6. Indolent Lymphomas

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

G. Oehl1, P. Fariñha1, W. Lam1, R. deLeuwl, K. Young2, E. Kjeldsen3, S. G. deNexit-Dutot, F. Amore1, W. Chan1, R. Gascoyne1

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 the 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. 7q,12p13,17p11-13 and deletion of e.g. 3p, 4q21, 13q14 were observed. By hierarchical clustering two sets of genes were identified, which were either over- or underexpressed upon transformation. Of these genes, 5-fold up- (7genes) or down-regulated (11genes). Among unregulated genes were: AIMIL (1p36), SCN11A (3g22), CALM3/CPL (1p15), CPNI (1q24), and ARF4L (17q12); among the down-regulated ones: DKFZp761B107 (4p15), LOC118182 (10q24), NAPIL1 (11q15), EBI-1 (12q22), C14orf135 (4q23), SIRT2 (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)

E. Camacho1, R. Arranz-Sáez1, A. Caleo1, J. Cannata1, L. Cerceda1, L. González1, M. Pérez-Martín1, L. Sánchez-Verde1, C. Montalban1, J. García1, M. Prijs1, C. Bellas3

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 clinical-biological, 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. Singly nuclear markers were scanned using the Bliss system and the quantitative expression was measured using the TMA scor e 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 K6eters 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. 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

HMD5, 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 samples, taken at presentation and first relapse were studied. Cases with morphological evidence for 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/IgH fusion, BCL6 rearrangement and deletion of P53 were assessed for the presence of the t(14;18). 84% of 38 analyzed cases a total of 469 genomic aberrations were detectable. The mean number of aberrations per case was 4.4. Genetic gains (n = 249) were more frequent than deletions (n = 216). Besides the identification of new chromosomal aberrations, for a large number of imbalances 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 DADD, which plays a crucial role in FAS mediated apoptosis. In this context we were also able 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 pronounced 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.
rearrangement was observed in 6/65 patients at presentation and 10 further patients at relapse. PS3 deletion was observed in 2/47 patients at presentation and 7 further patients at relapse. In summary 26% of patients had additional abnormalities at presentation and this increased to 57% at the time of relapse. The presence of additional abnormalities may be a useful predictor of the development of resistance to therapy over time. Newly presenting patients who have additional genetic abnormalities may have had a longer period of asymptomatic disease and consequently a shorter overall survival.

**Results:**
One hundred and thirty-nine regions of homozygosity were identified, 54 in the FL samples, 85 in TxDLBCL. In the 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 ≥90% homozygosity in at least 50 contiguous SNPs.

**Methods:**
Using the 10K SNP array (Affymetrix) whole lymph node tumours.

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

**Results:**
One hundred and thirty-nine regions of homozygosity were identified, 54 in the FL samples, 85 in TxDLBCL. In the antecedent FL such regions were present in 83% (20/24) of cases (mean 2.2 abnormalities 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 1 case. A mean of 3.5 abnormalities were observed in TxDLBCL (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 2/4 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 homozygous regions 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 not identified by the above algorithm, including in one case a 4.6 Mb 2p amplicon 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 TxDLBCL 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.

**FLIPI AND HISTOLOGICAL SUBTYPE ARE THE MOST IMPORTANT PREDICTING FACTORS OF HISTOLOGICAL TRANSFORMATION (HT) IN FOLLICULAR LYMPHOMA (FL)**
E. Gineì , A. López-Guillermo1, S. Montoto1, F. Bosch1, N. Villamor1, A. Mintáriolí1, L. Arenillas1, L. Colomo1, E. Campo1, E. Montserrat1
1Institute of Hematology and Oncology, Department of Hematology, Hospital Clinic, Barcelona, Spain; 2Hematopathology 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, ILI and FLIPI scores. Median overall survival was 11.3 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 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 5). 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 multivariate analysis, grade III histology, grade II histology P = 0.04; RR 2.0) and FLIPI in the multivariate analysis, grade III histology (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. Michels1, V. Foria1, G. Packham2, B. Mead1, 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). Centrocytes 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 (hpf) correlated well with overall survival (r = −0.31, p = 0.001), and high numbers of Mcl-1+ centroblasts (>200/hpf vs < 200/hpf) predicted for poor median survival (24 mo, 95% CI 7.3–40.3 vs 98.6 mo, 95% CI 85.4–112.8 mo, P < 0.001), 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.

238

COMBINED EFFECT OF SMOKING HABITS AND HEPATITIS C VIRUS ON NON-HODGKIN LYMPHOMA RISK
R. Talanini1, J. Polese1, L. Dal Mazz1, M. Montella1, A. Crispì1, M. Spina1, S. Franceschi1, M. Crovatto1, C. La Vecchia2
1Epidemiology and Biostatistics, Aviano Cancer Center, Aviano, Italy; 2EU, ‘Pascale’ Cancer Institute, Naples, Italy; 3Oncology, Aviano Cancer Center, Aviano, Italy; 4ICE, IARC, Lyon, France; 5Immunology and Virology, ‘S.Maria’ Hospital, Pordenone, Italy; 6Epidemiology, ‘Negri’, Milan, Italy

Introduction: Tobacco smoking is a risk factor for several cancers, but the role of smoking in the etiology of non-Hodgkin lymphoma (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-tobacco-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 1.1–4.1) compared with never smokers. The association between smoking and NHL was consistent across strata of sex and age. In respect to histological 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 25.8 (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-positive 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.

239

GENE EXPRESSION PROFILING OF FOLLICULAR LYMPHOMAS: IDENTIFICATION OF POSSIBLE BIOMARKERS
U. Andreasson1, 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 promised 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, purified by flow cytometry, and normal B cells were analyzed for their expression of more than 12,500 genes available on the HU95v2 array. Two main data analyses approaches were performed, either comparing FLs to all the 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.

240

NEW APPROACH FOR QUANTIFICATION OF IGH-BCL2 REARRANGEMENT IN FOLLICULAR LYMPHOMAS
C. Settegrana1, K. Beldjord1, V. Asnafi1, C. Fernandes1, D. Canioni2, R. Delarue1, M. Delfau1, F. Davi1, C. Bastard1, G. Salles1, E. Macintyre1, 1Laboratoire d’Hématologie, Hôpital Necker-Enfants malades, Paris, France; 2Laboratoire d’anatomie-pathologie, Hôpital Necker-Enfants malades, France; 3Hématologie clinique, Hôpital Necker-Enfants malade, France; 4Laboratoire d’Immunologie, Hôpital Henri Mondor, 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 (RQ-PCR) may facilitate exploitation of individual patients ’ results. The sensitivity of RQ-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 amongst 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 RQ-PCR using Biomed2 based JH primers. We compared quantification using 3A primers 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’ (10E-2 to 10E-5, majority 10E-4) and was proportional to the size of the rearrangements, although relatively large amplicon size not prevent adequate sensitivity. Comparison of quantification relative to an individual or a universal calibrator demonstrated a 1 log difference for 23/53 (43%) samples and a 2 log difference for 8/53 (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 consider the impact of variable amplicon size, if individual stratification is to be based on RQ-PCR bone marrow levels, as recently proposed by Rambaldi et al. (2005).

241

SIGNIFICANT DIFFERENCES IN THE IGHV AND BCL6 MUTATION STATUS IN AGGRESSIVE B-CELL LYMPHOMAS WITH AND WITHOUT MYC BREAKPOINTS
C. Pot1, G. German Cancer Aid (Deutsche Krebshilfe) Network Molecular Mechanisms in Malignant Lymphomas (MMML)2 et al. 1Second Department of Medicine, University Hospital, Kiel, Germany; 2Study central, University of Göttingen, Göttingen, Germany
**Introduction:** Somatically mutated IGHV 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 DHPLC and direct sequencing was applied to determine the somatic hypermutation status of clonal IGHV 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 VH 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=0.020). 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 significant differences in the VH and BCL6 mutation frequencies independently from histologic subtypes.

**4 GY INVOLVED FIELD RADIOTHERAPY INDUCES RAPID AND LASTING REMISSIONS WITHOUT SIGNIFICANT TOXICITY IN 200 B-CELL LYMPHOMA PATIENTS**

R. Haas1, P. Poortmans2, B. Aleman1, L. Dewit1, D.de Jong3, M.Verheij1, H. Bartelink1

1Radiotherapy, The Netherlands Cancer Institute, Amsterdam, Netherlands; 2Dept. Radiotherapy, Bernard Verbeeten Institute, Tilburg, Netherlands; 3Pathology, The Netherlands Cancer Institute, Amsterdam, Netherlands

**Purpose:** To study the efficacy of low dose involved field radiotherapy (IF-RT) in recurrent and/or chemotherapy refractory B-cell lymphoma patients (pts).

**Patients and methods:** IF-RT to a dose of 4Gy (either 1 fraction of 4Gy or 2 fractions of 2Gy, interval 48 hours) was given in 200 patients with B-cell lymphomas (123 follicular lymphomas, 23 CLL/SLL, 18 marginal zone lymphomas, 17 mantle cell lymphomas, 13 diffuse large B-cell lymphomas and 6 lymphocyte predominance Hodgkin lymphomas). This group included 102 males and 98 females with a median age of 61 years (range 31–93 years). Median time since primary diagnosis was 49 months (range 3–358 months). Patients were pretreated by a median of 2 chemotherapy and/or radiotherapy regimens (range 1–11). Bulky disease (≥5 cm) was present in 122 patients. Endpoint of the study was in-field lymphoma control.

**Results:** IF-RT resulted in 56% CR (n=111), 34% PR (n=69), overall response rate (RR) 90%. RR was independent of sex, age, intensity of prior treatment, time since diagnosis, histology or tumour size. Median time to progression (MTP) was 15 months and the median time to local progression (MTLP) was 24 months. As expected, toxicity was very mild to absent.

**Conclusion:** low dose IF-RT up to 4 Gy induces excellent RR in recurrent and/or chemotherapy refractory B-cell lymphoma patients even in pretreated patients. Toxicity is very mild.

**LIMITED STAGE FOLLICULAR LYMPHOMA, A CURABLE DISEASE?**

N. Voss1, T. Pickles1, J. Morris1, J. Barbeau1, M. Chhanabhai2, R. Gascoyne3, J. Conners2

1Radiation Oncology, BCCA, Vancouver, Canada; 2Pathology, BCCA, Vancouver, Canada; 3Medical Oncology, BCCA, Vancouver, Canada

**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, 53 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 1996 the BCCA has maintained a prospective database.

**Results:** 191 patients (M/F=87/104) were treated with RT alone. Median age was 63y (range 29–89). 147 presented with stage IA or IAE, 44 with stage IIA or IIAE. Median follow up was 4.9 years. The 10 and 20 year progression free survivals (PFS) for all patients were 51% and 48%, median 10.75 y. No patients relapsed later than 10.75 years after diagnosis. Overall survival (OS) at 10 and 20 years were 64% and 47%, median 14.24y. 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, IMMUNOCYTOMA, AND MANTLE CELL LYMPHOMA: RANDOMISED EVALUATION OF CURATIVE RADIOTHERAPY IN LIMITED STAGE NODAL DISEASE**

M. Engelhard1, M. Stuschke2, M. Hansmann3 et al.

1Radiooncology, Medical School, Essen, Germany; 2Pathology, Medical School, Frankfurt, Germany

**Introduction:** Follicular lymphoma grade I-II (FL), Immunocytoma (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, RDT), 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, <10 cm), 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 <3 cm, or plus 14 Gy to those of 3–10 cm. 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 diaphragm. In pts >75 ys with FL, IC, and MCL, involved field (IF) radiotherapy is applied (all doses as in the RDT).

**Results:** From 2000–2004, 211 pts were recruited, 162 to the RDT, median age 53 (23–65) yrs; 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 leuko-(0%/16%) and thrombocytopenia (5%/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) PRESENTS A HOMOGENEOUS DISEASE ENTITY**

Y. Guo1, K. Karube2, J. Suzumiya2, M. Kikuchi2, K. Ohshima1

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

Follicular lymphoma (FL) is morphologically classified into grades 1,2,3a 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(t(14;18) and Bcl6(t(3q27)) in all cases.

(Results and discussion) The grade distribution of FL was, grade 1: 26%; grade 2: 48%; grade 3a: 17% and grade 3b: 9%, 93%, 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 FLs 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 aberrances including Bcl2 translocation, Bcl6 translocation, Bcl2 amplification or other unknown gene abnormality. Our findings indicate that typical FL presents a homogenous 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

E. Deconinck 1, P. Colombat 1, C. Foussard 1, N. Milpied et al. 1
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 stem cells.

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, and interferon, patients treated with high-dose therapy had a higher survival rate due to an excess of secondary malignancies after transplantation. 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.

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)

C. Moreno, A. López-Guillermo, S. Montoto, E. Domingo-Domènech, J. Ribera, A. Labés, C. Estany, J. Besalduch, L. Escoda, C. Pedro, S.Gardella, A. Asensio, A. Fernández de Sevilla, D. Colomer, E. Montserrat Hematology, GELCAB (Grup per l’Estudi dels Limfomes a Catalunya i Balears), Barcelona, Spain

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

Methods: 110 pts diagnosed with advanced stage FL included in the FCM trial in whom DNA material was available for molecular assays. RQ-PCR analysis was performed either at diagnosis (peripheral blood [pb], bone marrow [bm] and lymph node [ln]), at the 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, 7% (56), no MBR/mcr, 32 (33%). There was a strong correlation between the RQ-PCR 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 (89%) in whom DNA material from pb, bm and ln was available, the results on bcl-2/JH were concordant between pb/bm and ln. Pb RQ-PCR initial values correlated with bulky disease and bm involvement. The clinical outcome and pb molecular assessment at different time-points are detailed in the table:

<table>
<thead>
<tr>
<th></th>
<th>Pre-FCM</th>
<th>FCMx3</th>
<th>FCMx6</th>
<th>6–12 mos. post FCM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 54)</td>
<td>(N = 32)</td>
<td>(N = 54)</td>
<td>(N = 38)</td>
</tr>
<tr>
<td>CR rate (%)</td>
<td>0</td>
<td>50</td>
<td>87</td>
<td>81</td>
</tr>
<tr>
<td>Molecular CR (%)</td>
<td>0</td>
<td>76</td>
<td>88</td>
<td>71</td>
</tr>
<tr>
<td>RQ-PCR (pb +)</td>
<td>3.2 ± 10</td>
<td>0.0003 ± 0.002</td>
<td>0.001 ± 0.004</td>
<td>0.006 ± 0.01</td>
</tr>
</tbody>
</table>

Conclusions: RQ-PCR is a high molecular CR rate, with BCL-2/JH assessment by RQ-PCR being a reliable method to assess it.

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

P. McNamara 1, M. Rodriguez 2, F. Hagemeister 3, J. Romaguera 3, A. Serrano 2, A. Youssef 1, A. Goy 1, F. Samanioue 3, M. Hess 3, M. Lee 2, L. Medeiros 2, L. Fayad 2, B. Pro 1, Y. Jiang 3, A. Ayala 1, F. Cabañillas 1
1Lymphoma/Myloma, 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 t(14;18) involving bcl-2 (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 survival, 92 vs 90%, 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 neuropenia 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. Methyleneplasia 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.
Introduction: We previously reported that the addition of rituximab to chemotherapy with cyclophosphamide, vincristine, and prednisone (CVP) improved time to progression in patients with untreated follicular lymphoma (Marcus R, Blood 2005). We present here the analysis of impact of pre-treatment prognostic factors on treatment effect.

Methods: 321 patients with previously untreated follicular lymphoma were randomized to receive 8 cycles of CVP with or without the addition of rituximab. Time to progression was one primary outcome. A multivariate analysis was performed using Cox regression including the following variables: Follicular Lymphoma International Prognostic Index (FLIPI) score, International Working Formulation (IWF) class, B symptoms, and presence of bulk.

Results: The median time to progression for all patients was 32 months vs. 15 months ($P=0.0001$). The median TTP was greater in the CVP-R arm across all FLIPI risk groups and across all IWF classes and was greater for all other variables with the exception of the subgroup of patients with a hemoglobin $<12 \mathrm{g/dL}$ (N=66) in which no statistically significant difference was identified.

Conclusion: The addition of rituximab to CVP for patients with previously untreated follicular lymphoma improves time to progression across prognostic groups and may abrogate the impact of common prognostic variables on outcome.

RITUXIMAB IN COMBINATION WITH CHOP RESULTS IN A SIGNIFICANTLY SUPERIOR RESPONSE RATE AND TIME TO TREATMENT FAILURE IN FIRST-LINE TREATMENT OF LYMPHOPLASMACYTOID/IC IMMUNOCYTOMA (LP-IC) — A PROSPECTIVE RANDOMIZED TRIAL OF THE GERMAN LOW GRADE LYMPHOMA STUDY GROUP (GLSG)

C. Buske1, M. Dreyling, H. Eimeracher, H. Boeck1, M. Pfreundschuh, B. Metzner, M. Unterhalt, W. Hiddemann

1Dept. of Medicine III, Univ. Hospital Grosshadern/LMU, Munich, Germany; 2Dept. of Medicine II, C.W. Zhang/University Hospital, Hagen, Germany; 3Practice, Hem./Onc., Offenbach, Germany, 4Dept. of Medicine I, Univ. Medical School Saarland, Homburg, Germany

Introduction: Advanced stage lymphoplasmacytoid/immune lymphoma (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 immuno-chemotherapy 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 lymphoplasmacytic lymphoma, 28% as lymphoplasmacytoid 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% (5% CR, 65% PR), respectively ($P=0.035$). 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 summary, 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 (R-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. Forstpointner2, W. Ludwig2, M. Gramatzki2, H. Boeck1, M. Haenel3, H. Wandt3, R. Parwaresch3, M. Unterhalt1, W. Hiddemann1

1Dept. of Medicine III, University Hospital Grosshadern – LMU, Munich, Germany; 2Robert-Rössle Hospital, Charite, Berlin, Germany; 3University Hospital, Erlangen, Germany; 4Practice of Hematology, Offenbach, Germany; 5Hospital Chemnitz, Chemnitz, Germany; 6Hospital Nuernberg-Nord, Nuernberg-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 lymphomas. 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 maintenance. 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. A total of 4 courses were given. After the statistical analysis confirmed the significantly improved response rate (20%/75%) and overall survival was confirmed by 45 patients subsequently designated to combined immuno-chemotherapy. Fifty-five patients subsequently designated to the combined study arm (R-FCM) confirmed the superior remission rates (36%/96%), progression-free and overall survival. Similarly, in 50 randomized MCL patients, R-FCM achieved a 20% increase in response rate (94% vs. 71%, \( P = 0.011 \)) as well as overall survival rate (74% vs. 4 years vs. median of 3.8 years, \( P = 0.033 \)) was improved after combined immuno-chemotherapy. Fifty-five patients subsequently designated to the combined study arm (R-FCM) confirmed the superior remission rates (36%/96%), progression-free and overall survival. Similarly, in 50 randomized MCL patients, R-FCM achieved a 20% increase in response rate (94% vs. 71%, \( P = 0.011 \)) as well as overall survival rate (74% vs. 4 years vs. median of 3.8 years, \( P = 0.033 \)) was improved after combined immuno-chemotherapy.

**Conclusion:** This is the first prospectively randomized trial which demonstrates the superiority of a combined immuno-chemotherapy in patients with relapsed follicular and mantle cell lymphoma, 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. Dreyling2, R. Forstpointner1, M. Kneba2, N. Schmitz2, R. Schmitz2, B. Metzner1, M. Reiser1, R. Parwaresch1, M. Unterhalt1, 1Department of Medicine III, University of Münster, Germany; 2University Hospital Kiel, Germany; 3Institute of Pathology, University Kiel, Germany

**Introduction:** The addition of Rituximab (R) to combination chemotherapy 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 prospective randomized comparison of R-CHOP versus CHOP alone followed by a second randomization in remission for Interferon 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 evaluable 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: 84% vs. 63%, \( P=0.0004 \)) whereas so far, 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 followed by PBCT). In contrast, in 122 MCL patients, 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 (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 RADIOMUNOTHERAPY (RIT) WITH IODINE 131 LABELLED RITUXIMAB IS FEASIBLE AND EFFECTIVE IN RELAPSED LOW GRADE NON-HODGKIN LYMPHOMA**

T. Hildge1, M. Baye2, M. Zivanovic1, B. Mead1, P. Johnson 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 radiolabeled anti-CD20 mAb \(^\text{131}\text{I}}\) tositumomab and \(^{90}\text{Y}\) ibritumomab tiuxetan in patients <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 followed by 2 fractions of \(^{131}\text{I}\) labelled rituximab given 8 weeks apart in relapsed “low grade” NHL and have included patients with higher 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 120Gy. A unique anti-rituximab idotype antibody has been generated enabling serial analysis of serum rituximab concentrations, 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 infusions of Rituximab, and two fractions of RIT has on the clearance and biodistribution of the \(^{131}\text{I}\) labeled rituximab. Sequential pharmacokinetic analyses have identified wide variation in the effective half-life of \(^{131}\text{I}\)-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 \(^{131}\text{I}\) rituximab increased from 43 hours prior to induction rituximab to approximately 106 hours prior to the second fraction of \(^{131}\text{I}\)-rituximab. A strong inverse correlation was found between the patients’ disease burden and the clearance 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 \(^{131}\text{I}\) Rituximab than can be given with a single dose of anti-CD20 (60% higher than is given with \(^{131}\text{I}\) 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 regimens. 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.

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

A. Davids1, S. Howell1, A. Rohatiner1, J. Matthews1, J. Clayton2, T. Massey2, S. Owens2, K. Britton1, J. Radford1, A. Lister1
1Cancer Research UK Medical Oncology Unit, St Bartholomew's Hospital, London, UK; 2Department of Medical Oncology, Christie Hospital, Manchester, UK

Between March 1996 and Feb. 2001, 90 patients (pts.) received Tositumomab and Iodine 131 Tositumomab (BEXXAR®) at St Bartholomew’s Hospital, London and Christie Hospital, Manchester either in open phase II trials (n=55)(JCO 2000;18(6):1316 and JCO 2004;22(8):1469) or on a compassionate release basis (n=35). Long-term follow-up is presented. The median age was 53 years (range 27–90); 37 pts. had bone marrow infiltration (<25%). Therapy was delivered a median of 3.5 yrs (range 2 months-8yrs) from diagnosis; Thirty three pts. were treated at 1st recurrence, 23 at 2nd and 32 at ≥3 (range 0–9, median 2nd). 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 45Cy. 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 (maximal) response rate (ORR) was 60% (CR/Cu(10%) 32%) and by histology, follicular lymphoma (n=60) ORR 73% (CR/Cu(42%), transformation to diffuse large B-cell lymphoma (n=18) ORR 35% (CR/Cu(17%)), lymphoplasmacytoid lymphoma (n=5) ORR 60% (1 pt. CR), 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/Cu(10%) and for pts. with recurrence after HDT the ORR was 45% (CR/Cu(18%). Median progression free survival (PFS) for all pts. was 6 months (95% CI 5.6–10.7). There was no significant decline in ORR or PFS by number of progressions. However, PFS was significantly superior for pts. in whom CR/Cu(10%) was achieved compared with those entering PR, median 5 years (95% CI 1.6 to NR) 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/Cu(10%) among responders, however pts. that were refractory to rituximab were less likely to achieve CR/Cu(10%) (P=0.01). To date 1 pt. has developed mDMS and 7 pts. have required thyroxine replacement. Achieving CR/Cu(10%) following Tositumomab and Iodine 131 Tositumomab is associated with durable remissions.

PHASE II TRIAL OF CLADRIBINE (2-CDA) AND RITUXIMAB (R) IN PATIENTS WITH WALDENSTROM’S MACROGLOBULINEMIA (WM) OR SMALL LYMPHOCYTIC LYMPHOMA (SLL): PRELIMINARY REPORT OF A MULTICENTER STUDY

D. Laszló1, G. Andreola1, C. Rabascio1, P. Mancuso1, R. Balzano1, S. Bassi1, P. Bertazzi1, P. Antoniotti1, C. Manz2, A. Pinto1, A. Billio1, G. Martellini1
1Hematology, European Institute of Oncology, Milano, Italy; 2Clinical Research and Development, Lipomed AG, Arlesheim, Switzerland; 3Oncology, National Tumor Institute, Napoli, 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 biological activity by immunophenotypic, molecular and pharmacogenomic studies. Newly diagnosed WM or SLL pts requiring systemic treatment due to Htc<50% or PLT<100,000 or symptomatic neuropaty/cryoglobulinemia, or not previously treated with either nucleoside analogues or R, were eligible for the study. The therapy consists of an infusion of R (375 mg/mq) on day 1 followed by 2-CDA 0.1 mg/kg (sc injection) for 5 consecutive days. Each cycle was administered monthly for 4 times. Before treatment, immunophenotypic (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 study include: sex (M/F) 8/7, median age 62 (52–70 yrs), 9 newly diagnosed (5 WM, 4 SLL); WM pts presented Htc<30% in 25%, PLT<100,000 in 12%, peripheral neuropathy in 50%, peripheral IgM>4 g/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 neuropenia), 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 a IgH rearrangement suggestive for post germinal centre status without any correlation with CD38 and ZAP70 expression. SLL pts presented a IgH rearrangement suggestive for pre germinal center status, 5/6 were positive for ZAP70 expression. Of 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 interesting rate of molecular response observed in WM pts needs to be confirmed in a larger number of pts and may justify to prolong accrual.

FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN WALDENSTROM MACROGLOBULINEMIA: RESULTS IN 49 PATIENTS

J. Tamburini1, V. Lévy, Carine Chateillex, Jean Paul Fermand, Alain Delmer, Laure Stalniewicz, Pierre Morel, François Dreyfus, Marie Jose´e Orange, Bernard Christin, Yannick Chourry, Yvonme Leblond
Service d’hématologie, Hopital Pitéé-Salpétrière, Paris, France

Purpose: Fludarabine therapy gives a response rate of about 30% in previously treated patients with Waldenstrom 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 (25 mg/mq IV D1-D3) and Cy (500 mg/mq 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/g/100ml (4.6–14.9), 39.6/g (23–60.2), 3/mg (1.4–7.6) and 24.7/g (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 of 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 main 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<40/g/l.

Conclusion: The FDR/Cy combination therefore gives a high response rate in WM, even in previously treated patients with factors of poor prognosis.
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 paratrabecular 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>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>38 (7.5)</td>
<td>396 (78)</td>
<td>44 (8.6)</td>
<td>508</td>
</tr>
<tr>
<td>PMBCL</td>
<td>0</td>
<td>47 (100)</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>FL</td>
<td>157 (42)</td>
<td>202 (54)</td>
<td>1</td>
<td>375</td>
</tr>
<tr>
<td>MCL</td>
<td>92 (83)</td>
<td>18</td>
<td>0</td>
<td>111</td>
</tr>
<tr>
<td>MZL Nodal</td>
<td>14 (42)</td>
<td>17</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>MZL Splenic</td>
<td>29 (91)</td>
<td>2</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>MALT</td>
<td>27 (19)</td>
<td>104</td>
<td>0</td>
<td>142</td>
</tr>
<tr>
<td>SLL</td>
<td>64 (94)</td>
<td>1</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>LPL</td>
<td>101 (94)</td>
<td>5</td>
<td>1</td>
<td>108</td>
</tr>
</tbody>
</table>