs, including stepwise blinatumomab dose escalation to 60 μg/m^2/day plus early dexamethasone prophylaxis. Over the course of the study, the overall patient incidence of CNS AEs regardless of causality was 71% (grade 3, 22%; no grade 4 or 5). The most common AEs during the study were pyrexia, fatigue, headache, weight increase, and weight decrease. In all 76 patients, the overall incidence of grade 3, 4, and 5 AEs regardless of causality was 90%, 66%, and 4%, respectively. At the dose of 60 μg/m^2/day ($n = 35$), the ORR (dose escalation and extension phase) was 69% across all NHL subtypes, with a median response duration of 404 days; the complete response rate was 37%. At the same dose, the ORR by NHL subtype was 80% (12/15), 71% (5/7), and 55% (6/11) among patients with follicular, mantle cell, and diffuse large B-cell lymphoma, respectively.
Conclusions: In this population of heavily pretreated patients with relapsed/refractory NHL treatment with blinatumomab at the MTD of 60 μg/m^2/day was feasible and showed antilymphoma activity across NHL subtypes.

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PROGRESSION-FREE SURVIVAL ANALYSES OF TWO PIVOTAL PHASE 2 STUDIES OF BRENTUXIMAB VEDOTIN IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA OR SYSTEMIC ANAPLASTIC LARGE-CELL LYMPHOMA

J. Radford,[1] A. Younes,[2] B. Pro,[3] A. Chi,[4] E. Westin,[5] D. Huebner,[5] A. Engert.[6]
[1]Department of Medical Oncology, The University of Manchester and the Christie NHS Foundation Trust, Manchester, UK; [2]Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, USA; [3]Medical Oncology, Thomas Jefferson University and Hospitals, Kimmel Cancer Center, Philadelphia, USA; [4]Biostatistics, Millennium: The Takeda Oncology Company, Cambridge, USA; [5]Oncology Clinical Research, Millennium: The Takeda Oncology Company, Cambridge, USA; [6]Department of Internal Medicine I, University Hospital of Cologne, Cologne, Germany.

Introduction: Brentuximab vedotin (ADCETRIS®) is a CD30-targeted antibody-drug conjugate. Notable clinical activity in terms of overall response rate and complete remission rate, and a manageable safety profile were demonstrated in two pivotal phase 2 trials: in relapsed/refractory (RR) Hodgkin lymphoma (HL) following autologous stem cell transplant (SGN35-003) and RR systemic anaplastic large-cell lymphoma (sALCL, SGN35-004; studies funded by Seattle Genetics, Inc. and Millennium: The Takeda Oncology Company). We report on a post-hoc analysis comparing progression-free survival (PFS) achieved with brentuximab vedotin versus PFS achieved with the last prior therapy for each of these patient populations.
Methods: For both studies, PFS as assessed by the investigator was determined with brentuximab vedotin and with the last prior systemic therapy for each patient in the intent-to-treat population as of the 02 April 2012 data cut.
Results: SGN35-003 enrolled 102 patients, median age 31 years (range, 15–77); median 3.5 prior therapies (range, 1–13). At the data cut (median

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follow-up 27 months), 62% of patients achieved longer durations of PFS with brentuximab vedotin than with their most recent prior therapy. SGN35-004 enrolled 58 patients, median age 52 years (range, 14–76); median 2 prior therapies (range, 1–6). At the data cut (median follow-up 22 months), 66% of patients achieved longer PFS with brentuximab vedotin than with their most recent prior therapy. Comparisons of the PFS achieved with brentuximab vedotin versus that achieved with last prior therapy will be presented.
Conclusion: In these heavily pretreated patients, treatment with brentuximab vedotin was associated with a longer duration of remission than that achieved with the last prior therapy in more than 60% of patients. Furthermore, breaking prior treatment paradigms, PFS with last prior therapy was not predictive of PFS with brentuximab vedotin, suggesting lack of cross-resistance to brentuximab vedotin therapy.

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OBJECTIVE RESPONSES IN RELAPSED OR REFRACTORY B-CELL LYMPHOMAS WITH SINGLE-AGENT BRENTUXIMAB VEDOTIN.

E. Jacobsen,[1] N. L. Bartlett,[2] S. M. Horwitz,[3] Y. Oki,[4] R. Advani,[5] A. R. Shustov,[6] A. Forero-Torres,[7] O. A. O'Connor,[8] T. Siddiqi,[9] L. E. Grove,[10] J. P. Sharman.[11]
[1]Department of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, USA; [2]Department of Medicine, Oncology Division, Washington University School of Medicine, St. Louis, USA; [3]Department of Medicine, Lymphoma Service, Memorial Sloan-Kettering Cancer Center, New York, USA; [4]Department of Lymphoma/Myeloma, University of Texas MD Anderson Cancer Center, Houston, USA; [5]Department of Medicine, Division of Oncology, Stanford University Medical Center, Stanford, USA; [6]Hematology Division, University of Washington Medical Center, Seattle, USA; [7]Division of Hematology/Clinical Oncology, Department of Medicine, University of Alabama at Birmingham, Birmingham, USA; [8]Department of Medicine, Center for Lymphoid Malignancies, Columbia University Medical Center/New York Presbyterian Hospital, New York, USA; [9]Hematology & Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, USA; [10]Clinical Affairs, Seattle Genetics, Inc., Bothell, USA; [11]Hematology Research, Willamette Valley Cancer Institute and Research Center/US Oncology Research, Eugene, USA.

Introduction: Brentuximab vedotin (ADCETRIS®) is a CD30-directed antibody-drug conjugate. A phase 2, open-label, single-arm study was initiated to evaluate efficacy and safety of brentuximab vedotin in relapsed or refractory CD30+NHL, including B-cell and mature T-/NK-cell neoplasms (NCT01421667). This subset analysis presents interim data for patients (pts) with CD30+B-cell neoplasms. Enrollment is ongoing.
Methods: Pts receive 1.8 mg/kg brentuximab vedotin every 3 weeks until disease progression or unacceptable toxicity. The primary endpoint is objective response rate (ORR) by Cheson 2007. A secondary endpoint is a correlation of CD30 expression with response.
Results: To date, 44 pts with B-cell lymphomas with variable CD30 expression (range 0–100%) have been enrolled. Median age was 59.5 years (range 16–83); 25 pts (57%) are male.Pts have received a median of two prior systemic therapies; 30 (68%) were refractory (including primary refractory or refractory to most recent prior therapy), and 14 (32%) were relapsed. Of note, the ORR for all DLBCL was 44% (see table), and median duration of response has not been reached (range 0.1 to 48.1). Related adverse events (AEs) (>25%) included neutropenia (36%) and fatigue (27%). AEs ≥Grade 3 in >2 pts each included neutropenia, anaemia, and hypokalemia.
Conclusions: In this interim analysis of pts with advanced B-cell lymphomas, an ORR of 44% was demonstrated in DLBCL; median duration has not been reached. Analysis of correlation of CD30 expression with response is ongoing. Safety data are consistent with the profile of brentuximab vedotin.

PEDIATRIC LYMPHOMA

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PROFILE OF PRETREATMENT PLASMA CYTOKINE LEVELS IN CHILDREN WITH ANAPLASTIC LARGE CELL LYMPHOMA

C. Damm-Welk, F. Knoerr, J. Lisfeld, S. Werner, M. Zimmermann, A. Reiter.
Department of Hematologgy and Oncology, University Children's Hospital and NHL-BFM Study Center, Giessen, Germany.

Introduction: Patients with ALK-positive anaplastic large cell lymphomas (ALCL) mount a humoural as well as a CD4 and CD8 cellular immune response against the tumour-antigen ALK. The strength of the immune response reflected by ALK-antibody titres before therapy inversely correlates with relapse risk. However, the underlying kind of T-cell response could not be analyzed so far. Pre-treatment serum cytokine levels and profiles may help in characterizing the underlying T-helper cell response, thereby possibly explaining differences in ALK-antibody titres.
Methods: Pre-treatment serum/plasma from 120 NPM-ALK positive ALCL patients included in the trials NHL-BFM95 and ALCL99 between 1996 and 2010 were analyzed. Twenty-five cytokines were measured using commercial flow cytometry-based multiplex arrays. The assays were also performed on plasma/serum from 15 of those patients in remission.
Results: Compared with remission controls, initial serum contained significantly higher levels of interleukins 6, 9, 10 and 17A, IFN-gamma, and TNF-alpha at diagnosis. The cytokine-profile was not indicative for the activation of a specific T-helper cell subset. Higher levels of IL-6, sIL-2R and IFN-gamma inversely correlated with lower anti-ALK antibody titres, as did low levels of IL-23. SIL-2R, IL-6, IL-10, MCP-1, HGF, sCD30 and IP-10 were significantly elevated in patients positive for minimal disseminated disease in blood or bone marrow. IL-6 and IL-10 levels were significantly associated with higher stages, B-symptoms, clinical risk factors and poor general condition. Elevated levels of IL-6, IL-10, IL-17A, IFN-gamma, TNF-alpha, MCP1 and MIG were associated with poorer 3-year event-free survival.
Conclusion: ALCL patients show a distinct serum cytokine profile at diagnosis. IL6, sIL-2R, IFN-gamma and IL-23 correlate with the strength of the anti-ALK specific humoural immune response.

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	Best clinical response, *n* (%)				ORR, *n* (%)
	CR	PR	SD	PD	
Disease diagnosis					
B-cell neoplasms (*n* = 42[a])	6 (14)	8 (19)	12 (29)	16 (38)	14 (33)
All DLBCL (*n* = 25)	5 (20)	6 (24)	3 (12)	11 (44)	11 (44)
DLBCL (*n* = 19)	3 (16)	6 (32)	3 (16)	7 (37)	9 (47)
EBV+ DLBCL of the elderly (*n* = 4)	2 (50)	–	–	2 (50)	2 (50)
Plasmablastic (*n* = 1)	–	–	–	1 (100)	–
T cell-rich B-cell (*n* = 1)	–	–	–	1 (100)	–
Other B-cell neoplasms (*n* = 17)	1 (6)	2 (12)	9 (53)	5 (29)	3 (18)
Grey zone[b] (*n* = 6)	–	2 (33)	3 (50)	1 (17)	2 (33)
PMBL[b] (*n* = 6)	–	–	4 (67)	2 (33)	–
Follicular (*n* = 3)	–	–	2 (67)	1 (33)	–
PTLD (*n* = 2)	1 (50)	–	–	1 (50)	1 (50)

DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; PMBL, primary mediastinal B-cell lymphoma; PTLD, post-transplantation lymphoproliferation disease.
[a]Efficacy-evaluable.
[b]In 'Other' due to provisional diagnosis status (grey zone) and differing treatment paradigms/outcomes (PMBL).

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ARE MONOCLONAL ANTIBODY NECESSARY IN THE TREATMENT CHILDREN's B-NON HODGKIN LYMPHOMA?

J. Konja, M. N. Anicic, B. Glavas.
Hematology and Oncology Department Childrens' Clinic, Hematologist Oncologist, Zagreb, Croatia.

Introduction: B-non-Hodgkin's lymphomas (B-NHL) are a group of highly aggressive malignant lymphoproliferative diseases that require rapid diagnosis and prompt therapy initiation. The aims of the study were to confirm excellent results recorded in the BFM-NHL trial in Croatia and to identify appropriate measures to prevent the life-threateningevents frequently accompanying the early phase of treatment.
Patients and Methods: During the 1990–2012 period, 41 children with B-NHL (23 male and 18 female) aged 2–16 (mean age 9.7) years were treated according to the NHL-BFM protocol. Therapy consisted of 5-day pretreatment (standard chemotherapy dosage) combined with two to six cycles of high-dose chemotherapy; in addition, 14 patients received monoclonal antibodies (Mabtera®) in 2006–2012. Patients were divided into three risk groups.
Results: Complete remission was achieved in all 41 (100%) patients; disease relapse and lethal outcome were recorded in four (9.75%) patients, that is, three patients on chemotherapy alone and one patient also administered monoclonal antibodies (meningeal relapse 2 months of treatment completion); in 37 (90.2%) patients, the first complete remission has been persisting to the present. Grade III and IV toxicity was mostly observed after the first and second cycles of chemotherapy. The level of toxicity associated with the first cycle of chemotherapy was considerably lower in patients administered monoclonal antibodies, but in nine with moderate hypogammaglobulinemia. In one patient, secondary tumour disease (AML) developed 4 years of treatment discontinuation.
Conclusion: Although referring to a relatively small number of patients, therapeutic results were very good and consistent with those reported from other European centres. However, many questions remain to be answered. For instance, should monoclonal antibodies be administered in all patients with B-NHL, or in the high risk group only, or during the first chemotherapy cycle only, or in patients with disease relapse only, or as maintenance therapy in patients with B-ALL/NHL?

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SURVEY OF TREATMENT RESULTS FOR PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA IN POLISH PEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP

E. Latos-Grazynska,[1] B. Kazanowska,[1] G. Wrobel,[1] D. Kulej,[1] H. Bubala,[2] E. Kamienska,[3] A. Koltan,[4] T. Luszczawska-Kutrzeba,[5] L. Maciejka-Kapuscinska,[6] L. Maciejka-Kapuscinska,[7] J. Maldyj,[8] A. Mizia-Malarz,[9] F. Pierlejewski,[10] K. Roslan,[11] B. Sopylo,[12] J. Stefaniak,[13] M. Woszczak.[14]
[1]Department of Paediatric Bone Marrow Transplantation, Haematology and Oncology, Medical University, Wroclaw, Poland, Wroclaw, Poland; [2] Department of Paediatric Haematology and Oncology, Medical University of Silesia, Zabrze, Poland; [3]Department of Paediatric Haematology and Oncology, Medical University, Szczecin, Poland; [4]Department of Paediatric Haematology and Oncology, Medical University, Bydgoszcz, Poland; [5]Polish-American Institute of Paediatrics, Department of Paediatric Haematology and Oncology, Jagiellonian University, Krakow, Poland; [6]Department of Paediatric Haematology and Oncology, Medical University, Gdansk, Poland; [7]Department of Paediatric Haematology and Oncology, Medical University, Gdansk, Poland; [8]Department of Paediatric Pathology, Medical University, Warsaw, Poland; [9]Department of Paediatric Haematology and Oncology, Medical University of Silesia, Katowice, Poland; [10]Department of Paediatric Haematology and Oncology, Medical University, Lodz, Poland; [11]Department of Paediatric Haematology and Oncology, Medical University, Bialystok, Poland; [12]Department of Paediatric Haematology and Oncology, Medical University, Warsaw, Poland; [13]Department of Paediatric Haematology and Oncology, Medical University, Lublin, Poland; [14]Department of Paediatric Haematology and Oncology, Medical University, Chorzow, Poland.

Background: Two hundred eighteen patients (pts) were enrolled in the B-NHL BFM 04 protocol in the Polish Pediatric Leukemia/Lymphoma Study Group (PPLLSG), between 2004 and 2012. Twenty-four (11%) were diagnosed with diffuse large b cell lymphoma (DLBCL). Three-year event-free survival (EFS) achieved for this group was 80±8%, whereas EFS reported by CCG/SFOP (LMB89)/BFM/FAB (LMB96) were as follows: 90%, 90%, 95% and 90%.
Materials and Methods: Out of 24 pts diagnosed with DLBCL, two pts were excluded from the assessment, according to the protocol criteria, because of confirmed Nijmegen syndrome (first: mediastinal tumour, central nervous system [CNS] positive, st IV, R4; second: lymph nodes, st III, R2). The predominant primary sites in the analyzed group were abdomen four (21%) pts, lymph nodes five (21%) pts and mediastinum four (18%) pts. All patients presented with localized disease: st I—1 (4%), st II—7 (32%) and st III—14 (58%). There was no CNS involvement. Pre-treatment high LDH level was found in six (27%) cases. The treatment group stratification was R2—13 (59%), R3—6 (27%) and R4—3 (17%).
Results: In 20 (90%) pts, CR was achieved. One PR and one NR were reported. There was one isolated, mediastinal relapse—after a successful second-line treatment alloPBSCT was performed. There were three deaths reported. The causes of death were the following: lack of response to the treatment, late (3 years after treatment) infectious complications (encephalitis) and septic shock following alloPBSCT. In Nijmegen syndrome group, one isolated CNS relapse with a subsequent alloPBSCT (second CR) and one death in CR due to anaphylactic shock after IVIG infusion were reported. Second line of the chemotherapy was introduced in patient with PR, succeeding in CR.
Conclusions: EFS achieved in PPLLSG for patients diagnosed with DLBCL is comparable with the results in other national and international groups (CCG, BFM and SFOP). Still, as an enrollment to the protocol is finished, the data are being thoroughly analyzed, with emphasis on histopathological evaluation of prognostic protein expression (bcl-2, bcl-6, MUM1 and Ki67).

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OUTCOME AND TOXICITY PATTERNS IN CHILDREN WITH NON-HODGKIN'S LYMPHOMA

O. Abla, L. Rodriguez, M. Zolaly, A. Naqvi, A. Punnett, S. Weitzman.
Division of Hematology/Oncology, Department of Pediatrics, The Hospital for Sick Children, Toronto, Canada.

Introduction: The incidence and biology of non-Hodgkin's lymphoma (NHL) vary according to age, and the outcome differs amongst children being inferior in infants and adolescents. The objective of this study is to analyze the outcome and toxicity patterns of children with NHL comparing children younger than 10 years of age versus those aged ≥10 years at diagnosis.
Methods: Data of children with NHL diagnosed at the Hospital for Sick Children, from January 1995 to December 2008, were retrospectively reviewed. Patients with immunodeficiency, Post-transplantation lymphoproliferation diseases or mycosis fungoides were excluded.
Results: The charts of 162 immunocompetent children with NHL were reviewed. Five-year overall survival (OS) was 100% and 90% in patients aged 0–4 and 5–9 years, respectively, vs 83% and 82% for patients aged 10–14 and 15–18 years, respectively ($p = 0.027$). Five-year event-free survival (EFS) was 89% and 87% in the 0–4 and 5–9 years of age groups, respectively, vs 78% and 77% in the 10–14 and 15–18 years of age, respectively ($p = 0.077$). Regarding histological subtypes, there was also a trend towards a better outcome in younger children, with a 5-year EFS of 93% in children <15 years of age with Burkitt's lymphoma vs 75% in patients ≥15 years ($p = 0.098$). Children ≥10 years of age were more likely to develop neurotoxicity (9.7% vs 0%) and gastrointestinal toxicity (36% vs 19%). The cumulative incidence of toxic death was 5.5% in patients ≥10 years vs 2.9% in those aged <10 years ($p = 0.45$).
Conclusions: Children <10 years of age with NHL tend to have better outcomes and less treatment-related mortality than older children.

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MANAGEMENT OF PEDIATRIC HODGKIN LYMPHOMA IN CROATIA

J. Konja, M. N. Anicic, B. Glavas.
Hematology and Oncology Departement Childrens' Clinic, Hematologist Oncologist, Zagreb, Croatia.

Introduction: In the past decade, considerable modifications in the managemant of Hodgkin's disease in children have resulted in a continuously rising rate of recovery. As the disease responds favourably to the use of cytostatic therapy and radiotherapy, attempts have been made to treat as many affected children as possible with the least possible rate of early and late side effects. Previous therapy is known to have been associated with the development of secondary malignancies in some of the successfully treated children. The aim of the study was to describe the experience in the management of children with Hodgkin's lymphoma in Croatia, staged and treated at a single institution.
Patients and Methods: During the 1990–2011 period, 75 children with Hodgkin's lymphoma (39 male and 36 female) were treated at the Department of Hematology and Oncology, University Department of Pediatrics, University Hospital Centre

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Medical Faculty Zagreb, Croatia. The patients were administered a combination of cytostatic therapy (OPPA, OEPA and COPP) and radiotherapy (involved field radiation). Patients were allocated to three treatment groups (TG) by disease stage: TG1, stages I and IIA; TG2, stages IIEA, IIB and IIIA; and TG3, stages IIEB, IIIB, IIIE and IV. All patients underwent initially two cycles of OPPA or OEPA. In TG1, no further chemotherapy was given; patients in TG2 and TG3 received additional two or four cycles of COPP. The distribution of the patients was as follows: TG1, 27 = 36%; TG2, 28 = 37.3%; and TG3, 20 =26.7%. Radiotherapy was administered to the initially involved sites. Standard dosage was 20 Gy.

Results: Remission was achieved in all patients; in six patients with relapse of the disease, highly aggressive cytostatic therapy and radiotherapy were introduced, in four of them in combination with autologous bone marrow transplantation; three patients died. Seventy-two patients are still alive in the first or the second remission. There were no severe side effects and no case of secondary malignancies in any patients.

Conclusion: Combined modality therapy using risk-adapted low dose, involved radiotherapy + chemotherapy is an optimal treatment for the majority of children with Hodgkin's lymphoma.

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RAPID EARLY RESPONSE TO HIGH-DOSE METHOTREXATE IN AN ADOLESCENT WITH MULTIFOCAL PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

O. Abla,[1] H. Branson,[2] C. Hawkins,[3] S. Weitzman.[1]

[1]Division of Hematology/Oncology, Department of Pediatrics, The Hospital for Sick Children, Toronto, Canada; [2]Diagnostic Imaging, The Hospital for Sick Children, Toronto, Canada; [3]Division of Pathology, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Canada.

Introduction: Primary central nervous system lymphoma (PCNSL) is very rare in children, and their outcome appears to be better than adults. Primary treatment with chemotherapy alone, particularly high-dose methotrexate (HD-MTX), has been associated with better overall response rates.

Methods: We report the case of a teenager with multifocal PCNSL who had complete resolution of his lesions after four cycles of HD-MTX.

Results: A 17-year-old boy presented with 4-week history of vomiting, dizziness and ataxia. Neuro-imaging showed multiple enhancing lesions at the posterior medulla (obex), frontal horns, corpus callosum, right cerebellar peduncle and pituitary stalk. Biopsy of the obex mass showed diffuse large B-cell lymphoma, diffusely positive for CD20 with MIB-1 index of 100%. Metastatic work-up was negative, but cerebrospinal fluid was positive for malignant cells. During his initial admission, he developed severe liver dysfunction (ALT/AST > 1500) that caused some delay in starting treatment. Liver biopsy showed mild non-specific hepatitis. After gradual resolution of his liver dysfunction, he started treatment with HD-MTX 8 g/m^2 IV (over 3 h with leucovorin rescue), vincristine 2 mg/m^2 IV (day 1) and dexamethasone 10 mg/m^2 (days 1–5) with intrathecal MTX/hydrocortisone and cytarabine. He had significant improvement of his symptoms after first cycle of chemotherapy, and repeat brain MRI after four cycles showed complete resolution of all previously enhancing lesions. He received two cycles of consolidation with HD-MTX (3.5 g/m^2) plus high-dose Ara-C (3 g/m^2 for 2 days), followed by two cycles of intensification with HD-Ara-C (3 g/m^2 for 4 days) plus etoposide (200 mg/m^2 for 4 days). Overall, he tolerated well his treatment except for one episode of fever/neutropenia, which required hospital admission and bilateral hip avascular necrosis. He is currently at 8 months off therapy, and his last brain MRI was completely normal. This case confirms the well-known benefit of high doses of MTX in treating young patients with PCNSL. The largest paediatric PCNSL series showed that primary treatment with chemotherapy alone was associated with better overall response rates than chemoradiotherapy combinations. Furthermore, there was a significant relationship between higher doses of MTX and response. The 2-year progression-free survival and overall survival rates were 61% and 86%, respectively, and an ECOG PS of 0 to 1 was associated with better survival. In addition, at least 15 patients from this series received HD-MTX and HD-Ara-C combination, which was found to be a very effective consolidation regimen for childhood CNS-positive systemic B-NHL.

Conclusions: Because young patients can tolerate higher doses of MTX more than adults, we recommend induction therapy with HD-MTX at 8 g/m^2 for young patients with PCNSL followed by HD-MTX and HD-Ara-c consolidation and intensification with Ara-C and etoposide. Large prospective multinational trials are needed to test this regimen in childhood and adolescent PCNSL.

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