6. Indolent Lymphomas

ANALYSIS OF GENOMIC IMBALANCES AND GENE EXPRESSION CHANGES IN TRANSFORMED FOLLICULAR LYMPHOMA (FL)

G. Oehl1, P. Farinha1, W. Lam2, R. deLeuwe2, K. Young2, E. Kjeldsen2, S. Hamilton-Dutoit, F. d’Anore1, W. Chari1, R. Gascogne2
1Hematology, Aarhus University Hospital, Aarhus c, Denmark; 2Pathology, British Columbia Cancer Agency, Vancouver, Canada; 3Cancer Genetics and Developmental Biology, British Columbia Cancer Agency, Vancouver, Canada; 4Cancer Cyto genetics, Aarhus University Hospital, Aarhus c, Denmark

Introduction: The transformation of FL to DLBCL is probably the result of a progressive series of non-random genetic alterations. To look for transformation-specific changes in DNA copy number and gene expression, we analyzed paired pre- and post-transformation biopsies from Danish and North American patients with transformed FL.

Methods: High-resolution BAC-array comparative genomic hybridisation (CGH) was used to detect genomic imbalances. Gene expression profiling was performed using cDNA microarrays (Affymetrix).

Results: Of 9 biopsy pairs identified so far, analysis results of the first 4 are presently completed. Upon transformation, amplification of e.g. 7p,12p13,17p11-13 and deletion of e.g. 3p, 4q21, 13q14 were observed. Clustering of two sets of genes were identified, which were either over- or underexpressed upon transformation. Of these genes, 18 were fold-up (7 genes) or down-regulated (11 genes). Among upregulated genes were: ARF1 (1p13), ID1 (10q22), CALM3IP1 (10q11.22), CRNN (1p34.2), and ARF5 (17q21); among the down-regulated ones: DKFZP766B1107 (4p15), LOC118812 (10q24), NAP1L4 (11p15), EB1 (12q23), C14orf135 (14q23), STK2 (19q13), and CHD6 (20q12).

Conclusions: The combined use of array-CGH and gene expression analysis will provide a more comprehensive picture of the transformation process in FL.

FOLLICULAR LYMPHOMA: DESIGN OF A PROTEIN-BASED SURVIVAL PREDICTOR USING TISSUE-MICROARRAYS (TMA)

F. Camacho1, R. Arranz-Sáez2, A. Cañedo1, J. Cannata2, L. Cereceda2, L. González1, M. Pérez-Martín2, L. Sánchez-Vende3, C. Montalban3, J. García1, M. Pirts1, C. Bellas1
1Biología Celular, Spanish National Cancer Center (CNIO), Madrid, Spain; 2Hematology Department, Hospital de la Princesa, Madrid, Spain; 3Hospital 12 Octubre, Madrid, Spain; 4Hematology Department, Hospital Ramón y Cajal, Madrid, Spain

Follicular lymphoma (FL) is the most common type of low-grade non-Hodgkin’s lymphoma. Clinical course in FL patients is highly heterogeneous. Some patients survive for long periods of time while others have a significant shorter survival or associate histologic transformation into high-grade NHL. Survival predictors for FL are currently based on clinicopathological, without enough accuracy for predicting survival among patients with advanced stage disease. The aim of this study is to build a survival predictor based on a set of biological markers using Tissue Micro Arrays (TMA). We have retrospectively analysed the expression of a group of 40 selected genes related with apoptosis, cell cycle, B-cell differentiation and signaling in 192 FLs. The association of these molecules with survival and among FLIPI groups was evaluated. Singularly nuclear markers were scanned using theiss system and the quantitative expression was measured using the TMAscore v.1.0 image analysis software (Bacus Laboratories, Inc.). Mean overall survival (OS) was 74 months, and 38 months for progression-free survival (PFS). Statistically significant differences in OS were found between the FLIPI score groups (P <0.01). No significant OS or PFS differences were observed between FL grades 1-3, between grades 3a and 3b, or using K67 expression. Univariate analysis of OS revealed several TMA markers. On multivariate analysis, a set of 4 apoptosis and cell-cycle markers was integrated into a FLIPI-independent clinical predictor, recognizing two groups of FL patients with statistically significant differences in OS (83% versus 43% of OS at 120 months; P <0.001). The protein-based survival predictor (PBS) was then used in the low-risk (FLIPI 0–2) and high-risk groups (FLIPI 3–5). This PBS allowed to discriminate FL patients with statistically different OS among the complete series. In the group of patients with high-risk FLIPI score, the PBS was able to discriminate two groups, with statistically significant differences in OS (79% versus 84% OS at 60 months, P<0.05). The model is now being validated in a blind set. These results suggest that an integrated use of the FLIPI and the protein-based model could reach a higher accuracy predicting survival in FL.

IDENTIFICATION OF NEW GENOMIC ABBERRATIONS IN FOLLICULAR LYMPHOMA USING MICROARRAY BASED GENOMIC HYBRIDIZATION (MATRIX-CGH)

C. Schwabscher1, A. Viardot1, S. Ruf1, M. Enz1, H. Köhlhammer1, H. Kestler1, T. Barth1, P. Moeller1, H. Doehner1, P. Lichter1, M. Bentz1, S. Wessendorf, on behalf of the german project MIMM1; 1Innere Medizin III, Medizinische Klinik der Universität, Ulm, Germany; 2Forschungszentrum Bioinformatik, Universität, Ulm, Germany; 3Abt. Pathologie, Universität, Ulm, Germany; 4Molekulare Genetik, Deutsches Krebsforschungszentrum, Heidelberg, Germany; 5Medizinische Klinik II, Klinikum, Karlshuhe, Germany

In cytogenetic and molecularcytogenetic studies analyzing Follicular Lymphoma (FL), in approximately 70%–100% of all cases genomic aberrations are present. However, due to the limited genomic resolution of the applied techniques, the exact chromosomal mapping and the description of candidate genes are still at the beginning. Moreover, in contrast to other hematologic neoplasms, no established genomic prognostic factor is applied for risk stratification so far. In this study, we used high resolution clones comprised (i) clones mapping to genomic regions or genes of possible pathogenic relevance in lymphoma and (ii) a large genome-wide cluster of clones covering the genome at a distance of approx. 2 Mbp. This chip represents the largest set of clones containing 1,5 Mbp and covers approximately 10% of the human genome. DNA from 84 FL samples were analyzed and the results were compared to clinical data sets. The sensitivity of array-CGH was considerably higher compared to published chromosomal CGH studies in FL. In 74 (89%) of the 84 analyzed cases a total of 469 genomic aberrations were detectable. The mean number of aberrations per case was 4.4. Genomic gains (n=249) were more frequent than deletions (n=216). Besides the identification of new chromosomal aberrations, for a large number of abnormalities we were able to delineate minimally altered chromosomal regions allowing the description of possible candidate genes. As one region of particular interest an interstitial deletion at 11q13 was identified. One gene affected by this deletion is FADD, which plays a crucial role in FAS mediated apoptosis. In this context we were able also to identify in 38 of 84 analyzed cases four additional genomic alterations interfering with the FAS mediated apoptosis. Moreover, a deletion on 9p21, affecting the tumor suppressor gene CDKN2A/B, with prognostic relevance for inferior survival, was identified in non-transformed FL. In conclusion, these data underline the potential of array-CGH for the sensitive detection of genomic imbalances in FL.

PROGRESSIVE ACQUISITION OF ADDITIONAL GENETIC ABNORMALITIES OCCURS IN FOLLICULAR LYMPHOMA IN THE ABSENCE OF MORPHOLOGICAL TRANSFORMATION

K. Turner, S. Barrans, R. Owen, A. Jack
HMDS, Leeds Teaching Hospitals, Leeds, UK

Patients with follicular lymphoma often have a protracted clinical course characterized by multiple relapses and increasing resistance to therapy. Disease progression may be associated with the acquisition of a range of genetic abnormalities in addition to the t(14;18). The aim of this study was to examine the rate of accumulation of additional genetic abnormalities in follicular lymphoma patients by comparing paired lymph node biopsies taken at presentation and at first relapse. 54 sets of paired lymph node biopsies, taken at presentation and at first relapse, were studied. Cases with morphological evidence of transformation were excluded. The median time between presentation and relapse was 26 months. Each biopsy was assessed for the presence of the t(14;18) and additional copies of the BCL2 Light Chain fusion. BCL2 rearrangement and copy number of P53 were assessed using interphase fluorescent in-situ hybridisation (FISH) on tissue touch preparations, nuclei extracted from paraffin-embedded tissue, or thin sections. The t(14;18) was observed in 45/54 patients. Of these 6/45 patients had t(14;18) multiple fusions at presentation with 4 further patients acquiring t(14;18) multiple fusions at relapse. BCL6

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HOMOZYGOSITY MAPPING BY GENOME-WIDE SNP ANALYSIS OF THE TRANSFORMATION OF FOLLICULAR LYMPHOMA (FL) TO DIFFUSE LARGE B-CELL LYMPHOMA (TDLBCL). A. Davies1, S. Bapat1, J. Fitzgerald1, M. Raghavans1, T. Chaplin1, A. Norton1, A. Lister1, B. Young1
1Cancer Research UK Medical Oncology Unit, St Bartholomew’s Hospital, London, UK; 2Department of Histopathology, St Bartholomew’s Hospital, London, UK

Introduction: The molecular mechanisms underlying transformation of FL to TDLBCL are poorly understood. The introduction of high-density array-based analysis of single nucleotide polymorphism (SNP) has provided a powerful genome-wide approach to detect deletions, mitotic recombinant and amplification by mapping regions homozygosity in tumours.

Methods: Using the 10K SNP array (Affymetrix) whole lymph node DNA extracted from 24 patient pairs comprising TDLBCL and their antecedent FL sample was used to determine the pattern of genomic aberration acquired upon transformation. Greater than 10,000 SNPs were called for each sample, and regions of alteration were defined by the presence of 99% homozygosity in at least 50 contiguous SNPs.

Results: One hundred and thirty-nine regions of homozygosity were identified, 54 in the FL samples, 85 in TDLBCL. In the antecedent FL such regions were present in 83% (20/24) of cases (mean 2.2 aberrations per case; range 0–6). Most frequently affected were 6p (29%), 6q (21%), 12q (21%), 13q (17%), X (8%), and 2p (8%). Homozygosity at 17p was observed in only one case. A mean of 3.5 aberrations were observed in TDLBCL (range 0–9) with the most frequent regions of homozygosity occurring at 6p (29%), 6q (29%), 9p (25%), 7 (25%), X (25%), 8p (21%) and 17p (17%). Homozygous missense mutations in TP53 have previously been identified in 24 of the cases with 17p homozygosity. Homozygosity at 8p (21%), 17p (13%), 7 (13%), 13q (8%), 6q (8%) and X (8%) was acquired most frequently upon transformation. Forty percent (56/139) of homozygosity were common to both the FL and DLBCL of a pair, in 9 cases the homozygosity maps were identical in both histological phenotypes. Higher resolution analysis however defined smaller regions of homozygosity identified by the above algorithm, including in one case a 4.5 Mb 2p amplion present only upon transformation which was subsequently confirmed by genomic RQ-PCR of the REL oncogene. In 7 cases some regions of homozygosity were identified that were unique to the FL. This indicates that the FL and TDLBCL may arise from a common precursor cell rather than by direct evolution.

Conclusions: SNP array profiling provides a new approach to the identification of critical regions implicated in the pathogenesis and phenotypic transformation of FL.

KARYOTYPIC AND MOLECULAR ANALYSIS OF THE CLINICALLY PROGRESSED FOLLICULAR NON HODGKIN LYMPHOMA M. Tibielli1, M. Taboret1, V. Martin2, B. Pozzi1, I. Prosproio3, A. Rinaldi1, L. Kowless2, F. Bertoni2, C. Capella2
1Osteo Anatomia Patoligica, Ospedale di Circolo-Universita dell’Insubria, Varese, Italy; 2Experimental Oncology Unit, Iosi, Bellinzona, Switzerland; 3Osteo Oncologia Medica, Ospedale di Circolo, Varese, Italy

Introduction: Follicular lymphomas (FL) and diffuse large B cell lymphomas (DLBCL) are the most common subtypes of non-Hodgkin lymphomas (NHL). Histologic transformation into the more aggressive DLBCL occurs in 10–60% of FL. Both FL and DLBCL are characterized by specific cytogenetic abnormalities such as the t(14;18) translocation involving BCL2 gene and promiscuous rearrangements of BCL6 at 3q27. FL and DLBCL also present a series of different secondary genetic lesions, mainly chromosomal changes. The clinical relevance of these abnormalities is still unknown. The characterization of the secondary chromosomal changes in a selected series of transformed lymphomas would help to identify genes involved in tumor progression and chemotherapeutic resistance.

Patients and methods: We analyzed serial tumor biopsies from 6 patients with follicular NHL using conventional and molecular cytogenetic approaches. Molecular cytogenetic was performed both on metaphases using FISH, on BCL2 and BCL6 genes. Serial whole genome profiling (arrayCGH) is being performed using the Affymetrix GeneChip 10k v. 2.0 arrays. Data mining is done with Affymetrix QDAS and CCNT software combined with algorithms developed within the R statistical package.

Results: Twelve serial samples of NHL from 6 patients were studied. A clonally related karyotype was shown in five paired biopsies, while the lymphomas appeared cytogenetically unrelated in one patient. The conventional cytogenetic demonstrated t(4;10) and del(6q) as constant aberrations present both at first diagnosis and in the following lymphoid node biopsies. On the converse, del(4)(p21pter), del(11)(q23pter) and del(3)(p21pter) were exclusively found in the second biopsies. Interphase FISH analysis performed on both lymphomas in each patient revealed an increasing number of cells showing specific rearrangements. ArrayCGH data will be presented.

Conclusions: Specific genomic regions involved in lymphoma progression have been identified.

FLI1 AND HISTOLOGICAL SUBTYPE ARE THE MOST IMPORTANT PREDICTING FACTORS OF HISTOLOGICAL TRANSFORMATION (HT) IN FOLLICULAR LYMPHOMA (FL) E. Ginei1, A. Lopez-Guillermo1, S. Montoto1, F. Bouchi2, N. Villamor1, A. Montana1, L. Arenillas1, L. Colomo1, E. Campo2, E. Montserrat1
1Instituto de Hematologia and Oncology, Department of Hematology, Hospital Clinic, Barcelona, Spain; 2Hematopatology Unit, Hospital Clinic, Barcelona, Spain

Objective: To analyze the incidence and risk factors for HT, as well as the outcome of transformed patients (pts) in a series of FL.

Patients and methods: 278 FL pts (139M/139F; median age: 54 years) consecutively diagnosed at a single institution. Main biological and clinical parameters were assessed and analyzed for HT, including IPI, ILL1 and FLIPI scores. Median overall survival was 11.2 years and median follow-up 6.5 years.

Results: 30 pts presented HT during the follow-up, with a risk of 15% and 22% at 10 and at 15 yrs from diagnosis, respectively. HT corresponded to a diffuse large B-cell lymphoma in all cases (tissue biopsy in 25 pts and cytology in 3). At the time of HT, 45% of pts presented with poor performance status, 68% with advanced stage and 79% with high LDH. Factors at diagnosis associated with HT were: grade III histology, nodal areas >4, high serum LDH and B2-M. FLIPI and IPI. In the multi-variable analysis, grade III histology (P = 0.04; RR 2.0) and FLIPI (P = 0.005; RR 2.1) retained prognostic significance. 28 of 30 pts (90%) received salvage therapy: 14 pts reached CR (50%) and 3 PR (9%). 25 pts have died during the follow-up, in 23 cases due to progression, with a median survival from HT of 1 yr. Early stage and CR achievement after HT were favorable features for survival.

Conclusions: HT is not infrequent in the natural history of FL, but the risk of such a phenomenon concentrates in pts with high-risk features at diagnosis (i.e., grade III histology and advanced FLIPI score).

EXPRESSION OF THE ANTI-APOPTOTIC MCL-1 IS A MARKER OF GRADE AND PROGNOSIS IN FOLLICULAR LYMPHOMA (FL) J. Michelis1, V. Fora1, G. Packham2, B. Mead3, P. Johnson1
1Cellular Pathology Department, Southampton General Hospital, Southampton, UK; 2CRUK Oncology Unit, Southampton General Hospital, Southampton, UK
Introduction: Mcl-1 is an anti-apoptotic member of the Bcl-2 gene family which shows reciprocal expression to Bcl-2 in normal germinal centres. The role of Mcl-1 and Bcl-2 during progression of FL to higher grade and transformation is poorly understood.

Methods: We investigated the expression of Mcl-1 and Bcl-2 by immunohistochemistry in 85 consecutive patients (pts) with FL, including cases with transformation, treated at one institution between 1993 and 1998.

Results: The median follow-up time of 85 evaluable pts was 94.5 months (mo). At the time of analysis, 59/83 pts had progressive disease and 40/85 pts had died. FL was graded 1-2-3-transformed (23-34-10-18 pts, respectively). In all cases, centroblasts expressed high levels of Mcl-1 and mostly low levels of Bcl-2 (62/85). Centrocyes had high Bcl-2 and low Mcl-1 expression. Numbers of Mcl-1+ centroblasts correlated highly with morphologic grade of FL. The number of Mcl-1+ centroblasts/high power field (hp) (hp) correlated with overall survival (r = -0.31, p < 0.001) and independent of the prognostic index.

Conclusion: Deregulated Mcl-1 expression in centroblasts is implicated in the progression of FL, and grading of FL by number of Mcl-1 expressing centroblasts may be a more accurate means to predict clinical outcome.

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COMBINED EFFECT OF SMOKING HABITS AND HEPATITIS C VIRUS ON NON-HODGKIN LYMPHOMA RISK


Epidemiology and Biostatistics, Aviano Cancer Center, Aviano, Italy; E.U. 'Pascale' Cancer Institute, Naples, Italy; Oncology, Aviano Cancer Center, Aviano, Italy; I.C. E.I.A.R.C. Lyon, France; Immunology and Virology, 'S. Maria' Hospital, Pordenone, Italy; Epidemiology, 'Negri', Milan, Italy

Introduction: Tobacco smoking is a risk factor for several cancers, but the role of smoking in the etiology of non-Hodgkin lymphomas (NHL) is inadequately understood. Hepatitis C virus (HCV) has been consistently associated with NHL risk, but the interaction between HCV and smoking habits has never been explored.

Materials and methods: Between 1999 and 2002, we conducted in Aviano and Naples (Italy) a case-control study on the association of smoking habits, HCV, and NHL. Cases were 225 patients with a new diagnosis of NHL. Controls were 504 patients, admitted to the same hospitals as cases, for non-smoking-related conditions. Odds ratios (OR) and 95% confidence intervals (CI), were computed by unconditional multiple logistic regression including terms for age, sex, center, education, place of birth, and HCV-positivity.

Results: Current heavy smokers (≥20 cigarettes/day) had an OR of NHL of 2.1 (95% CI 1.1-4.4) compared with never smokers. The association between smoking and NHL was consistent across strata of sex and age. In respect to historical type, compared to never smokers, current smokers of ≥20 cigarettes/day had ORs of 1.1 (95% CI 0.4-3.6) for B-cell-low-grade, 2.1 (95% CI 0.9-4.7) for B-cell-intermediate and high-grade, and 25.5 (95% CI 2.0-342.2) for T-cell NHL. OR was 2.6 (95% CI 1.6-4.3) for HCV-positivity and OR was 4.0 (95% CI 1.7-9.5) for HCV-positve current smokers.

Conclusions: Our study suggests that tobacco is associated with a moderate increase in NHL risk. Tobacco smoking and HCV seem to have an independent effect on NHL risk, leading to a 4-fold elevated OR in current smokers who were HCV positive. A paper describing the present study in details is in press with IJC.

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GENE EXPRESSION PROFILING OF FOLLICULAR LYMPHOMAS: IDENTIFICATION OF POSSIBLE BIOMARKERS

U. Andreasen, S. Ek, C. Borrebaek

Department of Immunotechnology, Lund University, Lund, Sweden

Follicular lymphoma (FL) is one of the most common forms of malignant lymphomas and depending on the severity of symptoms different treatment strategies are used. Chemotherapy is in many cases the first-line therapy, but during recent years combination therapy with anti-CD20 antibodies has shown promise for FL patients. However, the efficacy can still be significantly improved and our strategy is to find targets specifically expressed on the malignant B cells, in contrast to all mature B cells. In this study, gene expression profiling was used to identify genes differentially expressed in FL compared to five different populations of normal B cells. FL cells, parfit by flow cytometry, and normal B cells were analyzed for their expression of more than 12,500 genes available on the Hu18s v2 array. Two main data analyses approaches were performed, either comparing FLs to all five different B cell populations or to only the germinal centre (GC) B cell populations. The different aims were to find (i) general differences comparing FL and non-malignant B cells, and (ii) specific differences between the highly proliferative GC B cells, which are the normal counterpart of FLs. In the different analyses, 42-277 genes were found to be significantly (P<0.05) deregulated. The differentially regulated genes were analyzed with emphasis on extracellular expression for the corresponding proteins. Approximately 25 genes, encoding membrane bound proteins, were found to be differentially expressed in FL and these gene products are now being investigated with regards to functionality and as potential targets for immunotherapy.

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NEW APPROACH FOR QUANTIFICATION OF IGH-BCL2 REARRANGEMENT IN FOLLICULAR LYMPHOMAS

C. Sattleriani, K. Baldjord, V. Asnat, C. Fernandes, D. Canioni, R. Deluca, M. Delfia, F. Davi, C. Bastardi, G. Sallei, E. Macintyre

1Laboratoire d’Hématologie, Hôpital Necker-Enfants malades, Paris, France; 2Laboratoire d’anatomie-pathologie, Hôpital Necker-Enfants malades, France; 3Hématothérapie clinique, Hôpital Necker-Enfants malades, France; 4Laboratoire d’Immunologie, Hôpital H. Mondor, France; 5Laboratoire d’Hématologie, Hôpital Pitié-Salpêtrière, France; 6Laboratoire de Génétique Oncologique, Centre H. Bcquerel, Rouen, France; 7Hématothérapie, CHU Lyon Sud, France

Introduction: The place of BCL2-IGH molecular follow-up (FU) in follicular lymphoma (FL) by qualitative PCR has long been controversial. It is possible that quantitative real-time PCR (Q-PCR) may facilitate the quantification of individual patients’ results. The sensitivity of Q-PCR amplification is inversely proportional to the size of the amplicon, which complicates comparison of molecular data between patients whose BCL2-IGH PCR amplified products vary considerably in size, even if the MBR-JH informative cases. We therefore undertook to assess within the context of the GELA patients enrolled on the GELA/GOELAMS FL2000 study whether amplicon size variability impacts on residual positivity levels.

Methods: BCL2-JH was assessed by Q-PCR using Biomed2 based IH probes. We compared quantification using log dilutions from either the patient’s diagnostic tumoral material or a universal cell line (RL) or patient material as calibrator. 53 MRD blood, bone marrow or peripheral stem cells samples from 24 patients were analysed.

Results: Sensitivity varied between patients (10E2-2 to 10E5, majority = 10E4) and was proportional to the size of the rearrangements, although relatively large amplicon size not prevent adequate sensitivity. Comparison of quantification relative to a calibrator or a universal calibrator demonstrated a 1 log difference for 23/35 (66%) samples and a 2 log difference for 8/35 (15%).

Conclusions: These data demonstrate that the choice of calibrator can impact on molecular quantification of MRD samples in FL. While universal calibrators have several practical advantages, it will be necessary to compare the impact of variable amplicon size, if individual stratification is to be based on Q-PCR bone marrow levels, as recently proposed by Rambold et al. (2005).

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SIGNIFICANT DIFFERENCES IN THE IGHV AND BCL6 MUTATION STATUS IN AGGRESSIVE B-CELL LYMPHOMAS WITH AND WITHOUT MYC BREAKPOINTS

C. Pohl, G. Gerdes (Deutsche Krebsforschung) Network Molecular Mechanisms in Malignant Lymphomas (MMML) and et al.

1Second Department of Medicine, University Hospital, Kiel, Germany; 2Department of Radiology, University of Göttingen, Göttingen, Germany
Introduction: Somatically mutated IGVH regions are a hallmark of germinal center (GC) B-cells. Moreover, aberrant somatic hypermutation (SHM) of oncogenes, like changes in the 5' non-coding region of the BCL6 gene occurring in 75% of DLBCL, are a mechanism of oncogene activation independently of chromosomal translocations.

Methods: Large scale mutational screening using DPLC and direct sequencing was applied to determine the somatic hypermutation status of clonal IGVH as well as several oncogenes like BCL6 and MYC in a series of more than 140 aggressive B-cell lymphomas included in the Deutsche Krebshilfe funded network “Molecular Mechanisms in Malignant Lymphoma”. Mutation patterns were correlated with the results of molecular cytogenetic and gene expression profiling (GE) analyses.

Results: MYC breakpoints detected by FISH as well as the presence of the MYC gene expression (GE) signature (see abstract by Siebert et al.) clearly differentiated two groups of aggressive B-NHL with significantly different VH mutation status. Independent from the histologic diagnosis, MYC-positive lymphomas by FISH or GE carried VH1 genes with a mutation frequency significantly lower compared to aggressive lymphomas lacking these features (median 4.8% vs. 11.0%, P < 0.0001) and 4.8% vs. 11.2%, P < 0.0001, respectively). Similarly, the mutation rate of BCL6 was significantly lower in MYC-positive than in MYC-negative lymphomas (FISH and GE: median 0.13% vs 0.25%, P = 0.02). GC and ABC-DLBCL classified according to immunohistochemistry or GE showed comparable VH and BCL6 mutation rates. We observed a bias in VH gene usage in both groups with an overrepresentation of VH4 (40% in both groups) and VH3 gene (24% in MYC-positive, 40% in MYC-negative).

Conclusion: Molecular classification of aggressive B-cell lymphomas according to MYC breakpoints distinguishes subgroups with significantly different VH and BCL6 mutation frequencies independently from histologic subtypes.

Introduction: Radiotherapy (RT) is the widely accepted treatment of limited stage follicular lymphoma. We present a 24 year experience of 2 consecutive RT regimens.

Methods: Eligible patients had limited stage (I or II, no B sx, bulk <10 cm, ≤3 contiguous lymph node regions) follicular lymphoma (all subtypes), treated at the BCCA. Treatment policy until July 1996 was involved region RT (IRRT), subsequently involved field RT (IFRT). RT dose was 30 Gy in 10 fractions for small fields, 35 Gy in 20 fractions for large fields. Since 1986 the BCCA has maintained a prospective database.

Results: 191 patients (MF/F = 87/104) were treated with RT alone. Median age was 62y (range 29–89), 147 presented with stage I/IA or IAE, 44 with stage II or IIAE. Median follow up was 4.9y. The 10 and 20 year progression free survivals (PFS) for all patients were 51% and 48%, median 10.7y. No patients relapsed later than 10.75y after diagnosis. Overall survival (OS) at 10 and 20 years were 64% and 47%, median 14.2y. There was no significant difference in PFS when comparing grades 1 v. 2 v. 3 and E v. not E. A further 36 patients were treated with RT plus chemotherapy (CT). Similar results with no late relapses were obtained with this group.

Conclusions: The absence of late relapses argues for a proportion of patients who may be cured by moderate dose and volume RT. The addition of CT for a small sub-group did not provide an additional benefit.

FOLLICULAR LYMPHOMA, IMMUNOCYTOLOGY, AND MANTLE CELL LYMPHOMA: RANDOMISED EVALUATION OF CURATIVE RADIOTHERAPY IN LIMITED STAGE NODAL DISEASE

M. Engelhardt1, M. Stuschke1, M. Hansmann1 et al.

Radiology, Medical School, Essen, Germany; 2Pathology, Medical School, Frankfurt, Germany

Introduction: Follicular lymphoma grade I-II (FL), immunocytooma (IC), or Mantle cell lymphoma (MCL) patients (pts) with nodal early stage disease can potentially be cured by radiotherapy alone. This multicenter study evaluates adequate radiation volumes in FL pts (randomised trial, RTE), and standardised radiotherapy in IC and MCL pts (prospective observation trial, OBS).

Methods: In FL stage I-II and limited stage III disease (<4 involved regions, <10cm), pts aged 18–65 years (ys) are randomised to Extended field (EF) or Total lymphatic irradiation (TLI), dosis are 30 Gy, plus 10 Gy to macroscopic lymphoma <3cm, or plus 14 Gy to those of 3–10cm. Pts aged 66–75 ys are treated exclusively by EF.

In IC or MCL, stage I-II pts aged 18–75 ys receive EF limited to one side of the diaphram. In pts >75 ys with FL, IC, and MCL, involved field (IF) radiotherapy is applied (all doses in the RTE).

Results: From 2000–2004, 211 pts were recruited, 162 to the RTE, median age 53 (23–65) y; stage I, II, and III were 58%, 32%, and 10%, resp. In the OBS, 39 pts with FL, 2 with IC, 8 with MCL, median age 69 (30–85) ys, were included. EF/TLI were generally well tolerated, WHO IV toxicity occurred only as reversible leucopenia (5%/16%) and thrombocytopenia (3%/12%, resp.). The total complete remission rate is 92%, total relapse rate 16%, overall survival is 98%, median observation time 19 months.

Conclusions: In early stage FL disease, this ongoing randomised trial contributes to the crucial questions of curability by radiotherapy and to the determination of adequate treatment volumes necessary. This is also valid for standardised radiotherapy in early stage IC and MLC pts.

Histological, Immunological and Genetical Analysis of 147 Follicular Lymphoma: Low Grade Follicular Lymphoma with T(14;18) Preserves a Homogenous Disease Entity

Y. Guo1, K. Karube1, J. Suzukiyama2, M. Kikuchi3, K. Oshihama1

1Pathology, Fukuoka university, Fukuoka, Japan; 2Internal Medicine, Fukuoka university, Fukuoka, Japan

Follicular lymphoma (FL) is morphologically classified into grades 1, 2, and 3b by the World Health Organization. Bcl2, Bcl6 and CD10 are phenotypic markers of FL while the Bcl2 t(14;18) and Bcl6 t(3q27) gene
translocations are common genetic changes. However, to date, there has been no integrated analysis based on phenotype, grade and genotype from large numbers of FL cases. (Cases and Method) We evaluated grade of 147 FL. Bcl2, Bcl6 and CD10 expression was analyzed by immunohistochemistry, and fluorescent in situ hybridization (FISH) was performed about Bcl2/IgH (t14;18) and Bcl6 (t3q27) in all cases. (Result and discussion) The grade distribution of FL was, grade1: 26%; grade2: 48%; grade3a: 17% and grade3b: 9%. 95%, 71%, and 81% cases are positive for Bcl2, Bcl6 and CD10 respectively. Bcl2/IgH translocation and BCL6 translocation was detected in 81% and 10% of the cases respectively. From these findings, we classified FL into typical and the others types. The typical group, which includes 69% cases of FL, is characterized by low histological grade (Grade 1,2), co-expression of Bcl2 and CD10 and Bcl2/IgH gene translocation. The rest comprises a small part of low grade FL without Bcl2 gene translocation and high grade (Grade 3a and 3b) FL. These FL include some heterogeneous disease entities. They are characterized by high histological grade (87%), no definite expression of Bcl2 or CD10 and several kinds of gene aberrations including Bcl2 translocation, Bcl6 translocation, Bcl2 amplification or other unknown gene abnormality. Our findings indicate that typical FL presents a homogeneous disease entity whereas the rest comprises heterogeneous disease entities.

HIGH DOSE THERAPY WITH AUTOLOGOUS PURGED STEM CELL TRANSPLANTATION AND DOXORUBICIN BASED IMMUNOCHEMOTHERAPY IN PATIENTS WITH ADVANCED FOLLICULAR LYMPHOMA: A GOELAMS STUDY

D. Deconing1, P. Colombat1, C. Fournier1, N. Milpied et al. 2
1Hematology, CHU Jean Minjoz, Besançon, France; 2 for the GOELAMS, France

Background: Doxorubicin containing chemotherapy with or without interferon is the referential treatment for advanced follicular lymphoma. High dose chemotherapy with autologous stem cell support is highly effective in follicular lymphoma in relapse but remains controversial in first response. In a prospective randomized study we compared these two therapeutic approaches in patients with advanced follicular lymphoma, using autologous stem cell transplantation with purged or non-purged autologous stem cell transplantation.

Patients and methods: One-hundred and seventy-two newly diagnosed advanced follicular lymphoma patients were randomly assigned either to a conventional chemotherapy regimen (cyclophosphamide, doxorubicin, teniposide, prednisone and interferon) or to high dose therapy followed by purged autologous stem cell transplantation.

Results: Compared with the patients with conventional chemotherapy and interferon, patients treated with high-dose therapy had a higher overall response rate (69% vs. 81%; P=0.045) a longer median event-free survival (not reached vs. 45 months). This did not translate into a better survival rate due to an excess of secondary malignancies after transplantation.

Conclusions: Autologous stem cell transplantation cannot be considered as the standard first-line treatment of follicular lymphoma patients less than 60 years old with a high tumor burden except for some patients with a poor FLIPI.

STAGE IV INDOLENT LYMPHOMA: A RANDOMIZED TRIAL OF CONCURRENT VS. SEQUENTIAL FND (FLUDARABINE, MITOXANTRONE, DEXAMETHASONE) AND RITUXIMAB, WITH INTERFERON MAINTENANCE

P. McLaughlin1, M. Rodriguez1, F. Hagemeister1, J. Ronagueri1, A. Sarri1, A. Younes1, N. Dang1, A. Goy1, F. Samaniego1, M. Hess1, M. Loé1, L. Medeiros2, L. Fayad3, B. Pro1, Y. Jiang1, A. Ayala1, F. Cabanillas2
1Lymphoma/Myeloma, Univ. of Texas MD Anderson Cancer Center, Houston, USA; 2Hematopathology, Univ. of Texas MD Anderson Cancer Center, Houston, USA

Introduction: FND, rituximab (R), and interferon (IFN) are all effective in indolent lymphoma.

Methods: Between 1997–2002, 161 patients (pts) received concurrent FND + R or sequential FND followed by R; all received IFN. Pts with t14;18 involving bcl2-1 (mbr and mcr) were monitored by PCR.

Results: The median follow-up is 49 months. The outcomes, respectively for R-FND and FND-R, are: overall response rate, 100 vs 96%, p value not significant (NS); complete response, 88 vs 85%, NS; 4-yr failure-free survival (FFS), 70 vs 59%, P=0.22; 4-yr FFS, follicular lymphoma, 76 vs 58%, P=0.06; molecular response at 6 months, 89 vs 60%, P<0.01; molecular response at 12 months, 89 vs 68%, P<0.01. Tolerance has been good, with slightly more neutropenia with FND + R but no excess infections (Semin Oncol 2000; 27 [Suppl 12]: 37). 19% of patients did not complete all 8 courses of FND. Myelodyplasia occurred in 6 of these pts (Blood 2004; 104 [Suppl]: 391A).

Conclusion: Both FND + R and FND-R, with IFN, attain high rates of durable remission. FND + R results in a quicker and significantly higher rate of molecular remission, and a trend for superior FFS.

MOLECULAR ASSESSMENT BY BCL-2/JH QUANTITATIVE PCR TECHNIQUE OF PATIENTS (PTS) WITH FOLLICULAR LYMPHOMA (FL) IN ADVANCED STAGE TREATED WITH FCM (FLUDARABINE, CYCLOPHOSPHAMIDE AND MITOXANTRONE)


Hematology, GELCAB (Grup per l’Estudi dels Linfomes a Catalunya i Balears), Barcelona, Spain

Objective: To assess the efficacy of R-FCM by predicting clinical and molecular remissions in patients with FL treated with FCM.

Patients and methods: 110 pts diagnosed with advanced stage FL included in the FCM trial in whom DNA material was available for molecular and/or FCM analysis performed either at diagnosis (at peripheral blood [pb], bone marrow [bm] and lymph node [ln]), at end of treatment, and during the follow-up.

Results: The distribution of pts according to the bcl-2/JH breakpoint was: MBR, 67 (61%), mcr, 8 (7%), no MBR/mcr, 35 (32%). There was a strong correlation between the R-FCM values in pb and bm (R: 0.82, P<0.001). Discordant bm and pb cases were as follows: bm>pb, 2 pts; bm<pb+, 5 pts. In 47 of 53 pts (99%) in whom DNA material from pb, bm and ln was available, the results on bcl-2/JH were concordant between pb/bm and ln. P value R-FCM was correlated with bulk disease and bm involvement. In the clinical outcome and pb molecular assessment at different time-points are detailed in the table:

<table>
<thead>
<tr>
<th></th>
<th>Pts-FCM (N=54)</th>
<th>FCMc5 (N=32)</th>
<th>FCM66 (N=54)</th>
<th>6–12 mo. post FCM (N=38)</th>
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<tr>
<td>CR rate (%)</td>
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<tr>
<td>Molecular CR (%)</td>
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<tr>
<td>RQ-PCR pb*</td>
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<td>0.001±0.004</td>
<td>0.006±0.01</td>
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Conclusions: FCM results is a high molecular CR rate, with BCL-2/JH assessment by R-FCM being a reliable method to assess it.

CVP PLUS RITUXIMAB COMPARED TO CVP ALONE IN PREVIOUSLY UNREMTREATED PATIENTS WITH FOLLICULAR LYMPHOMA: IMPACT OF BASELINE PROGNOSTIC FACTORS

K. Imrie1, A. Belch1, R. Pettengell1, A. Rueda1, J. McKendrick2, P. Solal-Céligny2, F. Offner2, J. Bence-Backer3, I. Walewski1, J. Raposo4, R. Marcus5

1Medical Oncology, Northern General Hospital, Sheffield, UK; 2Hôpital Saint-Joseph, Lyon, France; 3The Christie Hospital, Manchester, UK; 4NHL Biostatistics, The Netherlands Cancer Institute, Amsterdam, The Netherlands; 5The Royal Marsden Hospital, Sutton, UK
**HOW MUCH RITUXIMAB IS NEEDED FOR PATIENTS WITH FOLLICULAR NON-HODGKINS LYMPHOMA: A MULTICENTER, RANDOMIZED TRIAL COMPARING 1.3 OR 6 INFUSIONS OF RITUXIMAB ADDED TO 6 CYCLES OF CHOP CHEMOTHERAPY (HD2000-TRIAL)**


**Purpose:** The combination of chemotherapy with the chimeric anti-CD20 antibody Rituximab has been reported to be highly active in the treatment of follicular lymphoma. The frequency and dosage of rituximab required to induce the maximum effect in follicular NHL is not defined. To evaluate how often rituximab should be added to standard chemotherapy to achieve maximum response, a multicenter, randomized phase II study was conducted. Data from a first interim analysis are presented here.

**Methods:** Patients (pts) with stage II/IV CD20 positive follicular NHL who were chemotherapy-naive were randomized to receive 6 courses of a standard CHOP-21 chemotherapy, accompanied by rituximab 375 mg/m² at day 0 only with the first CHOP course (arm A), with the first 3 CHOP courses (arm B) or with all 6 CHOP courses (Arm C). The major endpoint was the rate of molecular remission in bone marrow and peripheral blood in initially (14;18)-positive pts, assessed by PCR. Other endpoints of the study were overall and complete response rates, toxicity and time to progression.

**Results:** Since September 2000, 112 pts with a median age of 57 years (range 30–80) were recruited. 36 pts were randomized to arm A, 40 to arm B and 36 to arm C. So far 69 pts have been documented completely after all 6 cycles and are evaluable for side effects. All three treatment arms were well tolerated. The incidence of adverse events and Grade 4 toxicity was similar in all groups. The overall response rate (OR) of the whole group, which was evaluable in 72 pts so far, was 86%, with 20 complete and 42 partial remissions. Response rates are comparable in the three treatment arms (arm A 84%, arm B 96%, arm C 97%, P=0.1).

**Conclusion:** This multicenter, randomized, phase II trial addresses for the first time the optimal frequency and dosage of rituximab infusions in the combined immuno-chemotherapy. In the first interim analysis, similar toxicity and response was observed in all treatment arms.

**RITUXIMAB IN COMBINATION WITH CHOP RESULTS IN A SIGNIFICANTLY SUPERIOR RESPONSE RATE AND TIME TO TREATMENT FAILURE IN FIRST-LINE TREATMENT OF LYMPHOMAS MOYOCYTOIDIC IMMUNOCITOMA (LP-IC) – A PROSPECTIVE RANDOMIZED TRIAL OF THE GERMAN LOW GRADE LYMPHOMA STUDY GROUP (GLSG)**

C. Buske1, M. Dreyling5, H. Eimermacher1, H. Bock1, M. Pfreundschuh1, B. Metzner1, M. Unterhalt1, W. Hiddemann1
1Dept. of Medicine III, Univ. Hospital Grosshadern/LMU, Munich, Germany; 2Dept. of Medicine II, Catholic Hospital Hagen, Hagen, Germany; 3Practice, Hem./Onc., Offenbach, Germany; 4Dept. of Medicine I, Univ. Medical School Saarland, Homburg, Germany

**Introduction:** Advanced stage lymphomas/myelocytic immunocytoma (LP-IC) according to the Kiel classification is an indolent lymphoma, which cannot be cured by conventional treatment. Thus, there is an urgent need to analyze new treatment approaches in this lymphoma subtype.

**Methods:** The GLSG investigated the efficacy of a combined immunochemotherapy consisting of Rituximab (375 mg/m²) and combination chemotherapy CHOP (R-CHOP) versus CHOP alone as first line treatment of advanced LP-IC in a multicenter prospective randomized phase III trial.

**Results:** Of 75 patients 72% were classified as lymphomas/myelocytic lymphoma, 28% as lymphomas/myelocytic subtype by central pathology review. The median age was 61 years; 23% of the patients had an intermediate-high to high-risk IPI score. The overall response (OR) rate was significantly improved by R-CHOP compared to CHOP alone with an OR of 92% (11% CR, 81% PR) and an OR of 70% (56% CR, 65% PR), respectively (P=0.02). Patients treated with R-CHOP showed a significantly prolonged time to treatment failure (TTF) with a median not reached compared to an estimated median TTF of 1.8 years in the CHOP arm after a maximum follow up of 4 years (P=0.003). There was no major difference of the toxicity in both treatment groups.

**Conclusion:** In conclusion, these data demonstrate, that R-CHOP is significantly superior to CHOP alone in patients with advanced stage LP-IC, characterizing R-CHOP as a highly effective regimen for the first line treatment of this distinct lymphoma subtype.

**THE ADDITION OF RITUXIMAB TO A FLUDARABINE COMBINATION (RF-FCM) SIGNIFICANTLY IMPROVES REMISSION RATES AND OVERALL SURVIVAL IN RECURRENT FOLLICULAR AS WELL AS MANTLE CELL LYMPHOMA – FOLLOW-UP OF A PROSPECTIVE RANDOMIZED TRIAL OF THE GERMAN LOW GRADE LYMPHOMA STUDY GROUP (GLSG)**

M. Dreyling1, R. Forstpointner4, W. Ludwig3, H. Gramatzki1, H. Bock4, M. Haene1, H. Ward1, R. Parwaresch1, M. Unterhalt1, W. Hiddemann1, R. Robert-Rüssle Hospital, Charite, Berlin, Germany; 3University Hospital, Erlangen, Germany; 4Practice of Hematology, Offenbach, Germany; 5Hospital Chemnitz, Chemnitz, Germany; 6Hospital Nurnberg-Nord, Nurnberg-Nord, Germany; 7Institute of Pathology, University, Kiel, Germany

**Introduction:** Rituximab monotherapy has shown a high activity in relapsed follicular lymphomas. Recently, phase III studies indicated that its addition to chemotherapy may further improve the progression-free survival substantially.

**Methods:** In 1998 a multicenter national trial was initiated in patients with relapsed or refractory follicular and mantle cell lymphoma. As
most patients had received CHOP for first line treatment, a fludarabine-
containing regimen (FCM) was chosen for salvage therapy (fludarabine 25 mg/m² d 1–3, cyclophosphamide 200 mg/m² d 1–3, mitoxantrone 8 mg/m² d 1, repeat day 28) followed by an optional Rituximab maint-
enance. A total of 4 courses were given. After the statistical analysis confirmed the significantly improved response rate of the combined study arm (R-FCM), 111 subsequent patients were designated to immuno-chemotherapy.

Results: 122 of 244 currently evaluable patients (50%) had follicular, 95 patients (39%) mantle cell and 24 patients (10%) other indolent lymphomas. In 67 randomized patients with follicular lymphoma, complete (39% vs. 23%, P = 0.12) as well as overall response rates (44% vs. 71%, P = 0.011), progression-free survival (median: 3.9 vs. 1.7 years, P = 0.029) and overall survival (74% at 4 years vs. median of 3.8 years, P = 0.033) was improved after combined immuno-chemo-
therapy. Fifty-five patients subsequently designated to the combined study arm (R-FCM) confirmed the superior remission rates (36% vs. 96%), progression-free and overall survival. Similarly, in 50 randomized MCL patients, R-FCM achieved higher complete (29% vs. 0%, P = 0.004) and overall response rate (58% vs. 46%, P = 0.282) as well as overall survival (median: 3.0 vs. 0.9 years, P = 0.031). Again, the improved remission rates (20% vs. 75%) and overall survival was con-
firmed by 45 patients subsequently designated to combined immuno-chemotherapy.

Conclusion: This is the first prospectively randomized trial which demon-
strates the superiority of a combined immuno-chemotherapy in patients with relapsed follicular and mantle cell lymphomas, both in terms of response rates but most importantly also in terms of overall survival.

COMBINED IMMUNO-CHEMOTHERAPY (R-CHOP) HAS A LONG LASTING IMPACT ON SUBSEQUENT CONSOLIDATION IN REMISSION IN FOLLICULAR LYMPHOMA BUT NOT IN MANTLE CELL LYMPHOMA

W. Hiddemann1, M. Dreyling1, R. Fussmeister2, M. Krebs1, N. Schmitz1, R. Schmitz1, B. Metzner1, M. Reiter1, R. Parwaresch1, M. Unterhalt1

1Department of Medicine III, LMU, Munich for the GLSG, Germany; 2University Hospital Kiel, Germany; 3Institute of Pathology, University Kiel, Germany

Introduction: The addition of Rituximab (R) to combination chemother-
apy has been shown to increase the remission rate and to prolong the time to treatment failure (TTF) both in follicular lymphoma (FL) and mantle cell lymphoma (MCL). However, the impact of the combined immuno-
chemotherapy on subsequent consolidation strategies in remission remains unclear.

Methods: The GLSG embarked on two parallel studies in FL and MCL comprising a prospectively randomized comparison of R-CHOP, CHOP alone followed by a second randomization in remission for Inter-feron alpha maintenance (IFN) versus myeloablative radio-chemotherapy with subsequent stem cell transplantation (PBCT) in patients <60 years while older patients received IFN maintenance.

Results: In 428 pts, with FL, R-CHOP revealed a significantly longer TTF (median not reached vs. 2.6 yrs, P = 0.0001). From 347 patients eval-
uable for consolidation in remission, 79 younger patients received PBCT, and 121 patients were designated to IFN maintenance. Additional 122 older cases received IFN. For IFN maintenance, a significantly longer progression-free survival (PFS) was observed after R-CHOP (estimated PFS at 2 years: 94% vs. 63%, P = 0.0004) whereas no differences were encountered after PBCT (estimated PFS at 2 yrs for the total group: 86%). Hence, in FL initial therapy with combined immuno-chemotherapy seems to have a long lasting impact on PFS which may be comparable to other multimodal approaches (chemotherapy boosted by PBCT). In MCL, current, R-CHOP achieved a 20% increase in response rate (94% vs. 75%, P = 0.005) whereas only minor differences in PFS were observed after R-CHOP vs. CHOP and subsequent therapy with IFN or PBCT.

Conclusion: These data implicate a differential effect of combined immuno-chemotherapy administration (R-CHOP) in FL and MCL. While in FL, the addition of Rituximab to CHOP has a long lasting beneficial effect with a substantial impact on subsequent consolidation in remission, in MCL the major improvements are restricted to the remission induction period only.

FRACTIONATED RADIOIMMUNOTHERAPY (RIT) WITH IO-D131 LABELLED RITUXIMAB IS FEASIBLE AND EF-FICACIOUS IN RELAPSED LOW GRADE NON-HODGKIN LYMPHOMA

T. Bilde1, M. Bayne2, M. Zivanovic3, B. Mead4, P. Johnson5 et al.

1Clinical Oncology, Christie hospital, Manchester, UK; 2Oncology, Southampton University Hospitals, UK; 3Nuclear medicine, Southampton University Hospitals, UK

RIT produces high rates of durable complete responses in "low grade" NHL. A single infusion of a radionuclide-anti-CD20 mAb (131I tiotu-
omab and 125I itibritumomab tiuxetan in patients with <25% bone marrow involvement has become established clinical practice. In this study, we have tested the safety and efficacy of 4 weekly infusions of Rituximab fol-
lowed by 2 fractions of 131I labelled rituximab given 8 weeks apart in relapsed "low grade" NHL and have included patients with higher levels than 25% bone marrow involvement of lymphoma. Whole body dose (WBD) calculations have been used to allow the total dose given in 2 fractions to be increased to 120Cq. A unique anti-rituximab idiotype mAb has been generated enabling serial analysis of serum rituximab concen-
trations, that can bind to Rituximab already bound to the surface of lymphoma cells (Cragg et al. Blood 2004 104(8):2540). This tool has allowed us to accurately quantify the serum levels of rituximab during the entire treatment schedule. In this study we show the effects that 4 weekly infu-
sions of rituximab, and two fractions of RIT has on the clearance and biodistribution of the 131I labelled rituximab. Sequential pharmacoki-
etic analyses have identified wide variation in the effective half-life of 131I-rituximab not only between patients but also within the same patient over the course of the treatment protocol. We found that the mean effective half-life of 131I-rituximab increased from 43 hours prior to induction rituximab to approximately 106 hours prior to the second fraction of 131I-rituximab. A strong inverse correlation was found between the patients’ disease burden and the clearance rate of rituximab, both in the same patient as the tumor burden decreased and between patients. We have demonstrated that higher cumulative WBD doses can be safely delivered by two fractions of 131I Rituximab than can be given with a single dose of anti-CD20 (80% higher than is given with 131I Tositumomab) with all 14 patients in the first dose cohorts experiencing responses and response
durations equal to or in the majority of cases superior to that seen with their previous chemotherapy regimen. In conclusion, this RIT protocol allows some patients with >25% bone marrow infiltration to be treated safely. Fractionated RIT is feasible, efficacious, and enables larger WBD doses to be safely delivered than has previously been achieved with non-myeloablative RIT.

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LONG-TERM FOLLOW-UP OF PATIENTS RECEIVING TOSITU-MOMAB AND IODINE I 131 TOSITU-MOMAB FOR RECURRENT AND REFRACTORY B-CELL LYMPHOMA


1Cancer Research UK Medical Oncology Unit, St Bartholomew's Hospital, London, UK; 2Department of Medical Oncology, Christie Hospital, Manchester, UK

Between March 1999 and Feb. 2001, 90 patients (pts.) received Tositumomab and iodine I 131 Tositumomab (BEXXAR®) at St Bartholomew’s Hospital, London, Christie Hospital, Manchester either in open phase II trials (n=55) (JCO 2000;18(s):1316 and JCO 2004;22(8):1469) or on a compassionate release basis (n=35). Long-term follow-up (35 pts.) was performed. The median age was 53 yrs (range 27-90); 37 pts. had bone marrow infiltration (>25%). Therapy was delivered to a median of 3.5 yrs (range 2 months-18yrs) from diagnosis. Thirty three pts. were treated at 1st recurrence, 23 at 2nd and 32 at ≥3rd recurrence. Twenty-three pts. had not responded to their last chemotherapy (2 pts had primary refractory disease), 10 pts. were rituximab refractory and 11 pts. had relapsed following high dose therapy, the latter receiving an attenuated total body dose of 45Gy. Response was initially assessed at 7 weeks and 3 monthly thereafter. All 90 pts. were included in the analysis despite 4 pts. receiving only the dosimetric step (3 because of disease progression and 1 developed human anti-mouse antibodies). The overall (mixed) response rate (ORR) was 60% (CR/CRu 33%) and by histology, follicular lymphoma (n=60) ORR 73% (CR/CRu 42%), transformation to diffuse large B-cell lymphoma at the time of therapy (n=18) ORR 33% (CR/CRu 17%), lymphoplasmacytoid lymphoma (n=5) ORR 60% (1 pt. CRu, 2 pts. PR), mantle cell lymphoma (n=5) ORR 20% (PR 1 pt.). Two pts. with small lymphocytic lymphoma did not respond. For rituximab refractory patients the ORR was 60% (CR/CRu 10%) and for pts. with recurrence after HDT the ORR was 45% (CR/CRu 18%). Median progression free survival (PFS) for all pts. was 6 months (95% CI 15.6–10.7). There was no significant decline in ORR or PFS by number of progressions. However, PFS was significantly superior for pts. in whom CR/CRu was achieved compared with those entering PR after median 5 years (95% CI:1.6 to 11.6) vs 6 months (95% CI: 5.6 to 9.1, P<0.0001). Histological subtype, number of disease progressions, number of previous chemotherapies, BM involvement, response to therapy, total body dose and prior HDT did not correlate with an inferior chance of achieving CR/CRu among responders, however pts. that were refractory to rituximab were less likely to achieve CR/CRu (P<0.001).

To date 1 pt. has developed IMDS and 7 pts. have required thyroxine replacement. Achieving CR/CRu following Tositumomab and iodine I 131 Tositumomab is associated with durable remissions.

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PHASE II TRIAL OF CLADRBINE (2-CDA) AND RITUXIMAB (R) IN PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINAEMIA (WM) OR SMALL LYMPHOCYTIC LYMPHOMA (SLL): PRELIMINARY REPORT OF A MULTICENTER STUDY


1Hematology, European Institute of Oncology, Milan, Italy; 2Climical Research and Development, Lippeton AG, Arlesheim, Switzerland; 3Oncology, National Tumor Institute, Naples, Italy

Nucleoside analogues and R, alone or in combination, represent the main choice for the treatment of symptomatic WM and SLL. Aim of the study is to test the efficacy of the 2-CDA-R combination, investigating the clinical effect and the toxicity by immunocytometric, molecular and pharmacogenomic studies. Newly diagnosed WM or SLL pts requiring systemic treatment due to Hg<30% or PLT<100,000 or symptomatic neuropathy/criglobulinemia, or not previously treated with either nucleoside analogues or Rituximab, or were eligible for the study. The therapy consists of an infusion of R (375mg/mq) on day 1 followed by 2-CDA 0.1mg/kg (sc injection) for 5 consecutive days. Each cycle was administered monthly for 4 times. Before treatment, immunocytometric (CD38, ZAP70) and molecular (IgH rearrangement) study was performed in order to correlate the clinical response to the treatment. So far 15 pts (8 WM, 7 SLL) have been enrolled in this multicenter trial. Pts characteristics includes: sex (M/F) 8/7, median age 62 (52–70 yrs), 9 newly diagnosed (5 WM, 4 SLL); WM pts presented Hg<30% in 28%, PLT<100,000 in 12%, peripheral neuropathy in 50%, peripheral IgM>4g/dl in 12%. With the exception of 2 pts (1 discontinued R due to cardiac toxicity during the 1st infusion and in the other one 2-CDA dosage was reduced after 2 cycles due to G3 neutropenia), the treatment was well tolerated. 20% of pts developed G3 neuropenia and 27% G3 lymphopenia; no infection was noted despite the lack of antimicrobial prophylaxis. Among 9/15 pts evaluable for response, we observed: 2 CR (SLL), 4 uCR (WM) and 3 PR (SLL); All WM pts presented a monoclonal IgH rearrangement either in BM or PB; 5/8 showed an IgH rearrangement suggestive for post germal centre status without any correlation with CD38 and ZAP70 expression. SLL pts presented a IgH rearrangement suggestive for pre germal center status, 5/6 were positive for ZAP70 expression. Of 8 pts evaluable for molecular response at the end of the treatment, 50% of WM pts and 25% of SLL pts presented a molecular clearance in BM and PB. Basing on preliminary results, the combination of 2-CDA and R seems to be safe and active; the rate of molecular response observed in WM pts needs to be confirmed in a larger number of pts and may justify to prolong accrual.

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FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN WALDENSTRÖM MACROGLOBULINEMIA: RESULTS IN 49 PATIENTS

J. Tamburin, V. Lévy, Carine Chatelais, Jean Paul Perdman, Alain Devereux, Alain Lagrou, Pierre Morel, François Dreyfus, Marie José Grange, Bernard Christian, Sylvain Choquet, Yvonique Lohblond

Service d’hématologie, Hospital Pitié-Salpêtrière, Paris, France

Purpose: Fludarabine therapy gives a response rate of about 30% in previously treated patients with Waldenström macroglobulinemia (WM). The combination of fludarabine (FDR) and cyclophosphamide (Cy) has been shown to be effective in chronic lymphoproliferative disorders.

Patients and methods: we administered the combination of FDR (25mg/m² IV D1-D3) and Cy (300mg/m² IV D1-D3) to 49 patients. Median age was 64 years (54–83 y). The median (min-max) hemoglobin, albumin, beta 2 microglobulin and IgM levels were 9.9±10.0 g/dl (4.6–14.9), 39.6±22.2 g/l (23–60.2), 3.5mg/l (1.4–7.6) and 24.7±10.3 g/l (2–71.3) respectively. Fourteen patients (29%) had not previously been treated. FDR/Cy was administered every 4 weeks for a median of 4 cycles.

Results: Thirty-eight patients (77.6%) had partial responses, nine (18.9%) had stable disease and two (4.1%) had progressive disease. After a median follow-up of 25 months, six patients relapsed and two patients developed large-cell lymphoma. The median time to treatment failure was 27 months. The major toxicity was hematological. Twelve patients died, four from progression, one from large-cell lymphoma, three from infection and four from a second malignancy. In multivariate analysis, two factors negatively influenced overall and event-free survival, namely age >65 years and IgM<40g/l.

Conclusion: The FDR/Cy combination therefore gives a high response rate in WM, even in previously treated patients with factors of poor prognosis.

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BONE MARROW INVOLVEMENT IN B-CELL LYMPHOMA: A POPULATION-BASED REGISTRY ANALYSIS

M. Chabannah1, M. Saville2, D. Mattei, R. Gascoyne1

1Pathology, BC Cancer Agency, Vancouver, BC, Canada; 2Research and Development, Biogen Idec Inc, San Diego, USA

The aim of this study was to review the frequency, extent, and patterns of bone marrow (BM) involvement in a cohort of B-cell
non-Hodgkin's lymphomas (NHLs). A total of 1424 cases were included from our registry database between the dates 1/1/2000 and 31/12/2003.

Most patients with diffuse large B-cell lymphoma (DLBCL) had focal interstitial disease with a small reactive T-cell component, the exception being T cell-rich B cell lymphoma. Of note, approximately 9% of DLBCL had discordant low-grade lymphoma. Forty-two percent of follicular lymphoma (FL) BMs were involved at diagnosis and revealed focal paratrabeicular infiltrates as the most common pattern. Approximately 15% of FL patients had >25% BM involvement. The extent of disease was variable; in many cases reactive T cells outnumbered the neoplastic B cells. The percent of CD20+ B cells vs total lymphocytes in BMs of FL patients varied from 5 to 70%. Mantle cell lymphoma (MCL) commonly involved the BM (83%), with an interstitial pattern and infrequent reactive T cells. Nodal marginal zone lymphoma (MZL) showed BM+ in 42%, while splenic MZL (91%) commonly revealed an intrasinusoidal (IS) pattern. In conclusion, staging BM examination in FL prior to treatment planning requires an estimate of the percentage of BM involvement, accurate description of cell type, and immunostains to quantify the neoplastic B-cell component.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Pos (%)</th>
<th>Neg (%)</th>
<th>Discordant (%)</th>
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<td>44 (8.6)</td>
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<td>MCL</td>
<td>92 (83)</td>
<td>18</td>
<td>0</td>
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<tr>
<td>MZL Nodal</td>
<td>14 (42)</td>
<td>17</td>
<td>0</td>
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<tr>
<td>MZL Splenic</td>
<td>29 (91)</td>
<td>2</td>
<td>1</td>
<td>32</td>
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<td>MALT</td>
<td>27 (19)</td>
<td>104</td>
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<td>142</td>
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<tr>
<td>SLL</td>
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<td>LPL</td>
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<td>5</td>
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7. Hodgkin's Disease

TRENDS AND SOCIO-ECONOMIC INEQUALITIES IN CANCER SURVIVAL OF PATIENTS WITH HODGKIN'S DISEASE DIAGNOSED IN ENGLAND AND WALES BETWEEN 1986 AND 1999

J. Bohilis1, B. Rache1, M. Coleman1
1Department of Internal Medicine, University of Cologne, Cologne, Germany; 2Non-communicable Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, UK

Background: As with other cancers in England and Wales, survival from Hodgkin's disease is influenced by socio-economic background.

Objective: The present study analysed i) relative survival trends over time; ii) the survival gap by deprivation; iii) whether the survival gap by deprivation changed over time in patients with Hodgkin's disease diagnosed in England and Wales.

Methods: Anonymised data for 14,831 patients with Hodgkin's disease diagnosed in England and Wales between 1986 and 1999 and followed up until the end of 2001 were analysed. We estimated 5-year relative survival, defined as the ratio of the observed survival of cancer patients and the survival that would have been expected if the patients had had the same age- and sex-specific mortality in each time period as the general population. Time periods compared were 1986 to 1990, 1991 to 1995 and 1996 to 2001. Deprivation per area was categorized into five groups. Survival gradients across the five categories of deprivation were estimated with linear regression, weighted by the variance of the relative survival estimate. Men and women were analysed separately.

Results: For men relative survival improved consistently and statistically significantly throughout the 1980s and 1990s. On average, 5-year survival improved by 6.25% (95% CI 2.62 to 9.88) every 5 years. For women, there was no evidence for a statistically significant improvement in 5-year survival over time. In men diagnosed between 1996 and 1999 there was evidence for a survival gap between patients from affluent and deprived areas (−7.20%; 95% CI 3.04 to −11.36). For women diagnosed between 1996 and 1999 there was no evidence for a survival gap between patients from affluent and deprived areas. There is no conclusive evidence whether the survival gap by deprivation changed over time.

Conclusion: The present analysis showed that survival probabilities for patients with Hodgkin's disease improved consistently throughout the 1980s and 1990s. However, the existing deprivation gradient was shown to confirm that survival for Hodgkin's disease in England and Wales depends on socio-economic background. Research is required to assess whether inadequate access to effective diagnosis and therapies underpins these socio-economic inequalities.

FAMILIAL HODGKIN LYMPHOMA (FHL): PAEDIATRIC ONSET IN THREE OUT OF FIVE SIBLINGS

P. Kamper1, E. Kjeldsen2, N. Clausen2, K. Bendix1, S. Hamilton-Dutoit2, F. d'Amore2
1Department of Haematology, Aarhus University Hospital, Aarhus C, Denmark; 2Cancerubiotics, University Hospital Aarhus, Aarhus C, Denmark; 3Pediatrics, Aarhus University Hospital, Århus N, Denmark; 4pathology, Aarhus University Hospital, Aarhus C, Denmark

Introduction: This is a report on FHL describing a family with five children of whom three were HLA class I genotype identical. Within a period of six years, three of these siblings (one girl and two boys) were diagnosed with Epstein-Barr virus (EBV)-positive Hodgkin lymphoma (HL).

Methods: In situ hybridisation for EBV-encoded small nuclear RNAs 1 and 2 (EBER-ISH) was performed by standard non-isotopic technique. Germine mutation analysis was performed on peripheral blood lymphocytes.

Results: Two of the siblings were diagnosed at age 12, the other at age 5. All three cases were localised at diagnosis i.e. Ann Arbor stage I/1 (two cases) and IIA (one case), without evidence of bulky disease. All three were treated with chemoradiotherapy. Two are in a still ongoing complete remission (CR) at 3 and 8 years after end of treatment. One experienced a late relapse, but has now completed her second year in CR after ending second line treatment. The presence of EBV in tumour cells was demonstrated by EBER-ISH and immunohistochimical staining for latent membrane protein 1. The three siblings were also found to be HLA class I genotype identical (A26,B83,A9,B35). A genetic analysis performed on peripheral blood samples of all family members (the five siblings and their parents) did not reveal germine mutations. None of the children had overt immunodeficiency or autoimmune disease.

Conclusions: Genetic factors may be involved in HL. In fact, FHL cases may share HLA haplotypes and antibodies with the autoimmune lymphoproliferative syndrome. A polymorphism in the HLA class 1 subregion is associated with an increased susceptibility to develop EBV-positive HL. The present cases contribute to a further characterisation of FHL, and to the hypothesis of a role played by HLA class I antigens, intratumoural EBV genome and/or a combination of the two in the pathogenesis of at least some cases of HL.

HODGKIN'S DISEASE VARIANTS OF RICHTER'S SYNDROME: AN UPDATE OF THE MD ANDERSON EXPERIENCE

L. Faye1, J. Dang1, M. Keating1
1Lymphoma/Myloma, Univ. of Texas MD Anderson Cancer Center, Houston, USA; 2Medical Oncology, Univ. of Texas MD Anderson Cancer Center, Houston, USA; 3Leukemia, Univ. of Texas MD Anderson Cancer Center, Houston, USA

Introduction: Hodgkin's disease (HD) and Chronic lymphocytic leukemia (CLL) are rarely associated together, and this condition has been called HD variant of Richter's transformation.

Methods: A retrospective review of the CLL database over 33 years has shown 15 cases of HD. The median age at the time of the HD diagnosis was 71 years (range 51–78), male 80%, B symptoms (73%), rapidly progressive lymphadenopathy (86%), prior CLL therapy (87%), advanced Ann Arbor stage (80%) and marrow involvement (40%). The Reed–Sternberg cells were CD51+ in 12 of 13 tested and CD20+ in 1 of 12 tested. Mixed Cellularity HD was the most common subclassification type identified (8 of 9 determined). The median time to develop HD after a diagnosis of CLL was 6 months (range 0–96).

Results: Twelve evaluable patients were treated for HD with chemotherapy and achieved a response rate of 66% (4 partial responses, 4 complete responses). Four are alive without recurrence of their HD. The longest HD-free survival is 73 months.

Conclusion: Previous reports have suggested that these patients have a poor prognosis with a mean survival of just over one year. This update of the experience at this institution shows a more favorable picture than previously reported in a subgroup of those patients.

SALVAGE RADIOTHERAPY (SRT) FOR HODGKIN'S LYMPHOMA (HL) AFTER CHEMOTHERAPY FAILURE: LONG TERM FOLLOW-UP

A. Winch1, B. Campbell1, A. Miller1, M. Macnmanus1,1, D. Ryan1
1Radiology, Peter MacCallum Cancer Centre, East Melbourne, Australia; 2Centre for Biostatistics, Peter MacCallum Cancer Center, Australia

Introduction: The long-term results and prognostic factors for SRT after chemotherapy failure for HL are not well defined.

Methods: We performed a retrospective study of patients (pt) with HL, who had failed at least 1 prior chemotherapy regimen, and received SRT. pt with relapse in a previously irradiated field, and pt undergoing high-dose chemotherapy were excluded.

Results: Between 1974–2001, 81 eligible pt were treated: 62 SRT alone, and 19 SRT plus salvage chemotherapy. The median age was 31 years, and prior chemotherapy regimens included 12 MOPP/MOPP-like (99%), 2 OVP (3%), ABVD (9%), other (4%). After a median time of 7 years, 62 pt were still disease-free.

Conclusion: SRT produced long-term remissions in a proportion of pt with Hodgkin's disease after prior chemotherapy failure. These results support the incorporation of SRT with salvage chemotherapy/transplantation for HL. SRT is also an option for pt unuitable for further chemotherapy.
Conclusion: We confirmed the safety, tolerability and high response rate to SV, with comparable results to previous studies in this population. A phase III study to undertake the same comparison with power to compare FPS and OS is now in progress and has accrued a further 200 patients to date.

THE OUTCOME OF PATIENTS WITH RELAPSED HODGKIN'S LYMPHOMA: THE GILO EXPERIENCE


1 Dipartimento di Oncologia e Ematologia, University of Modena and Reggio Emilia, Modena, Italy; 2 Ematologia, A.O. “Bianchi Melacrino Morelli”, Reggio Calabria, Italy; 3 Oncologia Medica, A.O. “A. Manzoni”, Lecco, Italy; 4 Clinica Medica, Università di Pavia, Pavia, Italy

Background: Although Hodgkin’s Lymphoma (HL) ranks among the most curable neoplastic diseases approximately 15–20% of patients relapse after the achievement of a complete remission (CR1). These patients are treated with salvage therapies, but the chance of a further durable remission is usually disappointing.

Methods: We have conducted a retrospective analysis on patients with HL treated by the Gruppo Italiano Studio dei Linfomi (GILS) in different prospective trials and relapsed after CR1, with the aim of assessing the efficacy of salvage treatments in these settings.

Results: Nine-hundred and eighty cases out of 1080 newly diagnosed patients with HL entered into GILS’s prospective clinical trials from 1988 to 2002 achieved a CR1. The median follow-up of the whole series was 73 months (range 1–192). All cases were reviewed and updated between January and March 2004 allowing the identification of 115 relapses which are the subject of this analysis. Initial therapy of relapsed cases consisted of 6 courses of VBM based therapy plus IF-RT for patients at low risk (17%), 4–6 courses of EVPE, 4 courses of ABVD or EVPE plus IF-RT for those at intermediate risk (31%) and of 6 courses of ABVD, MOPPEV/VCAD, BEACOPP or 12 weeks of Stanford V plus RT on residual masses or on sites of previously bulky disease for those at high risk (52%). Median duration of CR1 was 19 months (range 1–123 mos), median age of all patients was 32 years (range 17–79). In univariate analysis, factors predicting a longer duration of CR1 were IPS (0–1 vs >1), histology (NS vs others), leukocytosis (WBC>15,000/mmc) and >1 ENS. After relapse 6% patients did not receive any further therapy and died <3 months. 51% received second-line chemotherapy, 33% responded BMT; 3% received RT alone. A second CR was achieved in 67% (61% to CR1 and 76% for BMT). For patients achieving a second remission the median Survival Until Second Relapse (SUSR) was 20 months (range 1 to 166 months) with a 5 year SUSR of 47%. Patients treated with RT did not do worse than those who received CT (5-year SUSR 26% vs 68%); this difference can be partly explained by a higher frequency of patients with favourable HL in the BMT group.

Conclusion: Based on the results of the present study the chance of achieving a second remission in relapse is less distant than expected. Moreover, our data suggest that HDT plus ASCT does not appear to be superior to conventional CT as salvage therapy.

COLONEG HIGH-DOSE SEQUENTIAL CHEMOTHERAPY IN RELAPSED AND REFRACTORY HODGKIN LYMPHOMA: 40 MONTHS FOLLOW UP AND FIRST RESULTS FROM THE HDR-2 TRIAL OF THE GHSG/EOORTC

A. Joost1, C. Rudolph1, M. Mapiura1, V. Diehl1, A. Engert1

1 Department 1 of Internal Medicine, University Hospital, Cologne, Germany; 2 Department of Internal Medicine, Carl-Thiem-Hospital, Cothen, Germany; 3 Section for Hematology/Oncology, University Hospital Charité, Berlin, Germany

Purpose: We designed a dose- and time-intensified high-dose sequential chemotheraphy regimen with a peripheral blood stem cell transplantation (PBSCT) for patients (pts) with treatment failure or relapse of Hodgkin lymphoma (HD).

Patients and Methods: Eligibility criteria included age 18–65 years, proven primary progressive or relapsed HD. Treatment consists of two cycles DHAP; pts with PR or CR received cyclophosphamide 4 g/m², followed by peripheral blood stem cell (PBSC) harvest; melphalan 30 mg/m² plus vincristine 1.4 mg/m², and etoposide 2 g/m². The final course was BEAM with PBSCT.

Results: 102 pts, median age 34 years (18–65) were enrolled. The response rate 100 days posttransplantation was 87% (72% CR, 8% PR). PBSC harvest was successful and toxicity was tolerable. With a median
follow-up of 40 months (range 3–184) freedom from second failure (FF2F) and overall survival (OS) were 52% and 73% for all patients, respectively. FF2F and OS for patients with early relapse were 61% and 85%; for late relapse 62% and 81%; for PD: 41% and 45% and for multiple relapse 39% and 44%, respectively. Response after DHAP and duration of first remission and for OS response after DHAP, duration of first remission and anemia.

Conclusion: This regimen is feasible, tolerable and effective. Based on these results a prospective randomized study was started comparing this regimen with two courses of DHAP followed by BEAM, first results of this study will be presented.

CLINICAL PRESENTATION AND TREATMENT OUTCOME IN LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA: A REPORT FROM THE GERMAN HODGKIN STUDY GROUP
Department I for Internal Medicine, University Hospital, Cologne, Germany

Purpose: To investigate the clinical characteristics, course, and treatment outcome of patients with lymphocyte-rich classical Hodgkin lymphoma (LRCCHL) in comparison to patients with other subtypes of classical Hodgkin lymphoma (CHL) and lymphocyte-predominant Hodgkin lymphoma (LPHL).

Methods: We performed a retrospective analysis using the database of the German Hodgkin Study Group (GHSG). Among 2715 assessable patients with biopsy-proven Hodgkin lymphoma (HL) treated within the trials HD7 to HD12 of the GHSG, we identified 100 cases of LRCCHL, 145 cases of LPHL, 1688 cases of nodular sclerosis (NS), 731 cases of mixed cellularity (MC), and 23 cases of lymphocyte depleted HL (LD).

Results: Patients with LRCCHL were predominantly male with a male to female ratio of 2:1. The median age at diagnosis was 39 years (range 16–74) years. Theses Patients typically presented with early stage disease (Stage I: 34%; Stage II: 46%). The median time of follow-up was 32.2 months (95% confidence interval CI 29.2–35.3). Complete remission was achieved in 96 (96.0%) and partial remission was achieved in 4 patients (4%). At 30 months the event free survival was 97% (95% CI 96.7–96.9). The overall survival at 30 months was 97% (95% CI 96.8–97.0). Only 3 patients died, all of them due to treatment related toxicities resulting in an Hodgkin-specific event free survival of 100%.

Conclusion: Patients with LRCCHL are on average older and usually present in early stages. They have an excellent treatment outcome with the current modern treatment modalities applied in the trials of the GHSG. Further strategies should focus on reducing treatment intensity.

OSTEONECROSIS AS A COMPLICATION OF TREATING HODGKIN'S LYMPHOMA AFTER BEACOPP CHEMOTHERAPY
J. Markova, M. Zidka, T. Kozak et al
University Hospital, Karlovo Vino Brnady, Prague, Czech Republic

Introduction: In this study we have analysed the incidence, risk factors, and morbidity for osteonecrosis (ON) as a complication in patients (pts) with HL treated with BEACOPP regimens.

Methods: The occurrence of symptomatic ON was investigated retrospectively in 124 pts with primary HL. 110 pts (93%) from this group was randomised to the 3rd and 4th generation trials of German Hodgkin Lymphoma Study Group (GHS) for intermediate and advanced stages (HD9, HD11, HD12 study) since 1995 until 2003. Median age at the time of treatment HL was 31 years (range 18–66).

Results: During the median observation interval of 48 months (range 26–110) ON was diagnosed in 16 pts (12%). 14 pts in advanced stages (HD9, HD11, HD12) and two pts in intermediate stages (HD11C). The incidence was higher for males (11 pts) versus females (5 pts). ON was diagnosed within 3 years of starting the HL therapy, median 22 months (range 10–30). Multifocal ON was revealed in 9 pts (56%). The cumulative incidence of prednisone dose was 8960 mg (range 3900–10000). At our institution dexamethasone was added as a component of combined antiemetic therapy, median dose was 148 mg (range 0–280). The mean duration of chemotherapy was 5.6 months (range 3–6). Symptoms of pain and/or immobility were chronic in 12 pts and 5 out of 16 pts have undergone orthopedic procedure (4 total hip and one knee replacement arthroplasty).

Conclusion: Dexamethasone may have additive or synergistic effects along with prednisone and may play a leading role in the development of ON.


ROLE OF HEMATOTOXICITY IN FEMALE PATIENTS WITH HODGKIN'S LYMPHOMA
D. Hodges, P. Petersen, M. Gospodarowicz, M. Pittilie, W. Wells, A. Sun, E. Holowaty, M. Crump, R. Tsang
1Radiation Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada; 2Medical Oncology, The Finsen Centre, Copenhagen, Denmark; 3Biostatistics, 4Oncology, The Finsen Centre, Copenhagen, Denmark; 5MOPP/ABVD (19).

Purpose: Several scores have described female gender as protective prognostic factor in patients with Hodgkin's lymphoma (HL). However, very little is known about gender-specific factors that influence treatment outcome and treatment-related variables and their influence on the outcome of HL patients.

Methods: This analysis comprises 4626 HL patients of all prognostic risk groups, who were enrolled into the multicenter studies HD4–HD9 of the GHSG. At a median observation time of 5.5 years, 2050 female (f) and 2576 male (m) consecutive patients were analyzed.

Results: Male and female patient characteristics were very similar in terms of age, performance status, stage, histological subtype, clinical risk factors and prognostic factors. However, there was acute hematotox- ery-related hematotoxicity in women. This difference was most obvious for severe leucopenia (WHO grade III/IV: 69.9% (f); 55.2% (m) for severe leucopenia, (p<0.0001)). Importantly, this did not translate into more infections. Female patients had similar response rates but fewer relapses and deaths. Female patients had similar response rates but fewer relapses and deaths. Severe leucopenia during chemotherapy was strongly associated with better outcome.

Conclusion: In this GHSG analysis, a better outcome was observed for female HL patients. The protective role of severe leucopenia supports more individualized therapy, that may be tailored in a response-adapted manner depending on the individual toxicity profile within the first cycles.

SECOND CANCERS IN HODGKIN’S LYMPHOMA SURVIVORS: UNDERESTIMATION OF RISK BASED ON INSTITUTIONAL VS. POPULATION-BASED REGISTRIES
A. Sun, P. Petersen, M. Gospodarowicz, A. Prisciandaro, R. Tsang, E. Holowaty, F. Croxford, A. Sun, E. Holowaty, M. Crump, R. Tsang
1Radiation Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada; 2Medical Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada; 3Oncology, The Finsen Centre, Copenhagen, Denmark; 4Biostatistics, 4Oncology, The Finsen Centre, Copenhagen, Denmark; 5MOPP/ABVD (19).

Purpose: To assess the long-term risk of second cancer (SC) in patients treated for stage I/II Hodgkin’s lymphoma (HL), comparing data capture from an institutional database with additional case-ascertainment from a population-based cancer registry. The data were updated regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. The Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly.

Methods: Patients referred from 1968–86 with stage I/II HD were included (n=865) in an institutional database (InstDB), which was updated regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly. Data linkage with the Ontario Cancer Registry (OCR) was performed regularly.

Results: Median age at diagnosis was 31 years (range 16–90 yrs), and the median follow-up was 20 years (range 0.4–36 yrs). At last follow-up, 547 patients were alive, and 318 died (130 died of HL, 1 died of treatment-related morbidity). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15).

Conclusion: The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15). The cumulative incidence of any SC was 10% (range 5–15).
and 187 of other causes). The InstDB identified 132 patients with SC. The OCR identified an additional 46 patients with SC. The 20-year cumulative risk of SC was 13% in the InstDB vs. 15% after adding cases from the OCR. The relative risks for all second cancers in InstDB and OCR were 3.8 and 5.0 for females, and 2.1 and 2.9 for males, respectively. The corresponding absolute excess risks per 10,000 P-Y follow-up were 88.1 and 125.4 for females, and 50.6 and 80.8 for males. The greatest increase in AER was seen in cancers of the breast (36.9 to 52.5), leukemia (3.5 to 8.7), and bronchus (7.6 to 14.2).

Conclusion: SC risk was substantially underestimated based on data from InstDB compared to a population-based registry.

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RISK OF BREAST CANCER AFTER HODGKIN’S LYMPHOMA: A 30-YEAR FOLLOW-UP STUDY

F. van Leeuwen1, W. Klokman1, M. van’t Veer2, B. Aleman3
1Epidemiology, Netherlands Cancer Institute (NKI), Amsterdam, Netherlands; 2Hematology, Daniel den Hoed Cancer Center (DDHK), Rotterdam, Netherlands; 3Radiotherapy, Netherlands Cancer Institute, Amsterdam, Netherlands

Introduction: Several studies have shown that female survivors of Hodgkin’s lymphoma (HL) have a strongly increased risk of breast cancer. It is not known, however, whether excess risk persists throughout the survivors’ lives.

Methods: We assessed risk of breast cancer in 624 female survivors of HL diagnosed before age 41 and treated in the NKI or DDHK between 1960 and 1987. Median age at diagnosis of HL was 25.3 years. 39% of the patients were treated with radiotherapy alone (RT), chemotherapy alone (CT) or combined chemotherapy and radiotherapy. Follow-up for breast cancer was complete for 95% of the cohort. The numbers expected on the basis of age and calendar period-specific incidence rates of breast cancer in the Dutch population were compared with the numbers observed in our cohort.

Results: In all, after a median follow-up of 18.1 years, 45 patients had developed breast cancer, while only 7.3 were expected [RR = 6.2 (95% CI 4.3–8.3)]. For women irradiated before age 21, at ages 21–30, and at ages 31–40, the RRs were 22.0 (95% CI 13.2–34.3), 5.5 (95% CI 3.1–8.5), and 2.9 (95% CI 1.4–5.3), respectively, compared with the general population. The RR was strongly increased in the 15–19, 20–24 year, and 25–29 year follow-up period, with RRs of 10.8, 9.5 and 12.2, respectively (all p < 0.001). The Absolute Excess Risk (AER) in the 25–29 year follow-up period amounted to 2.5 excess breast cancers per 100 patient-years. In 30-year survivors the risk appeared to decrease [RR = 5.2 (95% CI 0.6–19.0)], although the difference with previous follow-up intervals was not significant. The 25-year cumulative risk of breast cancer was 16.5%. The RRs slightly decreased with increasing age of the survivor cohort, with RRs of 16.8 (95% CI 5.4–39.2) and 6.3 (95% CI 4.0–9.4) for women with an attained age of less than 35 years and women who had reached ages of 45 years and above, respectively, but the AERs remained high at older ages.

Conclusion: Excess risk of breast cancer after irradiation for HL remains strongly increased for at least 30 years after HL treatment.

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BREAST CANCER AFTER RADIOTHERAPY FOR HODGKIN LYMPHOMA: IMPLEMENTATION OF THE UK RISK ASSESSMENT AND SCREENING PROGRAMME IN A LARGE REGIONAL CENTRE


Christie Hospital NHS Trust, Manchester, UK

Introduction: Young women with Hodgkin lymphoma (HL) treated with radiotherapy (RT) involving the breasts are at increased risk of breast cancer. In the UK, the Department of Health launched a national patient notification, assessment and screening exercise in November 2003. We report the implementation of this exercise at a large regional cancer centre.

Methods: Women aged 235 years when treated for HL in the NW region, 1960–2003 were identified using the Christie Hospital and North Western Cancer Registry (NWCR) databases. Those who had received a field of RT incorporating breast tissue were invited to an outpatient clinic for risk assessment and counselling. A telephone helpline was set up to aid the recall of appropriate patients and deal with urgent concerns. Women were interviewed and following informed consent, data extracted from the case notes, risk assessments performed and screening plans discussed (annual mammography, ultrasound or MRI as appropriate in those now >25 yrs since treatment).

Results: 405 eligible women were identified from the searches and 365 (90%) replied to the letter. 9 dedicated clinics were run and of the 240 women seen, 153 required immediate annual screening. 46 had screening deferred and 41 were already receiving mammograms as part of the existing UK screening programmes for women >25 yrs. The helpline took 216 calls, 65 women enquired about eligibility and 15 (3.6% of total) had not been identified by the searches. So far, 10 breast cancers and one breast sarcoma have been identified by history (3% of cohort).

Conclusions: There is feasibility of such an exercise has been demonstrated; response rates and levels of interest were high. The helpline was a valuable tool in the identification of eligible women and providing additional information/reassurance. The impact of screening will be evaluated in this population and the national results collated.

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SECOND MALIGNANCY RISK ASSOCIATED WITH TREATMENT STRATEGY FOR HODGKIN’S LYMPHOMA

J. Frankfurt, A. Pluetschow, M. Pan
KKSG, University Hospital Cologne, Koenig, Germany

Introduction: Despite several investigations, the risks of second malignancies (SM) following radiotherapy alone (RT), chemotherapy alone (CT) and combined chemo-radiotherapy (CRT) for Hodgkin’s lymphoma (HL) remain controversial. We performed a meta-analysis of SM incidence (and other outcomes) in randomised trials comparing these modalities.

Methods: We sought individual patient data for all identified, relevant, randomised trials ending recruitment before/after 2000 which compared RT vs. CRT, CT vs. CRT, RT vs. CT or involved-field (IF) vs. extended-field (EF) RT for untreated HL. SM, progression-free survival and overall survival were compared using PETo’s meta-analytic method as well as Cox regression techniques. Both overall SM and SM excluding events after HL-relapse were analysed.

Results: (1) RT vs. CRT (15 trials, 3343 pts, mainly early stages). Overall SM risk was lower with CRT than RT as initial treatment (Peto odds ratio (OR) = 0.78, 95% confidence interval (CI) = 0.62 to 0.98, P = 0.03). This effect was seen mainly in solid tumours and seems to be due to frequent relapse and hence salvage therapy after RT. (2) CT vs. CRT (16 trials, 2861 pts). Overall SM risk was higher with CRT than CT as initial treatment (OR = 1.38, CI = 1.00 to 1.89, P = 0.05). This effect was seen in both solid tumours and in AML/MDS. (3) RT vs. CRT (3 trials, 415 pts). Insufficient data. (4) EF-RT vs. EF-R (19 trials, 3221 pts, mainly early stages, mainly adjuvant to CT). No significant difference in overall SM risk.

Conclusions: Choice of first-line treatment strategy for HL significantly influences overall (including salvage-related) SM risk. SM should be considered when making this choice, alongside overall and progression-free survival.

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INCIDENCE OF CARDIOVASCULAR DISEASE IN LONG-TERM SURVIVORS OF HODGKIN’S LYMPHOMA (HL)

B. Aleman1, W. Klokman1, I. Mulder2, H. Bartelink1, F. van Leeuwen2
1Radiotherapy, Netherlands Cancer Institute, Amsterdam, Netherlands; 2Epidemiology, Netherlands Cancer Institute, Amsterdam, Netherlands; 1IKA, Comprehensive Cancer Center, Amsterdam, Netherlands

Background: Cardiovascular disease (CVD) causes excess mortality in survivors of HL. Data on long-term incidence of CVD are still scarce.

Objective: To assess the long-term incidence of CVD in 5-year survivors of HL after primary treatment stratified by treatment and age at treatment.

Methods: We assessed the incidence of CVD in 591 5-year survivors of HL treated before the age of 41 years in the Netherlands Cancer Institute between 1960 and 1987. Median age at diagnosis of HL was 25.5 years. Thirty-nine nine % of the patients were treated with radiotherapy (RT) alone, 3% with chemotherapy (CT) alone, 29% with initial combined modality treatment without treatment for recurrences, and 29% with initial RT, or RT+CT, followed by treatment for recurrences. Twenty-two % of all patients received anthracycline-containing therapy. Information on
medical status was complete for 95% of the cohort. We compared the numbers of CVs observed in our cohort with the numbers expected on the basis of age- and gender-specific Dutch incidence rates of CVs.

Results: In all, after a median follow-up of 18.1 years, 87 out of 591 patients developed one or more CVs, while only 22.8 were expected.

Conclusion: The incidence of several types of CVs was strongly increased after treatment for HL. This risk of CV remained elevated even after prolonged follow-up. The RR of CV was significantly lower in patients who were treated with protracted maintenance therapy or salvage therapy as compared to the RR in those treated with primary CT + RT only.

RELATIVE RISK OF CARDIOVASCULAR DISEASE AFTER TREATMENT FOR ADVANCED NON-HODGKIN'S LYMPHOMA

E. Moser1, J. Kluin-Nelemans2, E. Noordijk1 et al

Introduction: We described a marked increased risk of cardiovascular complications (22%) after therapy in long-term survivors of aggressive Non-Hodgkin's Lymphoma (NHL). However, as these patients are generally much older than patients with Hodgkin's lymphoma, the increased risk could be due to age instead of being treatment-related. To estimate whether the risk is truly increased, we therefore compared the incidence of cardiovascular disease in the normal population matched for sex and age using Dutch population data.

Methods: The relative risk of cardiovascular disease was determined in 476 (Dutch or Belgian) patients with NHL treated with doxorubicin-based chemotherapy in 4 EORTC (European Organization of Research and Treatment of Cancer) trials running from 1980 till 1999. Dutch population data were used to estimate the overall number of patients and subgroup relative risks (RR). Confidence limits were obtained using the Poisson distribution.

Results: Sixty-six cases of chronic heart failure were seen (RR 1.1; 95% CI 1.0 to 1.2; p = 0.03). In 14 patients due to coronary disease (RR 1.4; 95% CI 1.0 to 1.9; p = 0.03), 20 patients due to stroke (RR 1.6; 95% CI 1.0 to 2.8; p = 0.03), and 30 patients due to valvular disease (RR 1.9; 95% CI 1.3 to 2.8; p = 0.001). The risk was increased in patients aged 65 years or older.

Conclusion: Compared to population data, cardiovascular disease is indeed markedly increased during long-term follow-up after treatment for aggressive NHL.

FERTILITY IN MALE PATIENTS WITH HODGKIN'S DISEASE – RESULTS FROM THE GERMAN HODGKIN LYMPHOMA STUDY GROUP

M. Sieniawski, A. Josting, T. Reineke, V. Diehl, A. Engert

Introduction: Treatment results in Hodgkin Disease (HD) have improved over the last two decades. Therefore long term side effects of therapy as infertility are of growing importance. To investigate the influence of disease and therapy on the fertility status in pts with HD we performed semen and hormone analysis before and after treatment.

Methods: All analyses were performed in pts with first diagnosis of HD enrolled into trials of the GHSG between 1988 and 2002. Pts had no history of chemotherapy or radiotherapy.

Results: We included 243 male pts with a median age of 27 years (range 16–58). At diagnosis 138 pts were in clinical stage (CS) I/II, 105 in CS III/IV, and systemic symptoms were present in 106 pts. 202 pts underwent fertility screening before therapy: normospermia was diagnosed in 40 pts, 40 pts had inadequate semen quality. Normal values of FSH, LH and testosterone were found in 140/151, 99/125, and 84/101 of pts respectively. 112 pts underwent at last one fertility screening after therapy. Respectively, 56/112 pts received fertility treatment, BEACOPP/chemotherapy, with systemic symptoms and elevated BSG.

Conclusion: We confirmed that HD pts had inadequate semen quality even prior to treatment. The majority of pts had azoospermia after treatment but recovery of spermatogenesis was observed, in general 2 years after the end of therapy. Hormone values usually recovered in the first two years.

FEMALE GONADAL DYSFUNCTION AFTER HODGKIN'S LYMPHOMA (HL) IS INFLUENCED BY AGE AT TREATMENT, STAGE OF DISEASE, CHEMOTHERAPY REGIMEN, AND THE USE OF ORAL CONTRACEPTIVES DURING THERAPY: A REPORT FROM THE GERMAN HODGKIN STUDY GROUP (GHSG)

K. Behringer, K. Breuer, T. Reineke, L. Nogova, B. Klimm, V. Diehl, A. Engert

Introduction: The development of delayed consequences of the anti-tumour therapy is a very important problem in the treatment of patients with Hodgkin's lymphoma (HL). These include the injury to fertility. Depending on the type of the chemotherapy administered, premature ovarian failure develops in young women, causing clinical symptoms due to the lack of estrogen, and resulting in subsequent infertility.

Objectives: To investigate the prevalence of azoospermia and result in subsequent sterility.

Conclusion: A retrospective analysis of the fertility in female patients treated with the BEACOPP or ABVD and MOPP/COVP/ABVD chemotherapy. Their gonadal functions were evaluated during and after the therapy on the basis of their personal history and hormonal profiles.

DAMAGE TO OVARIAN FUNCTIONS IN YOUNG WOMEN TREATED WITH ABVD AND BEACOPP CHEMOTHERAPY FOR HODGKIN'S LYMPHOMA

L. Smarda, Z. Kral, I. Vasova, B. Wagnerova, J. Vorlicek

Internal Department of Gynaecology, Faculty Hospital Brno, Brno, Czech Republic

Introduction: The development of delayed consequences of the anti-tumour therapy is a very important problem in the treatment of patients with Hodgkin's lymphoma (HL). These include the injury to fertility.
Methods: A total of 16 patients underwent the therapy based on the BEACOPP regimen with baseline (n=6) or escalated doses (n=4), or a combination of 4 cycles of the baseline and escalated BEACOPP (n=6) regimen. The patients’ median age was 27 years (23–44). A total of 32 patients were treated with chemotherapy in the past, namely using the ABVD (n=25) or the hybrid MOPP/COPP/ABVD alternating regimen (n=7). The median of the patients’ age was 22 years (13–45). No adjuvant radiotherapy to the abdominal or minor pelvis region was applied in these patients. Except for one patient in whom the disease progressed despite the therapy, complete remission was achieved in all of them.

Results: 5/16 (31%) of the patients reported oligomenorrhea or complete amenorrhea during the BEACOPP therapy. After 6 months from the end of the therapy, half of the patients (n=8) did not menstruate; the examination of hormonal levels showed hypergonadotropic hypogonadism in all of them (FSH levels: 30.6–98.2 IU/l; LH: 24.9–59.0 IU/l; estradiol: below 0.04 nmol/l). One patient with normal hormonal levels has been trying to get pregnant unsuccessfully so far; the remaining patients do not plan pregnancy for now. The menses disappeared during the ABVD or MOPP (COPP)/ABVD chemotherapy in 6/36 (19%) of the patients. After the end of the therapy, all patients reported regular menses. Altogether, 14 cured patients delivered 17 healthy children, 3 women aborted spontaneously in the 1st trimester, and 3 women are currently pregnant.

Conclusions: The BEACOPP chemotherapeutic regimen poses a risk of early menopause and permanent sterility to young patients owing to the presence of several gonadotoxic agents. Our results corroborate the literary data that show that the ABVD chemotherapeutic regimen is gentler on the reproductive system of post-pubertal women.
THE PROGNOSTIC VALUE OF EARLY FDG-PET DURING TREATMENT STRATEGIES FOR LYMPHOMA AND POTENTIAL USE IN RESPONSE-ADAPTED TREATMENT STRATEGIES

M. Hutcheson, G. Mikhaeel, P. Fields, T. Nunan, M. O'Doherty, A. Timothy
1Dept. of Clinical Oncology, Guy's and St. Thomas' Hospital, London, UK; 2Dept. of Haematology, Guy's & St. Thomas' Hospital, London, UK; 3Clinical PET Centre, Guy's and St. Thomas' Hospital, London, UK

Background: Modern, risk-adapted lymphoma therapy regimens require an accurate prognostic stratification. FDG-PET has excellent properties for imaging tumour metabolism in malignant lymphomas. Several studies have shown that a PET scan performed early in the course of chemotherapy has prognostic value in non-Hodgkin lymphomas. With 10 years of experience in clinical PET-imaging in lymphoma, we present a large study of the predictive value of an early interim FDG-PET in Hodgkin lymphoma (HL) and high-grade non-Hodgkin lymphoma (HG-NHL).

Patients and Methods: 206 patients (85 HL and 121 NHL) treated for HL or HG-NHL from 1993 to 2004 underwent FDG-PET after 2–3 cycles of chemotherapy. The PET scans were performed in a full-ring, dedicated PET scanner. The data were analysed retrospectively.

Results: Survival analysis showed independent, highly significant associations between interim FDG-PET results, and PFS (P < 0.0001) and overall survival (OS) (P < 0.01). Among the 85 HL patients, 63 patients had negative interim scans, 9 patients showed minimal residual uptake (MRU) and 13 patients had positive scans. 5-year progression-free survival (PFS) was 92%, 88% and 42%, respectively. Among the 121 NHL patients, 50 patients had negative interim scans, 19 patients showed MRU and 52 patients had positive scans. 5-year progression-free survival (PFS) was 89%, 59% and 16%, respectively.

Conclusion: An interim FDG-PET scan performed early during treatment is a strong predictor of final-line treatment failure in HL and HG-NHL and an accurate and independent predictor of PFS and OS. An early assessment of chemotherapy response with FDG-PET could provide the basis for selection of poor-prognosis patients for alternative therapeutic strategies. If incorporated into the management of lymphoma, this method could improve the chances of a successful treatment outcome and lower the risk of late adverse effects. Prospective controlled trials are now required to confirm the mounting retrospective evidence.

FDG-PET AFTER 2 CYCLES OF CHEMOTHERAPY PREDICTS TREATMENT FAILURE IN Hodgkin lymphoma

M. Huczynas, A. Lof, M. Hansen, L. Pedersen, F. d'Amore, A. Bosen, S. Keiding, S. Buus, L. Specht
1PET and Cyclotron Unit, The Diagnostic Centre, Copenhagen University Hospital, Copenhagen, Denmark; 2Dept. of Haematology, The Finsen Centre, Copenhagen University Hospital, Copenhagen, Denmark; 3PET Centre, Aarhus University Hosp., Aarhus, Denmark; 4Dept. of Oncology, The Finsen Centre, Copenhagen University Hosp., Copenhagen, Denmark

Introduction: The response to Hodgkin lymphoma (HL) treatment is evaluated at mid-treatment and this monitoring is mainly based on a reduction in tumour size on CT. This criterion is a late and not always reliable predictor of outcome. FDG-PET has excellent properties for imaging tumour metabolism in lymphomas. The aim of this study was to investigate the ability of FDG-PET to predict treatment failure at an early stage during chemotherapy for HL.

Methods: 77 patients receiving chemotherapy for HL were prospectively included and had PET scans before treatment and after two cycles. Stage distribution (without PET information): I: 15, II: 33, III: 22, IV: 6.

B-symptoms: 17, ≥ 40. Histology: NS: 51, MC: 17. Gender: male: 49, female: 28. Age: median: 36 yrs, range 18–74 yrs. The PET scans were performed in a full-ring, dedicated PET scanner. The clinicians were blinded from PET results. Comparisons between PET-negative and PET-positive patients were made with log rank tests.

Results: All patients had pathological PET scans before treatment. After two cycles of chemotherapy 61 patients were PET-negative and 16 were PET-positive. Two of the 61 PET-negative patients experienced progression and 61 patients were alive at the most recent follow-up.

Conclusion: An early FDG-PET is a strong predictor of treatment failure in HL and should have a role in the selection of patients for treatment adaptation. This method could help improve the chances of a successful treatment outcome and lower the risk of late adverse effects. To our knowledge, this is the largest and most homogenous prospective material to be reported on the early prognostic value of FDG-PET in HL.

PET FINDINGS IN LUNGS IN PATIENTS TREATED FOR HODGKIN'S LYMPHOMA

H. Markova, J. Markova, O. Belohlavck, et al.
1Dept. of Clinical Haematology, University Hospital Prague, Prague, Czech Republic

Introduction: The aim of this prospective study was to assess the PET findings in lungs in patients (pts) with initial pulmonary involvement of Hodgkin's lymphoma (HL) after initial treatment and during follow-up.

Methods: 41 PET studies including pulmonary findings were evaluated in 22 pts with initial pulmonary involvement of HL after treatment according to DSHG trials HD12,15 and during follow-up.

Results: Median follow-up of this group is 34 (range 7–58) months. 16 pts with initial pulmonary involvement are in complete remission (CR) after PET and HCR revealed post-radiotherapy pneumonitis in two pts and this was still present 6–39 months after radiotherapy. Two pts progressed after initial chemotherapy, but without lung involvement at the time of progression according to PET. Two pts have persistent positive lesions on PET pulmonary scans after initial therapy. These pts are observed without further progression and treatment 49–53 months after initial therapy. One pt underwent lung biopsy, that confirmed postchemotherapy changes.

Conclusions: Although pulmonary changes on PET are relatively common in pts with HL after initial therapy, most pulmonary findings in our group of pts represent postchemotherapeutic and post-radiation effects. The prognostic significance of PET positive after initial treatment in HL is still not established and all PET positive findings should be carefully evaluated in a longer follow-up.

NEW TREATMENT STRATEGIES MONITORED WITH PET FOR PATIENTS WITH HODGKIN'S LYMPHOMA

1Department of Medicine I, University Hospital, Dresden, Germany; 2Department of Nuclear Medicine, University Hospital, Dresden, Germany; 3Clinic of Internal Medicine III, Chemnitz Medical Center, Chemnitz, Germany

Introduction: Prognosis of patients with Hodgkin's lymphoma (HL) has improved significantly over the last decade. Therefore, the impact of treatment associated late effects such as second cancers increased. The purpose of this prospective multicenter trial was to show the feasibility of risk adapted chemotherapy accompanied with IF-RT in case of residual mass.

Methods: Since May 2000 a total of 131 patients with early (n = 29), intermediate (n = 41) and advanced (n = 61) stage according to the current risk factors of the GHSG were enrolled. In the limited or intermediate stage, the patients received 4 or 6 cycles of ABVD, respectively, plus IF-RT in case of residual mass. In the advanced stage, the patients were treated with the etoposide-free BACOPP-D regimen, plus IF-RT in case of residual mass. PET scans were performed in 93/131 patients before as well as after chemotherapy for response evaluation.

Results: After finishing ABVD alone CT-based response designations were 49% CR and 51% PR. PET + CT-based designations were 74% CR and 26% PR in 50 out of all 70 patients. After completion of the total therapy including IF-RT 97% achieved CR/Cru. Five patients relapsed, four of these were monitored by PET, which showed an increased FDG-

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uptake in one patient with early relapse but missed the other three cases. One patient developed a hilar acute lymphocytic leukemia and died. The 2.5-year OAS and FFIT rates were estimated as 98% and 91%. In advanced stage CT-based response designations were 34% CR and 66% PR, PET+CT-based designations were 58% in 43 of all 61 patients. After completion of the initial therapy including IF-RT 95% achieved CR/CRu. One patient had progressive disease (PD), two patients relapsed. PD and one early relapse showed increased FDG-uptake, the PET scan of the late relapse was negative. No second cancer occurred. The 2.5-year OAS and FFIT rates were estimated as 93% and 87%.

Conclusions: In our interim analysis, ABVD alone is feasible in patients with limited and intermediate stage HL. In advanced stage HL BACOPPD was highly effective. The metabolic response corresponds to the good clinical outcome. Although, PET could not determine minimal residual disease in few cases.

PET STATUS AFTER STANFORD V CHEMOTHERAPY PREDICTS OUTCOME IN HODGKIN DISEASE
R. Advani, L. Maida, P. Lavore, R. Hoppe, S. Breslin, S. Rosenberg, S. Horig
1Oncology, Stanford University, Stanford, USA; 2Biostatistics, Stanford University, Stanford, USA; 3Radiation Oncology, Stanford University, Stanford, USA

We retrospectively analyzed 82 patients (pts) with Hodgkin disease with PET scans performed at baseline and after Stanford V chemotherapy. Of these, 70 had stage I/II disease (25 bulky mediastinal) and 22 had stages III/IV. Pts with favorable stage I/II (no bulky mediastinal disease) and those with stage III/IV or with bulky mediastinal disease were scanned after 8 and 12 weeks of chemotherapy, respectively. Radiotherapy (RT) fields were determined before starting chemotherapy based on baseline CT scans. RT, 20–30 Gy, was delivered to involved sites in favorable stage I/II pts and 26 Gy to sites ≥2.5 cm and macroscopic splenic disease for all others. After chemotherapy 6 of 82 pts had residual PET-positive sites. Among these 6 pts, 3 had bulky mediastinal disease of which 2 remained PET-positive after RT (scan not repeated after RT in 1). The remaining 3 pts became PET-negative after RT. Of the 6 PET-positive pts after chemotherapy, 4 relapsed: 2 of 2 bulky mediastinal and 2 of 4 others. Of the 76 PET-negative pt after chemotherapy, 4 relapsed. All relapses were biopsy confirmed. At a median follow up of 4 years, the FFP was 90% in PET-negative versus 20% in PET-positive pts. (P=0.001). In a multivariate analysis PET-positive status after chemotherapy (HR 12, p=0.0007) and pre-treatment International Prognostic Score ≥2 (HR 1.64 [per unit] p=0.045) predicted FFP. These data indicate that PET status after chemotherapy is strongly predictive of FFP with the Stanford V regimen.

POSITIVE PRE-TRANSPLANT PET/GA67 SCAN (GA) PREDICTS POOR OUTCOME IN REFRACTORY AND RELAPSE HODGKIN’S LYMPHOMA (HL)
E. Klabouli, C. Hosing, B. Pro, J. Khoumi, L. Fayad
1Lymphoma, Univ. of Texas MD Anderson Cancer Center, Houston, USA; 2Blood & Marrow Transplant, Univ. of Texas MD Anderson Cancer Center, Houston, USA; 3Lymphoma/Meduloma, Univ. of Texas MD Anderson Cancer Center, Houston, USA

Introduction: Stem cell transplantation (SCT) has become the standard of care in patients (pts) with relapsed or refractory HL. Chemosensitivity, assessed by conventional CT has been correlated with better outcome. Functional (PET/Ga) studies may provide more information.

Methods: From 1999 to 2004, 132 pts, male 57%, median age 30 years, (range 6–70), Ns type 90%, were diagnosed at stages I (5%), II (41%), III (33%), and IV (23%). 83% were treated with ABVD, 35% were 1st refractory. At relapse, B symptoms were present in 45%, extra-nodal disease in 22%, and relapse >12 months occurred in 65%. PET scan and Ga were done before SCT. 68 and 65 pts respectively.

Results: CN/BRu was observed in 57% of pts, PR in 38%, and 5% were stable by CT scan. PET scan and Gallium scan were positive in 25/68 (37%) and 14/65 (21%) pts respectively. Relapses occurred in 52 of 132 (39%) pts. The 2-year DFS was 65% for pts PET-negative vs. 30% for pts PET+ (P=0.00008), and 75% for Ga-negative vs. 37% for pts with a Ga+ (P=0.046). The positive predictive value of the PET and Ga were 68% and 65% respectively. The negative predictive values were 77% and 68% respectively.

Conclusion: Pre-transplant functional imaging is a strong predictive factor for patients. PET investigation should be considered for pts with positive functional studies.

18F-FUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG-PET) FOR THE EVALUATION OF PATIENTS (PTS) WITH FOLLICULAR LYMPHOMA (FL)
M. Vielletti, G. Cassini, P. Della Cioppa, G. Bresolin, A. De Chiarri, F. Russo, F. Frigeri, G. D’Arenna, L. Marcucci, A. Aloj, S. Lastoria, A. Pinto
1Hematology-Oncology, INT, Fondazione “G. Pascale”, IRCCS, Naples, Italy; 2Nuclear Medicine, INT, Fondazione “G. Pascale”, IRCCS, Naples, Italy; 3Pathology Unit, INT, Fondazione “G. Pascale”, IRCCS, Naples, Italy

Introduction: The role of FDG-PET for staging and monitoring of pts with FL has not yet been fully established. We conducted a single-institutional study to assess the sensitivity and the prognostic correlates of FDG-PET in FL.

Methods: Fifty-eight newly diagnosed (WHO criteria) FL pts who underwent FDG-PET scanning between January 2002 and December 2004 were reviewed. PET-FDG was performed before bone marrow biopsy and evaluators were blinded to the results of other diagnostic procedures. The characteristics of pts were: median age 54 years (range 27–82 yrs), CS I–II vs III–IV (28% vs 72%), G1 (30%), G2 (44%), G3 (24%), BM+ vs BM- (37% vs 63%), FL International Prognostic Index (FLIPI) score: low, 42%; intermediate risk, 24%; and poor risk, 34%.

Results: Overall FDG-avid disease sites were detected in 92% of cases, compared with a detection rate of 94% for CT scans of neck, chest, abdomen and pelvis, while the remaining 8% of cases were found PET negative. PET-FDG detected 16% more abnormal peripheral or thoracic nodal sites as compared to CT, while the two techniques appeared comparable as to the detection of extranodal disease sites. In 8% of cases PET identified ‘putative’ disease sites unconfirmed by CT, MRI and/or ultrasonography, being mostly related to skeletal sites and soft tissues. Conversely, PET sensitivity remained low (38%) for the detection of bone marrow infiltration. Interestingly, FDG-PET positivity was independent from histological grading (G1, 50%; G2, 59%; G3, 43%) and Ki-67 staining, and appeared to progressively increase among pts with higher FLIPI risk (low, 45%; intermediate, 54%; high, 57%).

Conclusions: FDG-PET represents a useful technique for evaluation of FL, it shows a higher detection rate of superficial and thoracic nodal involvement than CT scanning and may correlate with unfavorable prognostic features.

EARLY PROGNOSTIC VALUE OF 18F-FUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY IN AGGRESSIVE NON-HODGKIN'S LYMPHOMA: FINAL ANALYSIS ON 90 PATIENTS
1Hematologie Clinique, Hôpital Henri Mondor, Créteil, France; 2St. Louis Hospital, Paris, France; 3St. Antoine Hospital, Paris, France

Introduction: Assessment of early therapeutic response using metabolic imaging is potentially useful to determine prognosis in aggressive lymphoma.

Methods: Between January 2000 and January 2004, 90 patients with newly diagnosed aggressive lymphoma (median age 53 years, 94% diffuse large B-cell) were prospectively explored with FDG-PET prior to induction chemotherapy, after 2 cycles (“early PET”) and after induction completion. Therapeutic response was evaluated using conventional diagnostic methods (CDM) at 4 cycles. Induction treatment with an anthracyclin-containing regimen was administered in all patients, associated with rituximab in 41%. According to the International Prognostic Index, 37 patients and 53 patients belonged to the lower and higher-risk groups, respectively.
Results: At mid-induction, “early PET” was considered negative in 54 patients and positive in 36. After induction completion, 83% of PET-negative patients achieved CR based on CDM. Conversely, only 56% of PET-positive patients achieved CR. Event-free survival and overall survival differed significantly between the PET-negative and PET-positive groups (P < 0.001 and P = 0.006, respectively). The two-year estimates of event-free survival were 82% (95% confidence interval, 70 to 93%) for the PET-negative patients and 43% (95% confidence interval, 26 to 59%) for the PET-positive patients. The two-year estimates of overall survival were 90% (95% confidence interval, 81 to 98%) and 61% (95% confidence interval, 44 to 79%), respectively. The predictive value of “early PET” was observed in both the lower-risk and higher-risk groups.

Conclusions: FDG-PET at mid-induction therapy is a strong prognostic tool and should further guide first-line strategies.

EARLY WHOLE BODY F18-FDG POSITRON EMISSION TOMOGRAPHY (PET) RESTAGING HAS SIGNIFICANT PROGNOSTIC IMPACT IN DIFFUSE LARGE CELL LYMPHOMAS (DLCL-B)

M. Trusková1, U. Jaeger2, O. Belohlavek3, A. Becherer2, R. Pytlík1, C. Skrabánek4, P. Klenert1
11st Dept Medicine, Charles University General Hospital, Praha, Czech Republic; 2Dept Hematol Oncol, Medicine University Wien, Wien, Austria; 3Dept Nuclear Medicine, Nemocnice na Homolce, Praha, Czech Republic;

The most useful prognostic system currently used for NHL is International prognostic index (IPI). The response to therapy is a prognostic factor too, but it is still hard to exploit it for tailored therapy. The PET could be more informative than other conventional examination methods and probably earlier in the course of therapy. Eighty-four patients (pts) with diffuse large B-cell lymphoma (DLCL-B) diagnosed between January 1998 and August 2001 in our institutions and examined by PET during restaging were included to this study. There were 43 men and 41 females with median age 53 y (17–88) included. IPI has been found as low or low intermediate (IPI-L) in 51 (61%) pts and intermediate high or high (IPI-H) in 33 (39%) pts. All patients received anthracyclin based chemotherapy (CHOP has been used in 77 pts). PET restaging was performed either during the course of therapy (19 pts) or at the end (40 pts) or in both time points (25 pts). Out of 44 early examined patients 22 were found as PET negative and out of 22 pts PET positive 16 were PET restaged at the end and 6 became PET negative. Thirty eight pts out of 65 pts were found PET negative (58%). Thirty nine out of all 84 pts (46%) have been found at least once PET positive during restaging and 51 were found at least once PET negative (61%). Median follow up was 36 months.

The PFS (EFS resp.) probability at 3 years was found 82% (82% resp.) for early PET negative vs 39% (32% resp.) for early PET positive, P < 0.005 (P < 0.005 resp.). The difference was more significant for IPI-L pts (P < 0.003) than for IPI-H pts (P < 0.05). For pts examined at the end were PFS (EFS resp.) 77% (75% resp.) for PET negative vs 50% (38% resp.) for PET positive, P < 0.01 (P < 0.010 resp.).

Our data support the idea, that early PET restaging during the treatment course of DLCLB can be more useful than restaging at the end of chemotherapy, although both significantly discriminate two prognostic groups, and moreover it could be used as tool for tailored therapy.

UTILITY OF POSITRON EMISSION TOMOGRAPHY USING 18 FLUORINE FLUORODEOXYGLUCOSE (FDG-PET) IN POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLDS)

E. Ravelli1, V. Mancini1, C. Rossini1, G. Davanzo1, L. Gargantini2, G. Muci3, E. Morra3
1Division of Hematology, Niguarda Hospital, Milano, Italy; 2Nuclear Medicine Unit, Niguarda, Milano, Italy

Introduction: Several studies are evaluating the clinical usefulness of FDG-PET in staging, remission assessment and follow up in the different histopathological subsets of lymphomas.

Methods: Between February 2003 and December 2004, forty-one FDG-PET-CT scans were performed in 20 PTLDs in solid organ transplanted pts. The results of 22/41 PET were compared with those obtained from conventional imaging (CI), (CT, NMR, US) performed at the same time.

In 16/22 tests (73%) PET and CI were concordant. Moreover, in 1/5 pts with presence of disease, PET showed additional sites involved. PET sensitivity did not differ among PTLD histopathological subsets. Overall, PET demonstrated a good diagnostic value in 38/41 (93%) tests.

Conclusions: The role of PET in PTLDs is not yet known. This study suggests that PET may represent a useful choice in order to avoid contrast medium CT scan related nephrotoxicity in PTLD patients, whose renal function is frequently impaired because of chronic immunosuppressive treatment.
of the tests form a homogeneous entity with an activated B cell-like phenotype, in which downregulation of HLA class II genes is a dominant feature.

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**G-BANDING AND MOLECULAR CYTOGENETIC ANALYSES OF MARGINAL ZONE LYMPHOMA**

H. Vanveen Aa°1', F. Micci2', J. Delabie2', S. Heim1,3  
1Department of Cancer Genetics, The Norwegian Radium Hospital, Oslo, Norway; 2Department of Pathology, The Norwegian Radium Hospital, Oslo, Norway; 3Medical Faculty, University of Oslo, Oslo, Norway

**Introduction:** Marginal zone lymphomas (MZL) may be either extranodal, nodal, or splenic (SMZL). Their cytogenetic characteristics are incompletely known.

**Methods:** We analyzed the acquired chromosomal aberrations of 22 MZL by various genome-wide cytogenetic techniques such as G-banding, multicolor-FISH (M-FISH and RxFISH), and CGH (comparative genomic hybridization), as well as FISH with locus-specific probes.

**Results:** In the combined G-banding/FISH karyotype, chromosome 3 was the most frequently rearranged (ten cases). Chromosome 12 was abnormal in six cases. An extra X chromosome was seen in four cases, in addition to two cases with structural aberration of this chromosome. Trisomy 8 and trisomy 20 were seen in one MZL each and another two cases had other chromosome 8 and/or chromosome 10 aberrations. CGH analysis of altogether ten of the MZL showed only gains of chromosome material, namely of chromosome regions 3p12-25, 3q12-21, 3q23-28, 12q13-15, 12q22-24, 19p13, and 19q13 in two to four cases each (20% to 40%). In two MZL, the novel unbalanced translocation det(13)(3;13)(q11;p13) was detected as the sole karyotypic rearrangement, indicating that gain of 3q24-ter could be an important event in the pathogenesis of these lymphomas.

**Conclusion:** Several chromosomes were rearranged, especially chromosome 3 was nonrandomly involved, as already reported in previous studies. The novel rearrangements der(13)(3;13)(q11;p13) and der(4)(14;13)(q24;p13) may characterize subsets of MZL.

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**IMPROVED DEFINITION OF 7q DELETIONS IN SPLENIC MARGINAL LYMPHOMAS USING HIGH-RESOLUTION MULTI-COLOR BANDING**

S. Gazzì1, I. Chudoba1, P. Felmans1, F. Berger1, G. Salies1, B. Cottifè1, J. Magaud1, E.Celles-Bauchet1  
1Services d'Hématologie et d'Anatomie Pathologique, Centre Hospitalier Lyon Sud – Hospices Civils de Lyon, Pierre-Bénite Cedex, France; 2MetaSystems GmbH, Altlussheim, Germany

**Introduction:** The reported incidence of chromosome 7q deletions (7qdel) in splenic marginal zone cell lymphoma (SMZL) varies from 26% to 79%. Distinct commonly deleted regions (CDR) have been mapped between bands 7q21 and 7q35, and it has been demonstrated that the highest incidence of genetic loss involves bands 7q31-32. To improve the definition of breakpoints involving the 7q region in SMZL, we applied the high-resolution multicolor banding technique (Mband) in 28 patients displaying a 7qdel on conventional cytogenetics (Ck).

**Methods:** mBAND was performed using the chromosome 7-specific probe Xcen7, according to the manufacturer’s protocols (MetaSystems).**

**Results:** Based on Ck, chromosome 7q changes were interpreted as interstitial and terminal deletions in 15 and 13 patients, respectively. The abnormalities occurred as the sole chromosomal change in 12 cases. Proximal and distal breakpoints were distributed between the 7q21-1.1qter regions. All 7qdel were confirmed using the mBAND studies and the breakpoints determined by CK were redefined in 11/28 (39%) cases. No CDR was identified. However, deletions of the 7q32 region occurred in 26/28 (93%) cases. Unbalanced translocations that lead to a 7qdel were identified in 5 cases. Intrachromosomal changes were observed in 1 case.

**Conclusions:** Using the mBAND methodology, we unambiguously identified breakpoints involved in the 7q21-1.1qter region.
SMZL. Because this technique provides band-specific information, the precise description of intrachromosomal changes and unbalanced translocations was allowed in 6 cases. Even if a CDR was not observed, we confirmed that the most frequently deleted region involved the 7q32 band. This therefore suggests that this region may potentially harbor gene(s) relevant to the pathogenesis of SMZL.

A COMPARISON OF OUTCOMES IN PATIENTS WITH SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) OR MARGINAL ZONE LEUKEMIA (MZL) TREATED WITH IMMUNOTHERAPY, CHEMOIMMUNOTHERAPY, OR CHEMOTHERAPY

A. Tsimberidou, S. O’Brien, W. Wierda, G. Garcia-Manero, S. Lerner, M. Keating, Leukemia, M.D. Anderson Cancer Center, Houston, TX, USA

Introduction: The management of patients with splenic marginal-zone lymphoma (SMZL) or marginal-zone leukemia/lymphoma (MZL) remains controversial. To determine which treatment results in the best outcome, we retrospectively assessed the response, progression-free survival (PSFS), and overall survival (OS) rates in patients with SMZL and MZL.

Methods: We reviewed the records of 93 consecutive patients with SMZL or MZL who were referred to the leukemia service at The University of Texas M. D. Anderson Cancer Center between 5/95 and 10/04. The indications for therapeutic intervention were those used for chronic lymphocytic leukemia (CLL). Patients requiring treatment other than splenectomy were treated with immunotherapy, chemoimmunotherapy, or chemotherapy. CD20 surface antigen levels were assessed using flow cytometry.

Results: There were 56 men. The median age was 63 years (range 28–87). The median follow-up was 2.6 years. Patients were treated with immunotherapy (n = 35), rituximab (n = 34 patients, alemtuzumab 1 patient), chemoimmunotherapy (n = 15) or chemotherapy (n = 12). Thirty-one patients had splenectomy or were in the watch and wait approach and had not required any other therapy. The response rates were comparable between the three treatment groups (P = 0.14). The 4-year PSFS rates were higher in patients treated with immunotherapy (68%) than in those treated with chemoimmunotherapy (47%) or chemotherapy (17%) (P = 0.04, log-rank test). Patients treated with immunotherapy also had higher survival rates at 4 years (94%) than the two other groups (67% and 39%, respectively) (P = 0.004). These differences in PSFS and overall survival rates remained statistically significant when the analysis was limited to SMZL patients alone (P = 0.001 and P = 0.009, respectively). The median percentage of CD20 in marrow lymphocytes was 72% (range 1–96%), and the median number of CD20 molecules per cell was 65,224 (range 16,011–260,419). The fluorescent intensity of CD20 was 3+ in all patients tested.

Conclusions: These results demonstrate that immunotherapy induces durable responses and prolongs survival in patients with SMZL or MZL. The favorable outcome with rituximab immunotherapy may be partially explained by the high levels of CD20 molecules per cell in patients with SMZL or MZL. These levels appear to be higher than those reported in patients with CLL (mean 4067, range 540–46395). Rituximab should be considered the therapy of choice in patients with SMZL requiring treatment intervention other than splenectomy.

HIGH-DOSE METHOTREXATE FOR ELDERLY PATIENTS WITH NEWLY DIAGNOSED PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

I. Zhu, M. Mrugala, W. Nugent, F. Hochberg, T. Batchelor, Neurology, Stephen E. and Catherine Pappas Center for Neuro-Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, USA

Introduction: The introduction of methotrexate (MTX)-based chemotherapy has improved median survival for patients with primary central nervous system lymphoma (PCNSL). Older age is an independent negative prognostic factor in patients with PCNSL. We studied the response and adverse effects of HD-MTX in patients who were 70 or older at the time of diagnosis of PCNSL.

Methods: We identified 27 patients diagnosed with PCNSL at age 70 involving the brain, the eyes or both who were treated with HD-MTX (3.5–5 g/m²) as initial therapy from 1992–2004. The toxic effect of HD-MTX was determined by contrast-enhanced magnetic resonance imaging. Toxicity was analyzed by reviewing medical records. Parameters with potential prognostic value including performance status, CSF protein, serum lactate dehydrogenase and tumor location were analyzed.

Results: Twenty-seven patients with a median age of 73 (range 70–82) received a total of 296 cycles of HD-MTX as initial treatment. 72.7% of patients required dose reduction of the first cycle of MTX due to impaired creatinine clearance. Overall, 77.2% of the cycles required dose reduction because of impaired creatinine clearance. The median number of cycles per patient was 6 (range 1–21). In 26 evaluable patients the overall radiographic response proportion was 96.1%, with 16 complete responses (61.6%) and 9 partial responses (34.6%). Detailed, updated information on progression free survival and overall survival will be provided at the time of presentation. Grades 1-IV toxicities were observed in 22/27 patients and included gastrointestinal disturbances in 59% (5 grade III), hemato logic complications in 23% (4.5% grade III or IV) and renal toxicity in 32% (14 grade III).

Conclusions: We conclude that HD-MTX is associated with a high proportion of radiographic responses and only modest, usually reversible, grades IV and toxicity in patients 70 or older.
Results: Thirty pts (age ≤65y) have been enrolled in the study (median age 54, range 30–64y). 20 of 30 pts responded to HD-MTX (7 CR, 13 PR). 22/30 pts received high dose CT and autologous PBSC according to the protocol. Four pts refractory to MTX proceeded to RT directly and 1 patient died from PD subsequently. Beside cytopения no severe acute toxicities [WHO Grade 3 or 4] were observed. All patients that completed the protocol obtained CR (22/22). Two of these pts died, one due to relapse after one year, the other died after 25 mo due to progressive heart failure. With a median follow-up of 37 months (range 3–77 mo) the overall survival of all pts included and pts that fulfilled the protocol is 64% and 81%, respectively.

Conclusions: Sequential systemic application of high-dose differential acting cytostatic agents with consolidating hyperfractionated radiotherapy is very well tolerable with little acute toxicity. In a new multicenter II study, pts will be treated with more intensive high dose CT and PBSC with consolidating RT only for active residual disease.

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**PRIMARY CNS LYMPHOMA: RESULTS OF A MULTICENTER PHASE II STUDY INCLUDING HIGH-DOSE-CHEMOTHERAPY WITH AUTOLOGOUS PBSC AND HYPERFRACTIONATED RADIOTHERAPY AS FIRST-LINE-THERAPY**

G. Illert1, R. Marks1, G. Derig2, N. Frichthofen2, R. Gutenberg1, T. Kull2, G. Oertelt1, J. Finger2

1Dept. of Hematology and Oncology, University Medical Center Freiburg, Germany; 2Dept. of Hematology and Oncology, County Hospital Wiesbaden, Wiesbaden, Germany; 3Dept. of Radiotherapy, University Medical Center Freiburg, Freiburg, Germany; 4Dept. of Hematol. and Oncol., County Hospital Karlsruhe, Karlsruhe, Germany; 5Dept. of Stereotactic Neurosurgery, University Hospital Freiburg, Freiburg, Germany

Introduction: Primary NHL of the CNS (PCNSL) carries a poor prognosis despite initial response to steroids and radiotherapy (RT). Addition of methotrexate (MTX) to RT has improved the prognosis of patients (pts) with PCNSL, but a significant proportion of patients are still not cured. To improve relapse free survival and to reduce neurotoxicity we initiated a multicenter phase II study with early dose intensified chemotherapy (CT) and PBSC followed by hyperfractionated whole-brain radiotherapy (WBRT) for pts aged ≤65yrs. The use of high-dose (HD) lipopolysaccharide blood-brain-barrier-penetrating agents (BCNU, Thiopeta) in addition to maximum doses of water-soluble agents (MTX, AraC) is a novel approach in the treatment of PCNSL. To reduce the risk of delayed neurotoxicity, intra- or interstitial chemotheraphy was completely avoided and CT was administered before RT.

Methods: Induction treatment included 3 repetitive cycles of HD-MTX (8 g/m²). AraC (2x 3 g/m²) and Thiopeta (40 mg/m²) followed by R-CSF were used for stem-cell mobilisation. The conditioning regimen included BCNU (400 mg/m²) and Thiopeta (2x5 mg/kgBW) prior PBSC. Additional hyperfractionated WBRT (45 Gy, 2x1Gy/d) was administered as consolidation.

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**PHARMACOKINETICS AND EFFICACY OF IFOSFAMIDE AND TROFOSFAMIDE IN PATIENTS WITH INTRACRANIAL LYMPHOMA**

K. Jahneke1, T. Wagner2, N. Bechrakis2, G. Willerding2, S. Coupeland3, L. Fischer1, A. Kortel1, E. Thiel1

1Department of Hematology, Oncology and Transfusion Medicine, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany; 2Medical University of Lübeck, Section of Hematology and Oncology, Department of Internal Medicine, Lübeck, Germany; 3Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Department of Ophthalmology, Berlin, Germany

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**SALVAGE THERAPY WITH TOPOTECAN IN RELAPSED/REFRACTORY PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL): LONG-TERM RESULTS**

L. Fischer1, E. Thiel1, H. Klaes2, H. Kirchen2, K. Jahneke1, H. Poes3, J. Birkenmair2, A. Kortel1

1Hematology, Oncology, Transfusion Medicine, Charité Campus Benjamin Franklin, Berlin, Germany; 2Radiotherapy, Pius-Hospital, Oldenburg, Germany; 3Hematology, Krankenhaus Barnimerze Brüder, Trier, Germany; 4Medizin, Klinikum Nord, Nürnberg, Germany

Introduction: The majority of patients with PCNSL eventually will relapse, about one third is refractory to primary therapy. A standard therapy regimen in this setting has not been defined, however, salvage therapy prolongs survival. Topotecan reaches therapeutic levels in CNS.

Methods: Patients with refractory or relapsed non-AIDS-PCNSL were treated with topotecan 1.5 mg/m² d1–5 iv. every 3 weeks. Response was evaluated (MRI or CT) after the first and then at least after every second therapy course and every three months during follow-up.

Results: Twenty six pts. were included in this study. Histology was grade B-NHL in 24 pts., and low grade B-NHL in two. Pretreatment comprised chemotheraphy alone (CHT) in 14, CHT and whole-brain irradiation (WBI) in 11 and WBI alone in one patient. Up to three different regimens had been applied, and CHT always included high-dose methotrexate. Twelve pts. were refractory to last therapy, and 14 were in relapse. Lymphoma was confined to the CNS in all pts. including one isolated intracocular relapse. Median age was 51.5 years (26–76). One to six courses tocotepan were applied. Four complete and five partial remissions were observed, four pts. had stable disease and 11 progressed, one could not be evaluated. Overall response rate (CR/PoR) was 35%, and was higher in relapsed (50%) than in refractory pts. (17%). The median PFS was 2 months, in responders 5 months. Sixteen pts. received further therapy; either WBI alone (7), CHT and WBI (3, including high dose CHT with PBSC in one), or CHT alone (6). Median OAS from first diagnosis was 30 months. In three pts. a hematological remission without further therapy lasting 10, 13 and 28 months was achieved.

Conclusion: Topotecan is active in refractory and relapsed PCNSL and can induce long term remissions. The short median PFS reflects the overall poor prognosis in this collective. Future studies including tocotepan in combination therapy need to be performed.
Introduction: The prognosis of intracranial lymphoma (IOL) is poor, and the optimal treatment has not yet been defined. The study assesses ifosfamide (IFO) and treosulfamid (TRO) for treating IOL.

Methods: We prospectively evaluated the efficacy and aequous penetration of intravenous IFO, oral TRO, and their active 4-hydroxy (4-OH) metabolites in IOL. Doses varied between 1.500 and 2,000 mg/m²/day for IFO (days 1–3) and 150 and 400 mg/day (continuous or intermittent administration) for TRO. Four patients had newly diagnosed disease, and 6 had relapsed after pretreatment.

Results: All patients responded to first treatment with IFO or TRO, and two of three patients responded to retreatment with IFO on relapse. Progression-free survival from the first treatment with IFO or TRO was 1 to 18 months. Toxicity comprised anemia up to NCIC grade 2 in 5 patients, thrombocytopenia up to grade 2 in 3 patients, leukopenia up to grade 3 and neutropenia up to grade 2 in 2 patients each, as well as hemorrhagic cystitis grade 2 in one patient. In 6/6 patients, 4-OH metabolites were detected in the aqueous humor at a concentration of 0.71–1.56 μM immediately after IFO infusion, and patients who were diagnosed as orbital lymphoma histologically from Jan. 1990 to Oct. 2004 were treated with combination chemotherapy (cyclophosphamide, vincristine, and prednisolone). In cases of treatment failure, local radiotherapy were performed as a second-line treatment. We analyzed the efficacy of combination chemotherapy and the prognosis.

Conclusions: Twenty one patients of 15 men and 6 women were enrolled finally. The pathologic type and disease status of all patients were mucosa-associated lymphoid tissue (MALT) type and stage IE. Median age was 50, and 60% were under 50 years old, and median follow-up period was 38 months. Overall survival rate was 100% (complete remission of 76.2% and partial remission of 38%).

Epidemiological Research, Centro di Riferimento Oncologico, Aviano, Italy

Purpose: The prevalence of Chlamydia psittaci (Cps), Helicobacter pylori (Hp), and hepatitis C virus (HCV) infections was investigated in 54 OAL patients. Clinical and therapeutic implications were investigated.

Methods: Cps DNA was assayed in lymphoma biopsies from 45 pts; serum HCV markers were assessed in 54 pts and gastric Hp infection in 49.

Results: 14 pts had conjunctival lymphoma and 40 had intra-orbital (lacrimal gland, orbital soft tissues) lymphoma. Stage was IE in 42 (74%) cases, IIE in two (4%) and IV in 10 (19%, bilateral disease in 3). Cps, Hp, and HCV infections were detected in 30 (60.4%), 5 (10%), and 7 pts (15%), respectively. Concomitant Cps/Hp, Cps/HCV, and Cps/HCV/HCV infections were observed in 4 (7%), 5 (9%) and one (2%) pt, respectively. Three (6%) pts had no infections. Forty-three percent of HCV+ pts and 11% of HCV- pts had advanced disease (P < 0.005); no other associations between clinical characteristics and infectious agents were observed. Thirty-seven pts with disease limited to the orbits received a first-line therapy, obtaining CR in 27 (73%) and PR in 6 (16%; ORR: 89%). Among the 9 pts with extra-orbital disease, objective response was CR in 7 (78%) and PR in one (ORR: 89%). Sixteen pts were treated with Cps-eradicating antibiotic therapy (doxycycline 100 mg orally, bid, for 3 weeks), yielding a diagnosis (n = 7) or relapse, with two CRs, 6 PRs, 6 SD, and two PD (ORR: 50%). The 5 Hp+ pts received eradication combination of erthyromycin, tinidazole & omeprazole; no lymphoma response was detected in the four evaluable pts. Sixteen pts with advanced relapse, with a median TTP of 42+ mo, and a 5-yr OS of 69 ± 7%. HCV infection (71% vs. 23%, P = 0.001) and extra-orbital disease (78% vs. 22%, P = 0.002) were independently associated with a higher relapse rate. Fifty-two pts are alive at a median follow-up of 42+ mo, with a 5-yr OS of 97 ± 8%.

Conclusions: Cps infection is common in OAL, and provides the rationale for an active eradication therapy. Hpv-eradicating therapy is not active against OAL. HCV infection is associated with advanced disease and higher (systemic) relapse rate, with a consequent negative impact in pts managed with radiotherapy alone. A better knowledge of concomitant infections may play a critical role in further advancements against MALT lymphomas.