Further Abstracts

1. Virology/Epidemiology

A POPULATION-BASED STUDY OF INCIDENCE AND SURVIVAL OF HAIRY CELL LEUKEMIA
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Introduction: Hairy cell leukemia (HCL) is a rare but treatable indolent B cell neoplasm. In the era of treatment with nucleoside analogues 5-year survival above 85% can be expected. We performed a descriptive epidemiological study of HCL in a population-based national cohort. The Israeli Population is composed of a Jewish majority (~80%) and a non-Jewish, mainly Muslim, Arab minority (~20%). Cancer notification is required by law.

Methods: We assessed the incidence and survival of patients with HCL reported to the Israel Cancer Registry between 1991 and 2001. We measured crude and age-adjusted incidence rates and survival by gender, ethnicity and residential district.

Results: During the study period 181 cases of HCL were diagnosed in Israel: 147 (81.2%) in males and 34 (18.8%) in females. Age-standardized annual incidence rate for HCL in the Israeli population (per 100,000) was: 4.6 (95% CI 4.5-4.7) for women and 5.5 (95% CI 5.4-5.6) for men. The rate in Jewish men (7.1/105) was higher than in non-Jewish men (4.6/105), whereas for women the rates were 1.8 vs 0.5/105 respectively. Median age at diagnosis was 56 yrs for males and 62 for females (P<0.001). Median survival has not reached in any subgroup. Significant data were collected prospectively. Consecutive adult patients diagnosed to have NHL between Jan 2003 and Dec 2004 and seen at the SQUH were the subjects of the study. Over the study period, a total of 46 new patients were seen. The characteristics are as follows: Median age 53 (14-77) years. Male: Female 27:19. Histology (according to the Revised European American Lymphoma (REAL) classification), Diffuse large B cell lymphoma 31 (67%), Anaplastic large cell lymphoma 5 (11%), Marginal zone cell lymphoma 3 (one each splenic, cutaneous and intestinal), T-cell acute lymphoblastic lymphoma 2, others 6. Nineteen patients presented with primary extra-nodal disease; 31 had a high LDH; Performance status 0:1/2:3:4 = 3:17:9:8:9; Stage I:II:III:IV = 6:12:6:22; For aggressive lymphomas, International prognostic Index (IPI) score, Low risk 13, low intermediate 4, high intermediate 1, high risk 18. Three patients have been lost to follow up, and 16 patients have died. With a median follow-up of 8 months, the project 2-year survival for the entire cohort is 64%, 92% for low-risk, 71% for the low-intermediate and high-intermediate risk, and 28% for the high-risk group. Compared with the published literature, poor-intermediate present with aggressive disease (38%), stage IV disease (48%), poor performance status (56%) and with high-intermediate, and high risk disease (46%), including a high incidence of extra-nodal lymphomas (41%). Of the patients with aggressive lymphomas, 12 (29%) had a primary chemo-refractory disease.

ANTIVIRAL TREATMENT HEPATITIS C VIRUS INFECTION IN PATIENTS WITH B-CELL NON-HODGKIN'S LYMPHOMA
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Introduction: Several epidemiological studies in different parts suggest a link between hepatitis C virus (HCV) infection and some B-cell non-Hodgkin’s lymphomas. We undertook this study after a patient with low-grade non-Hodgkin’s lymphoma had a complete remission after antiviral treatment of HCV infection.

Methods: 21 patients who had non-Hodgkin’s lymphoma 8 with non-Hodgkin’s lymphoma and 5 with HCV infection were treated with interferon alfa-2b 3 million IU every days in combination with ribavirin 1000mg per day.

Results: Of the 5 patients with non-Hodgkin’s lymphoma and 2 patients with HCV’s lymphoma and 5 patients with HCV infection were treated with interferon alfa-2b 3 million IU every days in combination with ribavirin in serum. Patients had this therapy during 3 till 12 months. One patient relapse Hodgkin’s disease when the HCV RNA load again became positive.

Conclusion: Patients with lymphoma and HCV infection treatment with interferon 3 million IU every days in combination with ribavirin 1000mg per day can lead to regression of the lymphoma.

EBV POSITIVE NON-HODGKIN’S LYMPHOMA FOLLOWING ALCUMUTUMAB STUDY H THERAPY
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Camptan IH is effective therapy for T prolymphocytic leukemia(PDLL) and chronic lymphocytic leukemia(PLL). Opportunistic infections secondary to severe lymphopenia is the primary treatment-related adverse event. We report a case of an EBV-positive Non-Hodgkin lymphoma following repeated use of alemtuzumab. An 82/F was diagnosed with TPLL May, 2003. Alemtuzumab led to CR but was stopped due symptomatic CMV infection after 226 mg. Relapse was diagnosed 10 months later, which did not respond to chlorambucil or fludarabine/cyclophosphamide. Re-treatment with alemtuzumab induced a second CR and profound lymphopenia with CD4 levels of 129/μL. One month post-therapy the patient complained of low grade fever and painful enlargement of the right parotid gland. Trucut biopsy: diffuse large B cell lymphoma expressing CD30 and EBV-LMP with necrosis. PET CT: widespread involvement including multiple hepatic and splenic foci. Four courses of rituximab/IV methotrexate 250 mg resulted in rapid regression of the mass 5.5 cm to 1 cm. A literature search revealed four additional cases. Gharbou et al. (NEJM, 2003) reported a similar 80/F patient diagnosed as T-CLL with minimal or no response to chlorambucil or cyclophosphamide/fludarabine, and a good response to alemtuzumab. 6 months post alemtuzumab, the patient presented with diffuse large B cell lymphoma presenting as a 6x3.5 cm sole right parotid mass. G. Bruni et al. (Cancer, 2005) reported a young female CLL patients aged 55–58, treated with alemtuzumab who developed a large cell B cell lymphoma mixed with centrocytic T cells and a necrotic lung mass. All four patients had been treated with fludarabine prior to alemtuzumab. EBV-positive lymphoproliferative disorders are well recognized in immunosuppressed patients such as those with AIDS and solid organ transplantation and has been linked to fludarabine. After organ allografting increasing loads of EBV in the blood are associated with increases in the risk of PTLD. These tumors are also more common with low CD4 counts. Caution should be used when alemtuzumab is used alone or in...
combination with other therapies inducing T-cell immunodeficiency such as fludarabine. Monitoring CD4 counts and EBV viral load should be considered during alemtuzumab therapy.

EPSTEIN–BARR VIRUS ASSOCIATION IN HODGKIN’S LYMPHOMA IN HUNGARY


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Introduction: The occurrence of Epstein–Barr virus (EBV) associated Hodgkin’s lymphoma (HL) shows considerable variation from continent to continent and from country to country but in Hungary no such investigations have been performed so far.

Methods: We have analyzed paraffin sections from patients with histologically confirmed and untreated HL for the presence of EBV using PCR, in situ hybridisation and immunohistochemistry.

Results: Out of 109 cases, 61 patients (56%) showed virus positivity by PCR while LMP1 positivity was found in 47 cases (43%). As regards to gender ratio, 53% female and 58% male patients were virus positive by PCR. EBV association did not show any alteration in children (1–14 years) when compared to that of adults (out of 10 children 6 were positive by PCR). As regards to the lifestyle of EBV positive patients, the incidence of smoking and the ratio of poor social conditions were significantly higher. Mixed cellular subtype was the most frequent (65%) in EBV positive patients, primarily in age groups 11–20 and over 51 years. EBV PCR positivity was 52% in nodular sclerosis (negative cases cumulated in the age group 15–30 years), other histologic subtypes could not be evaluated due to the small number of cases. On examining HL and EBV association disease models, we could not categorise our patients into any of them though characteristic patient groups could be more or less observed also in our material. This may be explained by the socio-economic differences of the population living under different economic conditions.

Conclusions: Our results indicate that EBV infection may play an important role in the development of HL in Hungary, too.

LACK OF SV40-T-ANTIGEN IN EUROPEAN LYMPHOMAS

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Introduction: Simian virus 40 (SV40) transforms highly efficient human cells in vitro and induces malignant tumors in rodents. In vivo, in human tumors such as lymphomas, the role of SV40 infection is discussed controversially. Noteworthy, in initial Poliomyelitis vaccination campaigns from 1955–63 millions of people worldwide were exposed to SV40 contaminated vaccines. We therefore looked for the expression of the SV40-related large T-antigen in human lymphomas.

Material and Methods: Tissue microarrays (TMAs) containing more than 600 Non-Hodgkin-Lymphomas (NHL) and 300 classical Hodgkin-Lymphom (cHL) were analyzed for the expression of the large T-antigen by immunohistochemistry (SV40 T AG, Oncogene, 1:500, microwave pre-treatment). Archival samples encompassing the years 1974–2001 from Italian, Swiss and Austrian patients and representing the most frequent subtypes of NHL and different subtypes of cHL were included for the construction of the TMAs.

Results: Protein expression of the large T-antigen was not detected in 643 NHLs and 337 cHLS whereas tubular kidney cells as positive controls showed a distinct nuclear signal.

Conclusions: Our results do not provide evidence of an association of SV40 large T antigen and human lymphomas. Geographical differences might explain the discrepancy to some data from other countries.
ROLE OF ECTO-5’-NUCLEOTIDASE ACTIVITY IN LYMPHOCYTES IN PATIENTS OF LYMPHOPROLIFERATIVE DISORDERS
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Introduction: Ecto 5’ nucleosidase (Ecto 5’ NT) is a glycoprotein attached via a glycosylphosphatidylinositol linkage to the external plasma membrane of various cells including human lymphocytes. It catalyses dephosphorylation of deoxyribonucleotides to corresponding nucleosides. Ecto 5’ NT is believed to be a maturation marker of “B” and “T” lymphocytes. Reduced Ecto 5’ NT activity has been reported in chronic lymphocytic leukemia patients.

Method: In present study 10 patients of acute lymphoblastic leukemia and 10 patients of lymphoma admitted at PGIMS, Rohtak were subjected to Ecto 5’ NT estimation by Campbell, Fiske and Subbarow method. Twenty healthy normal subjects served as control.

Result: The activity was reduced both in leukemia and lymphoma patients. The enzyme activity increased after chemotherapy in these patients.

LATENT AND LYTIC EBV GENE EXPRESSION IN AKATA TUMOUR CELLS UPON REACTIVATION
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Introduction: Epstein–Barr virus (EBV) is linked to a number of B-cell malignancies (e.g. Burkitt lymphoma – BL). Independently of its role in tumor formation, the presence of EBV in all tumor cells offers a unique opportunity for targeted tumor therapy. Activation of the latent EBV into its lytic form to exploit the expression of EBV specific lytic proteins to induce specific tumor cell death are potential therapeutic approaches. Elucidation of the molecular mechanisms of lytic infection will facilitate the development of EBV targeted therapies.

Method: We have developed an oligonucleotide-chip with probes specific for 32 latent and lytic EBV genes. Real-time quantitative PCR (TaqMan) assays were also developed. Akata (BL) and B95.8 (lymphoblastoid cell line) cells were treated with TPA for induction of lytic infection. Akata cells were also activated by IgG-crosslinking. Cells were collected at different time points after activation and gene expression profiling was performed. Expression of the key lytic gene BZLF1 was also analysed at the protein level by Western blotting and immunofluorescence.

Results: IgG-crosslinking and TPA induced both EBV lytic infection in Akata cells with different kinetics: more rapid the first (peak of BZLF1 expression at 6h) and slower the latter (peak of BZLF1 at 48h). In both cases expression of BZLF1 was transient and declined 24h after peaking. TPA induced EBV lytic infection in B95.8 but activation was sustained and BZLF1 expression levels increased steadily throughout 96h. An up-regulation of latent genes EBNA1, EBNA2, following both IgG- and TPA-stimulation was observed in Akata cells, but not in B95.8 cells. Surprisingly, up-regulation of latent genes was concomitant with the decrease of lytic gene expression, and coincided with a numeric decrease of cells positive for the BZLF1 gene product Zta.

Conclusions: Our observations suggest an inhibitory mechanism by which undesired EBV reactivation is blocked. Counter regulation by latent EBV genes may hamper therapeutic efforts to induce lytic EBV infection in EBV-harbouring lymphomas.

CLINICAL CHARACTERISTICS OF B-CELL LYMPHOMA WITH 3q27 TRANSLOCATION
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About 20–30% of diffuse large B-cell lymphomas (DLBCL) have chromosomal translocations affecting 3q27/BCL6. Although the 3q27 rearrangements are the most frequent aberrations among DLBCL, the clinical impact on the prognosis remains to be defined. Patients: A total of 1388 patients with non-Hodgkin’s lymphoma were treated at ALTSG from 1998 to 2004. Chromosomal data were available in 435 of 823 patients with B-cell lymphoma.

Results: Among 39 patients having 3q27 rearrangements, 9 patients were follicular lymphoma (FL), 28 were diffuse large B-cell lymphoma (DLBCL). Translocation partners of 4 patients were an immunoglobulin gene (IG) type loci, 3 patients with 14q32 and 1 patient with 22q1. Two cases did not involve immunoglobulin gene (non-IG) type, (t(2),(3)(q23,q27) and (t(6),(6)(q27,p21)). The translocation partner was not identified in 3 patients. (t(14,18)(q32,q21.3) was observed in 3 of 9 patients, as an additional translocation. Among 30 patients with DLBCL, 14 patients had translocations with 14q32 and 3 patients with 22q11. Non-IG type was observed in 1 patient of (t(13)(p34;q27) and 12 of (del(3)(q27)). Besides 3q27 changes, 2 patients had (t(14,21), 2 had (t(8,21)(q35,p12), 3 had (t(14,14)(q32,q32), 6 had (t(7)(17)(p))7. 3q27 translocations were observed significantly higher among patients > 60 years and among patients with an advanced stage. Bone marrow infiltration was found in 8 of the 30 DLBCL patients. Complete remission (CR) was obtained in 22 (73%) of the 30 DLBCL patients; CR was achieved in 14 patients (82%) with the IG type and in 8 patients (62%) with the other types (P = 0.032). The CR rate among the 3q27-negative DLBCL was 42%, which difference was not significant from 3q27-positive patients. However, progression-free survival was significantly longer in the 3q27-positive DLBCL patients (P = 0.024).

Conclusions: DLBCL patients with 3q27 translocation had better prognosis despite the high-risk group according to the International Prognostic Index (IPI). These findings suggest that the presence of 3q27 translocation is a significant prognostic factor in DLBCL.

ABERRANT EXPRESSION OF PAX GENES IN LYMPHOMA MALIGNANCIES
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Introduction: The PAX gene family encodes transcription factors containing a paired box domain and some of them are known to be oncogenes. We analyzed the expression of PAX genes (1 to 9) and the DNA methylation status of PAX4 gene in lymphoid malignancies.

Methods and Results: Various normal hematopoietic tissues showed no expression of mRNA of PAX4 gene by RT-PCR method, but some of hematologic cell lines and affected lymph nodes of malignant lymphoma showed expression of PAX4 gene. The promoter region of this gene after bisulfite modification to evaluate the DNA methylation status. The DNA from lesions of lymph nodes in normal lymphocytes showed methylation status but the DNA from cell lines, which had PAX4 expression, contained demethylated sequences. The demethylated status of PAX4 gene was detected in 10 out of 18 primary malignant lymphomas by the combined methylation and restriction assay. The gene expression of other PAX genes was examined. PAX 6 genes also showed aberrant gene expression in some lymphoid cell lines but not in primary lymphoma and leukemia.

Discussion: These results indicated that PAX4 gene was activated in some hematologic cell lines and malignant lymphomas by aberrant DNA demethylation. Although the function of PAX4 gene in hematopoietic system is under investigation, PAX4 gene is thought to be a candidate oncogene in lymphoid malignancies.

BLYS SIGNALS THROUGH PI-3 AND PKC-DELTA IN NON-HODGKIN LYMPHOMA B-CELLS
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SECONDARY REARRANGEMENTS OF A TRANSLATED BCL6 GENE IN NON-HODGKIN B-CELL LYMPHOMAS

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Introduction: Biallelic BCL6 abnormalities have been repeatedly reported in non-Hodgkin B-cells lymphomas (NHL), and the chronology of the rearrangements and their implications are poorly understood. We describe here two tumors where a first BCL6 translocation was followed by a secondary rearrangement on the same allele.

Results: Two patients with a t(3;14) were studied. Both cloned der(14) indicate an involvement of BCL6 and of the IGH switch (S) γ3 region. Two der(3) were cloned first the tumor, corresponding to two related clones. The first der(3) is the expected der(14) reciprocal. The second has a three way junction (3μ-S-5γ-BCL6), with the S-3BCL6 junction identical to the one on the first der(3), indicating that a secondary class switch recombination took place on the translocated IGH locus. RT-PCR experiments indicate that this tumor expressed S specific BCL6 fusion transcripts from the 3μ-S-5γ-BCL6 fusion. For the second patient, the cloned breakpoints and the fusion transcripts indicate that the initial (3μ-S-14) was followed by a secondary inversion of BCL6 that involved a partner gene (E1F4A2) located downstream from the gene in a tail to tail orientation on the Xq27 region.

Discussion: We had previously showed that mutations can accumulate on the BCL6 gene after a translocation. Our results now indicate that an ongoing instability can induce secondary rearrangements. The structure of the translocations we describe indicate that two successive BCL6 promoter substitutions took place, suggesting that all BCL6 translocations may not have the same potential to induce transformation, and that secondary re-organizations of a translocated gene may play an important role during the progression of the disease.

COMPARATIVE ANALYSIS OF BONE MARROW RESPONSE KINETICS IN DISSEMINATED BURKITT'S LYMPHOMA AND B-CELL ACUTE LEUKEMIAS

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We also evaluated cytokines and drugs known to have NK cell immunomodulating activity in order to determine their impact on Fc γ RIla polymorphism dependent rituximab binding. NK cells were cultured with rHL-2 (100 U/ml), IL-12 (0.1 ng/ml), IFN-γ 2a (100 U/ml) and IFN-γ (100 U/ml) as well as with Thalidomide (10 μM) and Revlimid (10 μM) for 24, 48 and 48 h respectively. No remarkable changes in rituximab binding were observed, including among individuals with the FF polymorphism versus baseline controls. The above studies suggest that individuals with the Fc γ RIla – 158 VF and VF polymorphism demonstrate higher rituximab binding to their NK cells than individuals with FF polymorphism and may
account for the higher response rates observed to rituximab among indolent NHL patients expressing at least one valine at Fc-γ RIIA-158.

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DHPLC: A POWERFUL SCREENING METHOD FOR LARGE SCALE DISCOVERY OF SOMATIC MUTATIONS IN NHL
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Introduction: aberrant somatic hypermutation (SHM) is an important step during lymphomagenesis leading to oncogene activation independently of chromosomal aberrations. Determination of SHM status may provide insight in lymphoma development and functional activation of candidate oncogenes. Therefore we aimed to screen a large series of B-cell lymphomas for mutational status of several selected oncogenes. However, direct sequencing and cloning as the method of choice for mutation analysis is cost-intensive and time consuming.

Methods: DHPLC screening and sequencing was applied for discovering SHM in different oncogenes. Three DHPLC temperature profiles were tested and optimized for each target region.

Results: We established high-throughput DHPLC assays for mutation screening of REL, BCL6 and MYC. Serial dilutions of mutated in unmutated PCR-products showed a sensitivity limit of less than 5%, which depended on the DNA quality and the respective target region. We successfully screened a large series of 140 aggressive and 80 follicular lymphomas by DHPLC. Direct sequencing or cloning was performed in cases with aberrant elution profiles for confirmation and mapping of mutations. In our hands DHPLC provides a more sensitive option for mutation screening compared to direct sequencing (detective limit of 10-15%).

Conclusion: DHPLC technique allows sensitive large-scale screening for hypermutated oncogenes and provides an advantage especially in complex large genes without particular hotspots for sequence variations.

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A QUARTER CENTURY TREATING ORBITAL LYMPHOMA
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Aims: Primary orbital non-Hodgkin's Lymphoma is rare and comprises approximately 1% of all NHL presentations. A review of all cases treated within the Edinburgh Cancer Centre was performed to assess the management of this rare condition.

Methods & Materials: The clinical records of all patients diagnosed with stage I or II primary orbital lymphoma and seen at the EEC between 1975 and 2002 were reviewed. Information on patient characteristics, treatment, toxicity, response to treatment and survival was analysed.

Results: Thirty-three cases of localised primary orbital lymphoma were treated during the period assessed. Eighteen were graded histologically as indolent and eleven as aggressive. Eighty-eight percent of patients (n = 25) were treated with radiotherapy alone. The 5- and 10-year cause specific survival, overall survival, and disease free survival rates were 87% and 80%, 83% and 70%, and 69% and 39% respectively.

Local control rates were 100% for patients treated with radiotherapy and 97% overall. Seven patients (21%) developed systemic relapse. None of these patients had received chemotherapy as part of their primary management. Treatment with chemotherapy appeared to be associated with freedom from systemic relapse for patients with aggressive lymphoma but not for those with indolent disease. Late toxicity was seen in sixteen (55%) of the patients irradiated including six cases (21%) of cataract. Two patients (7%) developed punctate keratitis having been treated to biological equivalent radiation doses of 44Gy and 47.25Gy.

Conclusions: the prognosis of patients treated for localised primary orbital lymphoma in this centre is favourable. Radiotherapy is an effective treatment modality resulting in excellent local control rates.

Patients with high grade orbital lymphoma should be considered for combined modality treatment as primary management to reduce risk of systemic relapse. However, late toxicities were seen in a significant proportion of patients. This reinforces the opinion that a radiation dose of <34Gy is optimal for local control of orbital lymphoma whilst minimising morbidity.

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MULTICOLOR BANDING (MBAND) PROVIDES A DETAILED CHARACTERIZATION OF STRUCTURAL ABNORMALITIES OF CHROMOSOME 1 IN BURKITT'S LYMPHOMA
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Introduction: the genetic hallmark of Burkitt's lymphomas (BL) is a rearrangement of the CMYC gene by the chromosomal translocation t(8;14)(q24.3;q22) or its variants. The most frequent secondary chromosomal abnormality in BL is a partial duplication of the long arm of chromosome 1 involving variable bands. However, the chromosomal regions and genes involved in these duplications and the pathogenetic consequences are largely unknown.

Methods: In order to better characterize duplications of 1q in BL, we have applied chromosome 1-specific MBAND probes (Xceny-Metasystems, Altstettlen). Multicolor banding analysis allows the differentiation of up to 25 different regions/bands of chromosome 1 by its fluorescence color. The results of multicolor banding were compared with results from G-banded karyotypes obtained by Trpysin-Giemsa staining. Using these techniques, 5 BL cell lines (BL-70, MN-60, CA-46, Namalwa, Tanove) and three patients with BL, diffuse large B-cell lymphoma (DLBCL), and marginal zone B-cell lymphoma (MZBCL), respectively, cytogenetically characterized by duplications or insertions of 1q were analyzed.

Results: By conventional cytogenetic analysis the abnormalities of 1q were described as dup(1)(q21q31), dup(1)(q21q32), dup(1)(q22q31) and ins(1;?)(q21q21) in BL, as dup(1)(q25q31) in the DLBCL, and as trp(1)(q21q21) in the MZBCL. mBAND analysis revealed duplications of 1q21q21, 1q22q31, 1q21q32, and 1q23q31 in BL, 1q21q24 in the DLBCL, and trp(1)(q21q24) in the MZBCL. Furthermore, mBANDing identified additional structural alterations of 1q in 4 of the 8 cases.

Conclusion: Multicolor banding analysis could specify the results obtained by conventional cytogenetic analysis in all cases with duplications of 1q analyzed in this study. Supported by the Wilhelm Sander-Stiftung.

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CHROMOSOME 6Q DELETION PATTERN IN MALIGNANT LYMPHOMAS
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Introduction: Deletion of 6q is a frequent karyotypic alteration found in a variety of cancers and lymphoproliferative disorders including leukemia and lymphomas. In lymphomas, deletions of 6q can be detected at a frequency of 7% to 42%, depending on the histological types and the methodology applied. Recent studies using FISH show frequency of 6q deletions higher than previously reported by cytogenetic analysis and indicate two minimal deleted regions (MDR) in 6q16-21 and in 6q27.

No critical tumor suppressor genes have been identified in these regions.

Methods: To define the deletion pattern of 6q, we analyzed with conventional and molecular cytogenetic approaches a series of lymphomas including non-Hodgkin (NHL) and Hodgkin (HL) cases. Twenty-six cases were studied by conventional cytogenetics, while 35 cases where analyzed by FISH analysis. Four types of FISH probes were used: commercially available whole chromosome paints; subtelomeric probes specific for 6p-tel and 6q-tel; commercially available locus specific probes; chromosome 6q YAC clones.
Results: Conventional cytogenetic revealed a 6q deletion in 12 out of 26 (46%) lymphomas. Chromosome 6q deletion was the sole chromosome anomaly in two cases. Interphase FISH analysis demonstrated allelic loss of 6q regions in 33 out of 35 cases (94.2%). The deletions were discontinuous involving non-adjacent genomic regions. The deletion pattern analysis revealed distinct chromosome 6q MDRs covered by the following YAC clones: 856g2, 970a12, 962g10, 962g7 and 4h8. Individual histological subtypes showed different MDRs. Two specific 6q regions deleted in DLBCL but not in FL may be implicated in the clinical transformation.

Conclusions: Although 6q deletion is a common event in all lymphomas, specific deletion patterns seem to characterize different histological types. Different tumor suppressor genes might play different role in different types of lymphomas.

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CYTOGENETIC ALTERATIONS IN MANTLE CELL LYMPHOMA DETECTED BY G-BANDING AND FLUORESCENCE IN SITU HYBRIDIZATION (FISH)
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Introduction: Mantle cell lymphoma is characterised by t(11;14)(q13;q32). This translocation juxtaposes IGH sequences with the CCND1 (BCL-1), leading to the overexpression of CCND1. The presence of this alteration is important in the MCL diagnosis. The cytogenetic analysis of MCL based on conventional and FISH methods is more useful, due to low mitotic index and poor morphology of metaphases.

Methods: Cytogenetic analysis was carried out on lymphoid cells obtained from lymph nodes, bone marrow or peripheral blood. FISH was performed with the two probes: LSI IGH/CCND1 (Vysis) and BCL1/IGH (Qbiogene).

Results: Forty two patients with MCL were studied. Karyotyping was successful in 21 cases. Conventional cytogenetics confirmed the presence of translocation t(11;14) in 8 patients. One patient had the variant translocation t(2;11). In one case tetraploid side line was observed. The remaining 11 cases had normal karyotypes, since we investigated bone marrow or peripheral blood specimens. FISH analysis was performed in 28 patients. The typical 1R1G2Y of signals was observed in 20 cases, including 5 cases with normal karyotypes. Simultaneously, in 5 of them a subpopulation of cells with different signal pattern was observed. These patterns indicated tetraploidy or amplification of fusion gene. In two of the remaining 8 cases the signal pattern suggested the deletions in the CCND1 and/or IGH regions. In the next case the signal pattern 2R3G indicated a variant of translocation of CCND1 gene onto chromosome 2. In the last 5 cases hybridization demonstrated the lack of fusion signals.

Conclusions: The simultaneous karyotyping and FISH examination of MCL improved our knowledge on the clinicobiological significance of MCL cytogenetics and may have a prognostic meaning as well.

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DIFFERENT APOPTOTIC RATES OF B-CELL CELLS ACCORDING TO BCL-6 MUTATIONAL STATUS. PROTEOMIC PROFILES
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Introduction: In B-CLL, somatic mutation of IgVH genes defines a subgroup with a favorable prognosis. Our previous data suggest that BCL-6 mutations identify a subgroup of patients with high risk of progression despite the presence of mutated IgVH gene.

Objectives: To study the effects of Btz and Rtx on apoptosis of CLL cells in culture, correlating the responses to BCL-6 mutational status and to identify molecular markers with the characterization of proteomic profiles.

Material and Methods: PBMC from 35 B-CLL patients (Binet stage A) were in vitro exposed to Btz (0.1, 1.0 μM) and Rtx (10 μg/ml) during 24 hours. The cells were stained with Propidium Iodide and Annexin V–FITC and analyzed by flow cytometry. 100μg of total proteins were used for 2D electrophoresis and subsequent proteins identification by MALDI-TOF.

Results: Btz and Rtx induce apoptosis on B-CLL cells in a dose and time dependent manner. Table shows median apoptotic rates in percentages in the group of IgVH mutated cells.

<table>
<thead>
<tr>
<th>IgVH mutated</th>
<th>Btz 0.1 μM</th>
<th>Btz 1.0 μM</th>
<th>Rtx 10 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL-6 mut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 12)</td>
<td>48.0 (24.9–68.8)</td>
<td>66.4 (44.1–72.3)</td>
<td>45.2 (25.2–70.0)</td>
</tr>
<tr>
<td>BCL-6 unmot</td>
<td>66.7 (61.4–79.8)</td>
<td>72.0 (67.8–85.9)</td>
<td>27.5 (2.9–40.8)</td>
</tr>
<tr>
<td>P = (U. Mann–Whitney)</td>
<td>0.023</td>
<td>0.035</td>
<td>0.003</td>
</tr>
</tbody>
</table>

In the proteomic study (n = 12), the average number of spots/gel was 234. The comparison between the groups of patients, revealed 8 proteins exhibiting differential expression (P<0.01, Student’s t-test).

Conclusions: Btz and Rtx have in vitro pro-apoptotic activity. In IgVH mutated cells, the apoptotic rates show statistically significant differences according to BCL-6 mutational status. We found eight proteins with differential expression between those groups of patients. This proteins may have potential value in the biological and diagnostic distinction between subtypes of CLL. Supported by FIS P020289; GV048/339 and G03/179. E. Jantus Lewintan is a grantee of Fundación Carolina.

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PROGNOSTIC SIGNIFICANCE OF ANGIOGENESIS IN SPECIFIC LYMOPHOMA SUBTYPES
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Introduction: Angiogenesis is required for tumour growth. In solid tumours, the angiogenic activity often correlates positively with an unfavourable prognosis. In malignant lymphomas, the role of angiogenesis is as yet largely unknown. Aim of the study: To evaluate angiogenesis in different subtypes of malignant non-Hodgkin lymphomas (NHL) and to correlate angiogenic scores to clinical endpoints.

Methods: Pre-therapeutic diagnostic biopsies from 107 patients with follicular B-cell lymphomas (FL), 94 with diffuse large B-cell lymphomas (DLBCL) and 107 with peripheral T-cell lymphomas (PTCL) were studied. Microvessels were stained by CD34 antibody and scored according to the Chalkley and microvessel density (MVD) methods. These angiogenic scores were compared with histological subtype, International Prognostic Index (IPI), proliferation-index, response to primary treatment and survival (overall and event-free).

Results: Both methods showed, that the lymphoma subtypes differed significantly in angiogenic scores (P<0.001 between all subtypes). If measured within tumour areas, angiogenic scores were highest in PTCL, and lowest in FL. However, a remarkable high microvessel density was found in interfolllicular areas of FL. In FL, high interfolllicular MVD-scores were significantly correlated to higher IPI (P=0.039). Moreover, low interfolllicular MVD scores in FL correlated with better treatment response (CR and PR, P=0.003), while high MVD scores predicted progressive disease and poorer overall and event-free survival (P=0.024 and 0.013 respectively). High interfolllicular Chalkley scores correlated with transformation to DLBCL (P=0.04). In DLBCL and PTCL, angiogenic scores did not seem to have any prognostic impact and were not correlated to clinico-pathologic parameters.

Conclusion: Angiogenic scores were significantly different in the three histologic NHL subtypes. In FL, a clear correlation was observed between high interfolllicular MVD scores and poor outcome and high Chalkley scores predicted histological transformation. In PTCL and DLBCL, the angiogenic scores did not have a clear impact on outcome.
PREDICTION OF SENSITIVITY TO CHOP THERAPY AMONG DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATENTS BY GENOME-WIDE CDNA MICROARRAY ANALYSIS

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Introduction: The standered therapy for patients with DLBCL is CHOP chemotherapy, which achieves a complete response in more than 60% patients, but some cases are CHOP therapy refract. DLBCL is heterogeneous for chemotherapy. So we used cDNA microarrays to identify genes differentially expressed between CHOP refractory DLBCLs and CHOP effective ones.

Method: We compared seven CHOP refractory cases with six CHOP effective cases.

Result: In the cDNA chips, nine gene (PRAME, tyrosinase, caveolin1, trophoblast glycoprotein, cadherin2, bcl-2, keratin19) were up-regulated in CHOP refractory cases, but one gene, CD79a was down-regulated. Using RT-PCR, we confirmed cDNA expression of PRAME in 4/6 cases (66.7%) in tumor cells of CHOP refractory cases.

Conclusion: PRAME is useful to know the chemotherapy effect in DLBCL.

ABERRANT SOMATIC HYPERMUTATION AND EXPRESSION OF ACTIVATION-INDUCED CYTIDINE DEAMINASE mRNA IN MEDIASTINAL LARGE B-CELL LYMPHOMA

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Introduction: Mediastinal large B-cell lymphoma (MBL) is a subtype of diffuse large B-cell lymphomas (DLBL) with distinct clinicopathological characteristics. The histogenetic derivation and specific genetic alterations associated with the pathogenesis of the disease are not fully understood. A novel form of genetic instability, termed aberrant somatic hypermutation (ASHM) plays a role in the pathogenesis of more than 30% of DLBLs. Constitutive expression of activation-induced cytidine deaminase (AID) also plays a part in the pathogenesis of different subtypes of DLBLs by generating a loss of target specificity of physiological somatic hypermutation of Ig genes and therefore initiating ASHM.

Methods: To determine the possible role of ASHM and AID expression in the pathogenesis of MBL, we have analyzed the mutational status of genes affected by ASHM including c-MYC, Pax-5 and RhoH, and we have determined the expression level of AID mRNA by real-time polymerase chain reaction in tumour specimens from six patients with MBL.

Results and Conclusions: Mutations in one or more genes and high expression of AID mRNA were detected in all the six cases of MBL. These results suggest that ASHM and AID expression may have a role in the pathogenesis of MBL.

THE IMPORTANCE OF ACTIVATION OF MACROPHAGE FUNCTION IN THE TREATMENT OF MALIGNANT LYMPHOMA

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Introduction: Might be the addition of adjuvant BCG therapy to activate the macrophage function helpful in consolidate the chemo-radio-therapeutic effect on malignant lymphoma?

Methods: the BCG therapy was performed by scarification method, it might be administered singly or in combination with chemotherapy or/and radiotherapy. The therapeutic effects were monitored by the dynamic change of macrophage phagocytic capability and development of clinical disease course.

Results: Malignant lymphoma patients (n. 101) and non-tumor subjects (n. 194) showed a broad distribution spectrum of macrophage phagocytic capability, but the malignant lymphoma patients, especially the patient with clinical tumor, showed the marked tendency of depressed macrophage function as compared with non-tumor subjects. < 30%: 45.6% vs 30.9% and > 40%: 41.6% vs 53.6%. The macrophage function of patients in post treatment clinical disease free period recovered and reach to the levels of non-tumor subjects. 2. The dynamic change of macrophage function of malignant lymphoma patients with clinical tumor or in post treatment clinical disease free period might be markedly enhanced after BCG treatment, the pre-vs post BCG therapeutic level was < 30%: 71.8% vs 33.1% and > 40%: 23.1% vs 64.1%. The effective range increased over 30% and 40% to reach 56.4% and 33.3% respectively. 3. The successfully treated and long-term followed malignant lymphoma patients, who showed no standard effective treatment available, derived sub-sta-nstant therapeutic effect and a close relationship of macrophage function and the development of clinical disease course.

Conclusion: The addition of adjuvant BCG therapy to chemo-radiotherapy could efficiently activate the patient’s immune function of macrophage phagocytic capability. OT skin test reactivity and benefit the patient of malignant lymphoma lived in long-term disease free survival. It is help-ful to improve and consolidate the chemo-radiotherapeutic effect, it might give the chance to patient to reduce the treatment failure of chemo-radiotherapy.
STAGE I-II FOLLICULAR NON-HODGKIN’S LYMPHOMA (FL): IMPACT OF 18F FDG PET (PET) ON DISEASE STAGING AND PATIENT MANAGEMENT

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Introduction: Accurate staging is essential to identify patients (pt) with stage I-II FL who are most likely to benefit from involved field radiotherapy (IFRT). We performed a retrospective assessment of the impact of PET on the staging and management of pt with previously untreated stage I-II FL.

Methods: Eligible pt had stage I-II FL based on clinical examination, computerised tomography and marrow examination, and underwent PET scanning between 1996 and April 2003.

Results: 25 eligible pt were identified, median age 55, with 16 males. Histology was follicular grade 1–9, grade II-9, grade 3-1, not specified 6. Conventional stage was I-14, and II-11. Disease was PET avid in 21 cases- the remaining cases all disease had been excised (3) or consisted of a superficial scalp lesion (1). PET resulted in “upstaging” in 10 pt (40%): from I to II in 2 cases, and I-II to III in 8 cases. Potential impact on treatment was change from involved-field radiotherapy (IFRT) to systemic therapy or comprehensive lymphatic irradiation (CLI) in 8, and enlargement of IFRT treatment port in 2. Of 12 pt with stage I-II who received IFRT, 11 remain NED at a median follow-up of 23 months (range 10–48). Of 3 pt with stage III who had CLI, 2 remain NED at 33 and 51 months.

Conclusions: FL shows a high rate of PET avidity. In pt with conventional stage I-II disease, PET has an important impact on stage and treatment in 40% of cases. Outcome of PET staged pt treated with IFRT may be superior to historical series.

COMPARISON BETWEEN FDG-PET AND CT-SCAN IN THE MANAGEMENT OF NON HODGKIN'S LYMPHOMAS

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Introduction: The role of FDG-PET in the management of NHL is controversial. We show a comparison between FDG-PET and CT-Scan in a single centre experience.

Methods: We evaluated 96 patients with NHL who underwent whole-body FDG-PET and CT-Scan from Jun 2000 to Dec 2004 (193 studies). The studies considered mainly patients with DLCL in fact, out of 193 studies, only 32 concerned FL and 16 MCL. Both CT and PET were performed within 4 weeks, at different disease’s phase: staging (25), early response (18), restaging (75) and follow-up (75).

Results: The numbers in brackets show the outcome; median FU:24 mths.

<table>
<thead>
<tr>
<th>PET+</th>
<th>PET-</th>
<th>CT+</th>
<th>CT-</th>
<th>Sens%</th>
<th>Spec%</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 (24+)</td>
<td>1 (1+)</td>
<td>100/96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (7)</td>
<td>6 (5)</td>
<td>5 (4)</td>
<td>77/87</td>
<td>100/55</td>
<td></td>
</tr>
<tr>
<td>16 (16)</td>
<td>29 (28)</td>
<td>21 (20)</td>
<td>7 (3)</td>
<td>91/84</td>
<td>90/71</td>
</tr>
<tr>
<td>12 (12)</td>
<td>32 (32)</td>
<td>15 (14)</td>
<td>9 (1)</td>
<td>93/93</td>
<td>89/74</td>
</tr>
</tbody>
</table>

Conclusions: In staging, both PET and CT are reliable; PET seems to have a better diagnostic significance. The role of PET as early index predicting the outcome is an interesting target for future studies. PET is superior to CT in the evaluation at the end of the therapy (probably due to a better resolution on minimal residual disease in bulky masses). PET has probably a light superiority also in FU, but the number of false positive is not negligible. Results obtained in FL and MCL, though similar to these observed in DLCL, need to be confirmed in larger studies.
74% and 83% respectively (P<0.001). Patients with a positive CT / 67Ga scans pretransplant have a significant higher risk of relapse and death due to the disease [HR=0.03, CI(0.005–0.1), P<0.001 and HR=0.04 CI(0.007–0.02), P<0.001 respectively].

**Conclusion:** Gallium scan helps to discriminate residual mass pre-transplant. Autologous Stem Cell Transplant (ASCT) does not seem to be the best therapeutic approach for patients with a positive CT and 67Ga scan result in preTransplant.

**USE OF EARLY INTERIM GA67 OR HYBRID PET/CT SCINTIGRAPHY AND INTERNATIONAL PROGNOSTIC SCORE (IPS) FOR TAINORING CHEMOTHERAPY REGIMEN OF STANDARD AND HIGH RISK HODGKIN LYMPHOMA (HD)**

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**Introduction:** This study is trying to address change of chemotherapy dosage according to individual patient response to initial cycles of chemotherapy in order to reduce cumulative chemotherapy for early responders and maximize dose intensity for late responders.

**Method:** From 1999 to 2004 a prospective study was initiated for patients (pts) with classical HD age 18-65y. Patients were classified as below and received 2 initial cycles of standard BEACOPP (groups A and B) or escalated BEACOPP (group C). Group A: Stage I-II + more than 3 sites or age >50 or extranodal or ESR>50 or L.D. Group B: As in Group A but with B symptoms or bulky. Stage III or IV with IPI less than 3. Group C: as in Group B, but with IPI 3 or more. At the end of 2 cycles, the subsequent therapy was determined by the finding on PET/CT of Ga67 as outlined below: Those with positive scan had 4 more cycles of escalated BEACOPP, those with negative scan had 4 standard BEACOPP. 106 patients were enrolled.

**Results:** CR rate for groups A B C were 100, 92, 97% respectively. Three yrs PFS and OS is 87% and 93% respectively at a median follow up of 60 month (6-60). Disease progressed in 3/11 positive PET/CT pts versus 1/37 of negative scans P=0.03, and in 1/13 pts with early interim positive Ga67 versus 8/44 negative scan P=0.67. Negative predictive value for early interim PET/CT or Ga67 is 97% and 82% respectively.

**Conclusion:** PET/CT is a useful tool for early interim decision making regarding dose of therapy on an individual patient basis. Early PET enables clinician to change dose of chemotherapy and reduce dosage in 80% of high score patients. Only 17% of low score patients required dose intensification. Six cycles of tailored BEACOPP are effective.

**EARLY RESTAGING POSITRON EMISSION TOMOGRAPHY WITH 18F-FLUCRODEXYGLUCOSE (FDG PET) PREDICTS RESPONSE IN PATIENTS WITH HODGKIN'S DISEASE**

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**Introduction:** More than half of all patients with Hodgkin's disease (HD) are cured with standard chemotherapy. Therefore, it is important to distinguish between responders to standard treatment and non-responders who may benefit from an early change to a more effective therapy. This study was intended to assess the value of a FDG PET performed after two cycles of standard chemotherapy to predict final clinical response in patients with HD.

**Methods:** Between June 2003 and August 2004 in our institute, twenty-two newly diagnosed patients with advanced stage (IIIB-IVB) HD were consecutively treated with ABVD chemotherapy. Patients' characteristics were: sex M/F 9/13; median age 31 years (range 13–48); 13 NS, 1 LP, and 8 LD according the histopathology; 9 with bulky mass; and 5 with extranodal sites. All these patients underwent a specific staging and restaging (early and final): CT and PET at time 0, PET after 2 cycles, CT scan and PET after the completion of chemotherapy radiotherapy front-line program.

**Results:** In four of 22 (18%) positive FDG PET results obtained after the second cycle were associated with a persistence/progression of the disease at the completion of chemotherapy treatment (P = 0.0001). In these four patients the mean SUV values were: 13.2 (range 6.5–20) at time 0, 8.1 (range 3.7–12.9) after two cycles; 10.8 (range 8.4–15) at the chemotherapy completion. The remaining 18 patients showed a PET negativity after two cycles and at the end of front-line treatment.

**Conclusions:** Very early (after two cycles) FDG PET may be used to tailor induction chemotherapy in patients with HD.

**PREDICTIVE VALUE OF PET/CT SCANS AFTER CHEMOTHERAPY FOR HODGKIN'S LYMPHOMA**

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**Introduction:** Residual anatomic abnormalities in the initial sites of disease after chemotherapy for Hodgkin’s Lymphoma (HL) may be a simple scar in a cured patient or the sign of a still active disease. Moreover, even normalisation of Computed Tomography (CT) scans may hide occult disease.

**Material and methods:** A population of 28 patients affected with HL and with at least one negative prognostic factor, was treated at Umberto I Hospital of Mistre (Venice, Italy) between January 2002 and January 2004. Patients were accurately staged and treated according to updated protocols. Radiation Therapy (RT) when used, was directed on primary site of disease and dose optimised to visible residual disease and response assessment. Diagnostic CT with contrast and 18FDG PET/CT scan were done at staging, after 2 or 3 chemotherapy courses, at the end and later in the follow-up. PET/CT scans with proper setup were also used for direct RT planning. CT scans were scored in a semi-quantitative way, based on maximum diameter of any nodal involvement, assigning 0 for no evidence of disease (NED), 1 for dubious localisation, 2 up to 3 cm, 3 greater than 5 cm and up to 10 cm, 4 for greater or massive extralymphatic involvement (E). PET/CT scans were also scored in a similar way: 0 for NED, 1 for dubious localisations, 2 for 1 to 3 nodal areas, 3 for more areas and 4 for large active areas or massive E involvement.

**Results:** At the end of chemotherapy, twenty-three patients out of 28 (82%) had a negative PET/CT. Among these, 6 relapsed (26%), while all 3/8 (38%) patients with positive PET/CT had active disease (100%) (P<0.01). PET/CT shows a positive predictive value of 100%, a negative predictive value of 74%, with a sensitivity of 45% and specificity of 100%.

**Conclusions:** PET scan evaluation is not an easy task, and a high specificity requires skill. Positive PET at the end of chemo courses ask for salvage treatment. But also a substantial quote (26%) of negative PETs are still hiding disease, and we have to be aware of considering cured these cases. More, the further story of these patients shows just a nodal relapse in a never irradiated area, often resistant to more chemo and potentially curable with RT.

**WHOLE-BODY POSITRON EMISSION TOMOGRAPHY USING FLUCRODEXYGLUCOSE (FDG-PET) IN THE EVALUATION OF RESIDUAL MASS IN HODGKIN'S DISEASE: A MONOCEN-TRIC EXPERIENCE**

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**Background:** In approximately two-thirds of patients With Hodgkin's lymphoma (HL) a residual mass is present after treatment, whereas only
about 20% of these will relapse. FDG-PET has emerged as an important tool to monitor response to treatment in lymphomas.

Aims: To evaluate the sensitivity, specificity, positive and negative predictive value of FDG-PET performed at restaging in HL patients with a residual mass following treatment.

Methods: We retrospectively analysed the clinical data from 31 patients with HL in whom a residual mass was documented by CT-scan. At least one FDG-PET was performed. M/F ratio was 14/17; median age was 29 years (range 19–61); 2 patients (6%) were stage I, 12 (39%) stage II, 8 (26%) stage III and 9 (29%) stage IV. Chemotherapy consisted of ABVD, ABVD/OPP or BEACOPP + radiotherapy.

Results: In 19 out of 31 patients (61%), PET proved negative: of these, 18 patients (95%) are in continuous complete remission (CCR) at a median time of 4 months from completion of treatment and 1 patient (5%) has relapsed 3 months later. The remaining 12 patients (39%) resulted PET positive: 2 of them (16%) are in CCR 7 months after therapy, 5 patients (42%) underwent a biopsy that confirmed HD persistence of disease in 4 and fibrosis in 1; 5 patients (42%) underwent treatment because of progression confirmed by CT-scan. Sensitivity, specificity, positive and negative predictive value were 90%, 86%, 75% and 95%, respectively.

Conclusions: Our data confirm the experience of other groups that FDG-PET has a high sensitivity, specificity and negative predictive value, while the positive predictive value is lower (75%). Thus, a negative FDG-PET identifies patients who have an excellent prognosis, while FDG-PET positivity needs to be confirmed by further evidence of disease persistence.
4. Indolent Lymphomas

IDENTIFICATION OF UNIVARIATE AND MULTIVARIATE CLINICAL DATA PATTERNS PREDICTIVE OF MORTALITY IN FOLLICULAR LYMPHOMA

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We studied a clinical dataset of 90 individuals diagnosed with follicular lymphoma, covering 12 disease-related variables related to tumor stage and hematoloy. Using (a) conventional statistical, (b) continuous Bayesian, and (c) discrete probabilistic and information-theoretic data mining methods, we identified 4 variables and data profiles that were markedly associated with the terminal outcomes. Haemoglobin, B-Symptoms, Age and Relapse were each associated with mortal outcomes according to p-values ≤0.006, as revealed by F-test (ANOVA). Independently, these variables also provided the top 4 accuracy scores based on a Bayesian data mining method, IBIS (Integrated Bayesian Inference System). Hae-moglobin provided the best overall single variable predictive accuracy score (76%), with 91% accuracy for alive, and 36% accuracy for deceased outcomes. We found that Relapse paired with Age provided the best accuracies, i.e. 78% overall, 80% for alive and 72% for deceased, representing a clear improvement over any 1d model. Prediction of mortality becomes more refined when using the paired variables. For example, the probability of “deceased” is ~70% given a positive relapse score combined either with age >60years, or a positive B-symptoms score. Conversely, the probability of alive is >90% if the values for these 2 variable pairs are all negative. In conclusion, the application of complementary inference and predictive modeling methods allowed us to identify variables and patterns that may predict mortality in follicular lymphoma with 70%-90% accuracy, depending on whether one predicts death or survival. Further refinement based on combinatorial patterns, larger study samples and the addition of immunohistochemistry, FISH and gene expression data may help increase performance and validate these predictive models.

COEXISTENCE OF FOLLICLES OF DIFFERENT GRADES IN FOLLICULAR LYMPHOMA

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Introduction: Follicular lymphomas (FL) are graded by the number of centroblasts per high power field (hpf), and the presence or absence of centrocytes (WHO classification). The centroblast count is performed in 10 hpf and expressed as cells/hpf. However, heterogeneity of the neoplastic follicles with respect to grades is not well documented. Aims To determine whether different grades of FL coexist, and to document simultaneous diffuse large B cell lymphoma (DLBCL).

Materials and Methods: 135 cases coded as FL from 1992–2003 were retrieved via computerized laboratory reports. Small biopsies (21), fine needle aspiration specimens (8), infant (1), cutaneous FL (1), entirely diffuse follicle centre lymphoma (1) and cases with inadequate work-up (18) were excluded. Two observers performed counts in multiple neoplastic follicles to determine the grade(s) and the presence of DLBCL.

Results: 85 excision biopsies of lymph node were assessed. 33 (39%) had neoplastic follicles of more than one grade. Follicles with grade 3b histology (absent centrocytes on H&E slides) coexisted with follicles of other grades in 2 cases. The proportion of the associated grade/s varied between 5–50%. Three cases of grade 2 with DLBCL had a minor component of grade 3a.

Conclusion: 39% of cases of FL had components of more than one grade. Grade 3b was more likely to be associated with DLBCL. Coexistence of different grades in FL is likely to suggest a progression-related phenomenon. Diligent evaluation of several representative neoplastic follicles in FL samples is helpful in documenting these features.

GRADE 3 FOLLICULAR LYMPHOMA AUDIT

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Introduction: The WHO classification divides follicular lymphoma (FL) into 3 grades depending on the number of centroblasts per high power field and grade 3 cases can be further divided into a and b (the latter having solid sheets of centroblasts with no centrocytes). There is some evidence that the distinction between 3a and 3b is of biologic and possibly clinical significance. Most groups treat grade 3 patients with anthacycline based regimens. Whether all grade 3 patients merit anthacyclines is debatable.

Methods: Patients diagnosed with grade 3 FL in the West of Scotland in 2003 were identified using the Scotland and Newcastle Lymphoma Group database and analysed according to stage, extranodal disease, LDH, performance status, prognostic indices (FLIPI, IPPI) and treatment.

Results: 54 cases of FL were identified of which 17 (31%) were grade 3 (13 (26%) 3a and 3 (5%) 3b). 70% had a median stage and 41% had B symptoms. 50% (8 of 16) had a FLIPI of 2 or 3 i.e poor prognosis. Treatment details were available on 16 patients. Of the 3 grade 3a patients, 4 received “low grade” therapy—COP (3); chlorambucil + steroids (1) and 9 received “high grade” therapy—CHOP (3). R-CHOP (3), R-CHOP + radiotherapy (RT) (1), VAPEC-B (2). Of the 3 grade 3b patients, 1 received CHOP + RT, 1 CHOP, 1 CHOP + MTX. No grade 3b patient received rituximab.

Conclusions: Treatment of grade 3 FL is variable. Our current Scottish Blood Cancer Network guideline recommends treating grade 3a FL as grade 1 and 2 and treating grade 3b as diffuse large B cell lymphoma pending the results of a larger West of Scotland retrospective study of grade 3 FL.

CLINICAL, MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF GRADE 3 FOLLICULAR LYMPHOMAS (FL)

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Histological grading is believed to predict the outcome of FL. The WHO classification further subdivides the relatively uncommon grade 3 subset in types a and b (according to the presence of centrocytes) and recommends reporting the extent of the diffuse tumoral component; these distinctions may influence the clinical behaviour and be related to genetic characteristics. To determine the impact of the histological type on the clinical course and its relationship with the presence of the t(14;18) translocation, we reviewed 44 patients (pts) (22 women and 22 men, median age 61 yo, range 16 to 89) diagnosed with “de novo” grade 3 FL between July 1994 and December 2004. Twenty-nine were type a and 15 type b, of the 8 cases (19%) had a predominantly (>75% of tumor area) diffuse component. Seventeen pts had localized (stage I/II) and 27 advanced (stage III/IV) disease; according to the IPI, 19 pts had low, 16 pts intermediate and 6 pts high risk disease. The majority (77%) of pts received anthacycline-containing regimens; six had rituximab added to 1st line chemotherapy. Molecular analysis (with primers to discriminate between the MBR and MCR breakpoints) were performed in 37 cases and 23 (62%) were positive for the t(14;18) translocation. Cyrogenea analysis in 7 of the 14 negative cases, did not show recurrent abnormalities. No difference was found between the incidence of t(14;18) in types a and b FL, nor between predominantly diffuse and non-diffuse cases (P>0.05 Chi square test). With a median follow up of 26 (1–105) months

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and 5 pts not evaluable for response, the RR was 68% (59% CR and 18% PR), with a median duration of response and an overall survival of 12 (2 to 84) and 24 (1 to 1015) months, respectively. Although rituximab-receiving pts had responses not significantly longer than non-rituximab treated pts (22–10 to 36 and 10.5–2 to 84 months, respectively), all pts receiving the antibody are alive and in complete remission at the time of evaluation. Responses were unrelated to histological types (a or b) and to the predominance of diffuse areas (P = 0.05 Chi square test). The median duration of response and overall survival did not differ between types a and b nor between diffuse and non-diffuse cases. In conclusion, distinct clinical patterns related to genetic and histological characteristics were not identified in this series, which might suggest that other genetic mechanisms underlay the biology of the disease subtypes; the course of the disease is aggressive and 1st line rituximab-containing regimens may prove to be beneficial in grade 3 FL.

CLONAL SELECTION IN THE BONE MARROW INVOLVEMENT OF FOLLICULAR LYMPHOMA
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Approximately 50–80% of follicular lymphomas (FL) invade the bone marrow (BM) during the clinical course of the disease. To characterize the pathways of bone marrow involvement of follicular lymphoma, we performed morphological and immunophenotypical analysis of tumor cells from lymph nodes (LN) and corresponding BMs in 21 patients with FL. In these cases, genealogical trees were constructed based on the IgVH gene sequences of tumor clones from LNS and BMs. Our results showed that FLs within the BMs display identical or lower cytological grades than in the LNS. In the majority of cases, different number of tumor cells expressed bcl-2, CD10, K67 and p53 in LNS and BM. Tumor cells in the BM showed ongoing somatic hypermutation of the IgVH genes; the distribution of these mutations was highly consistent with antigen selection. The topology of the genealogical trees revealed that different subclones populate the LN and BM, and BM infiltration may occur at different points of the clonal evolution of FL. Early descendents of the original tumor clone and derivatives of diversified tumor clones may invade the BM. Our results suggest that the BM involvement of FL is associated with intensive clonal selection of tumor cells, and the BM provides a microenvironment, similar to the germinal centers of LNS, where tumor cells retain their biological nature.

INDOLENT NON FOLLICULAR NON HODGKIN LYMPHOMA: PROSPECTIVE EVALUATION OF GISS PROGNOSTIC CRITERIA FOR WATCH AND WAIT POLICY IN 109 PATIENTS
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Introduction: Indolent non follicular non Hodgkin Lymphoma (NHL), originally included in Working Formulation (WF) group A, according to REAL and WHO classifications set up a heterogeneous group of entities comprising small lymphocytic (SL), lymphoplasmacytic (LP), splenic marginal zone (SMZ), nodal marginal zone (NMZ), mucosa-associated lymphoid tissue (MALT) NHL. Since 1993 the Grupo Italiano per lo Studio dei Linfomi (GISL) designed a prospective study with the aim to evaluate in this NHL subset prognostic criteria directed to identify patients with an indolent non progressive clinical course eligible for a watch and wait policy.

Methods: Patients with a histological diagnosis of indolent non follicular NHL, not previously treated, could be enrolled in this prospective trial. These patients shouldn’t present any of the following features defining active disease according to GISS criteria: B symptoms, bulky disease (>5 cm), Hb level <10 g/dl, platelet count <100×10^9/l, diffuse pattern of neoplastic infiltration at bone marrow biopsy. The additional parameter of a short doubling time of the tumor burden was not evaluated in this preliminary analysis.

Results: Starting from 1993, 109 patients have been enrolled in this trial. After a median follow-up period of 35.6 months, the progression free survival of 91 evalable patients was 107 months and 85% of the patients are progression free. In fact, 17 (18.7%) patients progressed and required therapy. In order to possibly improve the prognostic system by the initial identification of cases more likely undergoing to progress, now we are evaluating additional variables including histological subtypes.

Conclusions: From this preliminary analysis GISS criteria for definition of indolent non follicular lymphoma are able to identify most cases with a truly indolent disease eligible for a watch and wait policy.

PRE-TREATMENT SERUM LDH LEVEL IS A PREDICTOR OF RESPONSIVENESS TO MONOCLONAL ANTIBODY (MAB) THERAPY IN PATIENTS (PTS) WITH LOW-GRADE LYMPHOMA (LG-NHL)
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Introduction: Mab therapy has become an established treatment modality for pts with LG-NHL. Responsiveness may depend on pre-treatment tumor cell biology or burden, as indicated by serum LDH.

Methods: We analyzed data from 3 international multi-center, phase II trials of combination mab therapy in pts with LG-NHL. Pts with relapsed or refractory disease received 360 mg/m^2 of etrapuzumab followed by 357 mg/m^2 of rituximab, both administered weekly over 4 consecutive wks. Median follow-up was 19.5 mo. in pts without disease progression.

Results: 108 pts with LG-NHL (82% follicular, 8% small lymphocytic lymphoma, 6% marginal zone lymphoma, 4% other) were treated. Median age was 61 yrs (range 31–87). Prior to treatment, 36% of pts had elevated LDH, 11% had ECOG PS ≥2, 92% had stage II or III disease, and 9% had ≥2 sites of extra-nodal disease. Among these variables, elevated LDH was the only significant adverse prognostic factor for complete remission (CR/Cru) and progression free survival (PFS) at 1 yr. CR/Cru was 33% for pts with nLDH and 14% for pts with elevated LDH (OR: 0.33; P = 0.04). PFS at 1 yr was 48% for pts with nLDH and 26% for pts with elevated LDH (OR: 0.38; P = 0.03). LDH remained a statistically significant predictor of CR/Cru (adjusted OR: 0.24, P = 0.04) and PFS at 1 yr (adjusted OR: 0.38, P = 0.03) even when adjusted for age, stage, and number of extra-nodal sites.

Conclusion: In pts with LG-NHL, serum LDH is a better prognostic factor for responsiveness to mab therapy than the other variables of the International Prognostic Index.

HIGH SERUM ß2 MICROGLOBULIN IS AN IMPORTANT INDEPENDENT PROGNOSTIC FACTOR FOR DISEASE PROGRESSION IN PATIENTS WITH FOLLICULAR LYMPHOMAS
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Introduction: The identification of risk factors is important to design risk-adapted treatment approaches in follicular lymphoma.
Methods: Characteristics at diagnosis were collected from 451 patients (pts) with follicular lymphoma, any grade, enrolled in clinical trials at our center from 1985–1992.

Results: At a median follow-up of 10 yrs, 10-yr OS and FFP for all patients were 60% and 43%, respectively. Corresponding results for pts with stage IV disease were 51% and 28%, respectively. By univariate analysis the following characteristics had a significant adverse influence on OS and FFP: age ≥60 y, low hemoglobin level, β2 microglobulin (B2M) ≥3 mg/L, LDH above normal, advanced stage. By multivariate analysis the most important prognostic features for FFP were stage, B2M, and LDH. For stage IV disease the FFP was significantly worse for pts with high B2M, shown in the figure below.

Conclusions: High B2M is an important prognostic feature for pts with follicular lymphomas and should be included in predictive models designed to select pts with low and high risks of relapse.

ACCURATE DETECTION OF CLONAL BCL-2/IGH MBR RERARRANGEMENT IN FOLLICULAR LYMPHOMA USING COMPARATIVE REAL-TIME PCR. A TECHNICAL REPORT

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Introduction: Clonal rearrangement of bcl-2 and IGH genes represents the most important molecular marker of follicular lymphoma (FL). In the majority of cases, bcl-2 breakpoints cluster to the major breakpoint region (MBR). Real-time PCR (RQ-PCR) is useful technique to evaluate efficacy of the treatment and/or minimal residual disease.

Methods: DNA derived from clinical samples was dissolved in Tris 10 mmol/L, pH 8.0. This stock DNA was then diluted in sterile water immediately before RQ-PCR assay. The results were normalized to a cellular gene for albumin. The assay was performed using TaqMan methodology. The primers and probe for bcl-2/IGH MBR were designed using the Primer Express 2.0. TaqMan system for albumin was described previously (Chiu et al., Clinical Chemistry, 2001). The bcl-2/IGH MBR and albumin gene sequences were cloned and used to construct the standard curves.

Results: We have used this RQ-PCR to determine the level of bcl-2/IGH positive cells pre, on, and post therapy in a panel of FL patients. We obtained 488 samples from 30 patients. Each new sample was analyzed simultaneously with two previously analyzed samples from the same patient. Such comparative testing served to standardize differences caused by suboptimal PCR efficiency. The normalized value of the bcl-2/IGH was expressed as the number of bcl-2/IGH copies per 10^6 cells. In our preliminary experiments a level of bcl-2/IGH carrying cells was normalized to the beta-actin or beta2-microglobulin copy numbers, but these housekeeping control genes are expressed at relatively high levels and their sensitivity to sample deterioration is low. Differences in amplification efficiencies between bcl-2/IGH and control gene result in inaccurate quantification.

Conclusion: We believe that the best method for reporting minimal residual disease in FL patients is a number of bcl-2/IGH copies compared with previous values, i.e. the serial samples post treatment. Our feasible method RQ-PCR with analysis of three following samples and simultaneously with amplification of albumin allow more accurate quantification of lymphoma cells.

MRD EXAMINATION OF BLOOD AND BONE MARROW MAY BE THE CLINICAL VALUE IN THE FOLLOW UP OF PATIENTS UNDERGOING HDT FOR TRANSFORMED FOLLICULAR LYMPHOMA

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Purpose: To examine in a prospective manner the value of minimal residual disease (MRD) detection for the early prediction of recurrence, in patients with follicular lymphoma, transformed to diffuse large B-cell lymphoma and treated with high dose therapy with stem cell support (HD). Methods: Tissues submitted for primary diagnosis were tested to find a clonal disease marker, either an IGH-BCL2 fusion gene or a rearranged IGCH complementarily determining region 3 (CDR3) fragment. The presence of either of these markers was validated by quantitative real-time PCR in blood and/or bone marrow samples submitted at diagnosis and during follow-up after treatment.

Results: In 22 patients, a positive tumour marker was found in the tumour specimen. 16 patients were examined adequately during follow-up. Median age was 45 years and median observation time from inclusion in the study to diagnosis was 30 months. Response after HDT: CR 15 patients; PR 0; Progressive disease 1. Status at last observation: Alive in CR 4; Alive in CR after new treatment 4; Alive with disease 2; Death from lymphoma 6. In seven of the 16 patients, MRD was detected either in the blood or BM before a clinical relapse while, also in seven patients, MRD was found at the same time as the clinical relapse. In two of these patients, only BM contained MRD. One patient developed a focal skeletal relapse as shown by radiography, without previous MRD in BM or blood. One patient never developed a relapse, was MRD positive at three months but later negative. The patient is in a new CR after treatment with local radiotherapy and rituximab. MRD levels one year after therapy were predictive of overall survival (Chi-square). Updated results will be presented at the meeting.

Conclusion: The results of this pilot study indicate that examination of MRD may be of value in the follow up of this patient population, but the results need to be confirmed in a larger study.

DUALCHIP HUMAN LOW GRADE B-CELL LYMPHOMA: A SPECIALIZED LOW DENSITY DNA MICROARRAY FOR THE DIFFERENTIAL DIAGNOSTIC OF SMALL B-CELL LYMPHOMAS

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Introduction: The differential diagnosis of lymphomas might be sometimes difficult, especially among small B cell lymphomas. Standardization of molecular approaches using multiple gene expression analysis on microarray should potentially contribute to the diagnosis. The aim of the present study was to develop a low density DNA microarray for the analysis of well known genes involved in B cell non-Hodgkin's lymphoma and to evaluate its performance in the diagnosis of small B-cell lymphoma.

Material and Methods: 10 B-cell chronic lymphocytic leukemias (B-CLL), 19 follicular lymphomas (FL), 9 mantle cell lymphomas (MCL) from 5 different institutions were evaluated; frozen tissue was available for each case and all cases were reviewed by a panel of three pathologists. A pool of 7 lymphoma B cell lines was used as reference sample. The microarray contains two arrays per slide with a range of 105 genes whose role in classification or lymphomagenesis of small B-cell lymphomas is known. Total RNA was extracted from frozen biopsies and cell lines using Trizol. Labeled cDNA were prepared using 25 μg of Total RNA. Hybridizations were performed in duplicate.

Results: Preliminary analysis of ratios of differentially expressed genes showed a down regulation of CD20 in B-CLL (2.43 +/- 0.10) versus FL (2.43 +/- 0.62) and MCL (2.05 +/- 0.43). We found a down regulation of
CD23 in MCL (−3.92 ± 0.08) and FL (−2.29 ± 0.17) versus B-CLL (2.91 ± 0.27), which was down regulated in FL (−4.39 ± 0.05) versus MCL (1.99 ± 0.04) and B-CLL (3.01 ± 1.73). Cyclin D1 was overexpressed in MCL (9.16 ± 4.8) versus FL (−2.40 ± 0.09) and B-CLL (−2.64 ± 0.22). CD10 and BCL6 were overexpressed in FL (2.59 ± 0.47) and 1.92 ± 0.23, respectively versus MCL (−2.62 ± 0.23 and −3.61 ± 0.08) and B-CLL (−5.64 ± 0.12 and −2.23 ± 0.25). These results allowed us to validate this molecular approach and a differential analysis of others genes is in process in order to evaluate which genes could be used to differentiate more deeply these entities.

Conclusions: We developed a new microarray tool which may be promising in the diagnostic approach of small B cell lymphomas and might be used easily in a near future on a routine basis in a department of pathology.

LOCALIZED FOLLICULAR LYMPHOMA: DEMONSTRATION OF B-CLL 2 POSITIVE CELLS IN THE BLOOD AND BONE MARROW AND POSSIBLE CLEARANCE AFTER LOCAL RADIOTHERAPY

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Background: Localized forms of follicular lymphoma (FL) are curable in 40–50% of cases with local radiotherapy. Polymerase chain reaction (PCR) of Bcl-2/JH rearranged cells provides for the majority of FL patients a sensitive measure of minimal NHL cell contamination in the peripheral blood (PB) and bone marrow (BM).

Aims: To evaluate the role of PCR detection of Bcl-2 positive cells in the PB and BM of patients otherwise considered stage I/II FL and the impact of treatment.

Methods: Between April 2000 and December 2003, 23 consecutive patients with follicular NHL, stage I/IIA according to the Ann Arbor classification, entered the study. Median age was 56 years, 11 were males and 12 females. Histology was centrally revised, confirming in all patients the diagnosis of FL according to the REAL/WHO classification. In 7 of the 23 patients, the diagnosis was FL grade L in 15 grade II and in 1 grade III. Involved field radiotherapy was performed in all patients (total dose ranging from 30 to 40 Gy). PCR was evaluated in the BM and PB of all patients at diagnosis, re-evaluated 3 months after treatment and, thereafter, every 6 months.

Results: At diagnosis, BcL-2+ cells were found in the BM and/or PB in 16 of the 23 patients studied; of these, 15 had a major breakpoint region (MBR) rearrangement and 1 a minor cluster region (mcr) rearrangement. After treatment, in 10 out of 15 BcL-2+ valuable cases, a disappearance of PCR positive cells was observed in the BM and PB, which still persists after a median follow-up of 38 months (range12–55), while 5 are still persistently positive. Three patients relapsed.

Conclusions: In the majority of localized FL patients PCR analysis can document the presence of viable BcL-2+ cells in the BM and PB. A durable disappearance of BcL-2+ cells from the BM and/or PB samples can be obtained in a high proportion of patients with local radiotherapy alone.

FLUDARABINE, MITOXANTRONE AND DEXAMETHASONE IN THE TREATMENT OF RELAPSED AND REFRACTORY LOW-GRADE NON-HODGKIN LYMPHOMA

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Introduction: The aim of the study was to evaluate response, duration of response, and toxicity of fludarabine (F), mitoxantrone (M), and dexamethasone (D) (FMD) in patients (pts) with relapsed or refractory low-grade non-Hodgkin lymphoma (LGNHL).

Methods: 26 pts with relapsed/refractory LGNHL exposed to previous chemotherapy (CHT) received 3–6 monthly cycles of FMD. The regimen consisted of F (25 mg/m² i.v., day 1–3), M (10 mg/m² i.v., days 1 and D (20 mg p.o., day 1–5). Parameters analyzed included response, toxicity and infection rates, number of previous CHT lines, age, sex, performance status (ECOG), Ann Arbor scale, LDH, International Prognostic Index score, freedom from progression (FFP) and overall survival (OS).

Results: FMD induced 25% complete and 37.5% partial response, with a total response rate of 62.5%. The median FFP and OS were 5 and 7 months. 10% of the cycles were associated with neutropenia and 11% with infection despite prophylaxis. Median number of previous CHT lines in pts without FMD-induced toxicity (1) was lower than in pts with its manifestation (2). Toxicity rate was not correlated with the remaining parameters. Among them, only ECOG >1 (P = 0.005) and presence of FMD-induced toxicity (P = 0.016) were predictive for shorter OS.

Conclusions: FMD is an active regimen for relapsed and refractory LGNHL. Toxicity rate is substantial and seems to predict survival. Identification of factors linked with susceptibility to FMD-induced toxicity warrants larger group of pts and longer follow-up.

ORAL FLUDARABINE/CYCLOPHOSPHAMIDE COMBINATION IN PRETREATED INDOLENT NON-HODGKIN'S LYMPHOMA

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Introduction: The efficacy of oral fludarabine (FLU) does not differ from that of the intravenous formulation and its safety profile is similar in patients with previously treatment chronic lymphocytic leukemia. A synergistic effect of cyclophosphamide (CY) with FLU has been demonstrated in vivo and in vitro. Based on this scientific background, in our Institute we have conducted a phase II clinical trial to evaluate the efficacy and safety of the combination of oral FLU and CY in pretreated indolent non-Hodgkin’s lymphoma (NHL) patients.

ANTHRACYCIN-CONTAINING REGIMENS (CHOP) ACHIEVE HIGHER RESPONSE RATES BUT NOT SUPERIOR OVERALL SURVIVAL IN FOLLICULAR AND MANTLE CELL LYMPHOMA

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Introduction: In advanced stage follicular lymphoma, conventional chemotherapy is non-curative and no major improvement in overall survival

ANTHRACYCIN-CONTAINING REGIMENS (CHOP) ACHIEVE HIGHER RESPONSE RATES BUT NOT SUPERIOR OVERALL SURVIVAL IN FOLLICULAR AND MANTLE CELL LYMPHOMA

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Introduction: In advanced stage follicular lymphoma, conventional chemotherapy is non-curative and no major improvement in overall survival
has been achieved by different regimens. Similarly, MCL, a lymphoma subtype with an especially poor clinical outcome, cannot be cured by conventional chemotherapy.  

Methods: In 1996, the German Low Grade Lymphoma Study Group (GLSG) initiated a randomized trial to evaluate the clinical efficacy of two different anthracycline/anthrachinone containing combinations: CHOP (cyclophosphamide 750 mg/m² day 1, vincristine 1.4 mg/m² day 2, adriamycin 50 mg/m² day 1, prednisone 100 mg/m² days 1–5) versus MCF (mitoxantrone 8 mg/m² days 1–2, chlorambucil 3·3 mg/m² days 1–5, prednisone 25 mg/m² days 1–5).  

Results: 415 patients with advanced stage indolent lymphoma were prospectively randomized and treated with 6–8 cycles of either CHOP or MCF. 277 patients (67%) had a follicular lymphoma (FL), 86 (21%) had a mantle cell lymphoma (MCL) and 52 (13%) patients showed another indolent lymphoma. Responders up to 60 years were subsequently assigned to either myeloablative consolidation and autologous stem cell transplantation or interferon-α maintenance (IFNo), all other patients received IFNα. As stem cell mobilization was hampered in the MCP arm, from July 1998 all younger patients were assigned to the CHOP arm. 86% complete and partial remissions (18% CR) were observed in the CHOP arm, whereas after MCP an overall response rate of 77% was obtained (14% CR, P = 0.0094). In subgroup analysis similar improvement of remission rates were detected in follicular lymphoma (91% vs 82%, P = 0.026) and mantle cell lymphomas (87% vs 73%, P = 0.008). No differences were observed, however, between both regimens for progression-free survival (PFS) in both lymphoma subtypes. Overall survival (OS) in FL was comparable in both study arms (74% vs 69% at five years, P = 0.29). In MCL, CHOP seems to achieve a higher overall survival rate (5y OS: 57% vs. 31%, P = 0.0576). However, multivariate analysis including the IPI score revealed no significant difference.  

Conclusions: CHOP achieves higher response rates in FL and MCL, but survival rates are not significantly improved. Thus, in elderly patients chemotherapy may be selected on an individual base whereas in patients qualifying for high dose chemotherapy CHOP should be preferred.

FLUDARABINE PHOSPHATE AND CYCLOPHOSPHAMIDE COMBINED WITH IMMUNOTHERAPY (RITUXIMAB) ONCE WEEKLY IS SAFE AND HIGHLY EFFECTIVE IN OLDER PATIENTS WITH INDOLENT B-NHL  

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Background: Treatment of advanced, indolent B-NHL has evolved to include different therapeutic approaches resulting in significantly increased disease-free survival. However, patients still relapse and further treatment options are needed.  

Aims: Tolerability and efficacy of a weekly regimen with fludarabine, cyclophosphamide and rituximab was assessed in older pts with advanced, indolent B-NHL. Chemotherapy is given once weekly in order to reduce toxicity in older patients.  

Methods: Pts received fludarabine (25 mg/m² iv) and cyclophosphamide (200 mg/m² iv) on day 1 of weeks 1–3 and rituximab (375 mg/m² iv) on Day 1 of Weeks 1–4, 9, 14 and 22. The overall duration of treatment was 5 months.  

Results: 22 pts with follicular lymphoma (n = 5), mantle cell lymphoma (n = 6), lymphoplasmacytic lymphoma (n = 5), chronic lymphocytic leukemia (n = 6) have been treated. The cohort comprised previously untreated (n = 8) and treated pts (n = 14, with 1–4 prior treatments). Median age was 73.5 years (range 66–81), most pts had advanced disease. Toxicity: Leukopenia: 8 pts (36%) grade 1–2, 10 pts (46%) grade 3–4. Thrombocytopenia: 5 pts (23%) grade 1–2. Anemia: 3 pts grade 1–2, 2 pts grade 3. 6 pts (27%) experienced fever, 8 pts (36%) infections and 3 pts (14%) mucositis (all limited to grade 1–2 severity). Treatment was discontinued in 6 pts, and was temporarily postponed in 4 pts due to persistent leucopenia. 2 pts (9%) required dose reductions due to thrombocytopenia or leucopenia. 2/22 pts (9%) required hospitalization. Efficacy: Overall response rate was 100%: 5/22 pts (23%) achieved partial response, 1/22 (4%) mixed response and 16/22 pts (73%) complete response. All pts remain in remission after a median follow-up period of 6.35 (range 2.6–35.6) months.  

Conclusions: These preliminary data suggest that the weekly FC/immuno-therapy combination is acceptably safe and highly effective in older patients with advanced B-NHL. A longer follow-up is needed to finally define the therapeutic value of this regimen.  

FLUDARABINE PHOSPHATE AND CYCLOPHOSPHAMIDE COMBINED WITH IMMUNOTHERAPY (RITUXIMAB) ONCE WEEKLY IS SAFE AND HIGHLY EFFECTIVE IN PATIENTS WITH INDOLENT B-CELL MALIGNANCIES  

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Background: Treatment of advanced, indolent B-cell lymphomas has evolved to include different therapeutic approaches, including single-agent chlorambucil, CHOP like chemotherapy and various fludarabine phosphate-based combination chemotherapies, resulting in significantly increased disease-free survival and a median survival expectation of 8–10 years. However, patients still relapse. The availability of anti-CD20 antibodies that target the B-cell lineage presents a potential opportunity to further increase the efficacy of fludarabine phosphate-based treatment.  

Aims: To assess the tolerability and efficacy of a weekly regimen with fludarabine phosphate, cyclophosphamide and the anti-CD20 antibody rituximab, in patients with advanced, indolent B-cell lymphoma. The chemotherapy is given once weekly in order to reduce toxicity in older patients.  

Methods: Patients received fludarabine phosphate (25 mg/m² iv) on Day 1 of Weeks 3–4, cyclophosphamide (200 mg/m² iv) on Day 1 of Weeks 3–4 and rituximab (375 mg/m² iv) on Day 1 of Weeks 1–4, 9, 14 and 22. The overall duration of treatment was 5 months.  

Results: To date, a total of 22 patients with follicular lymphoma (n = 5), mantle cell lymphoma (n = 6), lymphoplasmacytic lymphoma (n = 5), chronic lymphocytic leukemia (n = 6) have been treated. The cohort comprised previously untreated (n = 8) and previously treated patients (n = 14, range of prior therapies, 1–4). 3 pts (14%) achieved complete response, 3 pts (14%) partial response, 5 pts (23%) achieved partial response and 1 pt (5%) did not respond.  

Toxicity: Leukopenia was the predominant haematological toxicity. Eight pts (36%) developed grade 1–2 leucopenia and ten pts (46%) grade 3–4 leucopenia. Five pts (23%) developed grade 1–2 thrombocytopenia, three pts grade 1–2 anaemia and two pts grade 3 anaemia. Six pts (27%) experienced fever, eight pts (36%) infections and three pts (14%) mucositis. However, all cases of fever, infection and mucositis were limited to grade 1–2 severity. Treatment was discontinued in six pts, and was temporarily postponed in four pts due to persistent leucopenia. In CLL, 5/6 (83%) achieved complete response and 1/6 (17%) partial response, in follicular lymphoma 3/5 pts (60%) achieved complete response, in mantle cell lymphoma 2/6 pts (33%) achieved complete response, 2/6 pts (33%) achieved partial response and 1/6 pts (17%) mixed response. In the other indolent NHL, 3/5 pts (60%) achieved complete response and 2/5 pts (40%) achieved partial response. Two pts (9%) required dose reductions due to leucopenia. In addition, 2/22 pts (9%) required hospitalization. Efficacy: The overall response rate was 100%: 5/22 pts (23%) achieved partial response and 16/22 pts (73%) achieved complete response. Two pts with a previously treated Non-B-cell malignancy achieved partial remission at interim re-staging but subsequently died with progress. However, all other patients remain in response after a median follow-up period of 6.35 (range 2.6–35.4) months.  

Conclusions: These preliminary data suggest that the weekly FC/immuno-therapy combination is acceptably safe and highly effective in older patients with advanced B-cell malignancies. A longer follow-up is needed to finally define the therapeutic value of this regimen.  

FLUDARABINE PHOSPHATE, CYCLOPHOSPHAMIDE AND MITOXANTRONE (FCM) IN PREVIOUSLY UNPRETREATED PATIENTS WITH ADVANCE STAGE FOLLICULAR LYMPHOMA (FL)  

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Objectives: to analyze the efficacy and toxicity of FCM as first-line treatment in younger patients (pts) with advanced stage FL.

Patients and methods: 115 pts (53M/62F; median age: 53 yrs) with FL (grade 1, 57; grade 2, 52) in stages III-IV included in the trial between 1999 and 2003. The distribution according to FLIPI (n = 113) was: low-risk, 34 (30%); intermediate-risk, 48 (42%); poor-risk, 31 (28%). FCM consisted of 6 cycles of fludarabine (25 mg/m²/d, days 1–3), cyclophosphamide (200 mg/m²/d, days 1–3), mitoxantrone (6 mg/m²/d, day 1).

Results: 96 pts completed the 6 cycles of FCM. A total of 620 cycles were administered. Grade III-IV neurotoxicity and thrombocytopenia was seen in 6% and 0.6% of cycles, respectively. Six episodes of grade III-IV infection were observed (neutropenic fever, 4; pneumonia, 1; progressive multifocal leukoencephalopathy (PML), 1). 3 pts died during therapy (PML, meningoencephalitis and bronchoaspiration). No late toxicity, including myelodysplasia, has been observed. Among 108 pts with assessable response, 91 reached CR (84%), 13 PR (12%) and 4 failed to respond (4%). After treatment, 47 of 54 pts reached a RQ-PCR negative status assessed in peripheral blood and bone marrow. With a median follow-up of 2.5 yrs, 21 pts have relapsed, with a 4-yr failure-free survival (FFS) of 66%. ECOG >1, extranodal sites >1 and high b2-microglobulin predicted poor FFS. 11 pts have died during the follow-up (3 during FCM treatment and 7 after relapse due to progression or toxicity), with a 4-yr overall survival (OS) of 85%. ECOG >1 and high b2-microglobulin were associated with a shorter OS.

Conclusions: FCM produces a high CR rate, including molecular responses, with prolonged FFS in younger pts with advanced stage FL.

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TWO DOESES OF ATG IN FLUDARABINE PHOSPHATE-BASED RIC TRANSPLANT FOR LYMPHOPROLIFERATIVE DISEASES: A BHS STUDY


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BACKGROUND: Transplant related mortality (TRM) of allogeneic stem cell transplantation is a major problem in lymphoma (NHL) and Myeloma (M) patients. Indeed, this population has a higher median age and higher risk of GVHD. This TRM can be alleviated by the reduced intensity conditioning (RIC) approach but graft versus lymphoma (GVL) effect should be preserved.AIHS: We have compared two doses of ATG Freseniusio (10 mg/kg/x x 2 days) in a RIC regimen (F 30 mg/m²/d x 4 and CPA 1mg/m²/d x 3) in terms of engraftment, acute and chronic GVHD, tumor response, event free survival (EFS) and overall survival (OS) according to the age. GVHD prophylaxis consisted of C A (60%) and MMF (25%).

RESULTS: 74 patients (pts) with lymphoproliferative disorders (29MM, 26 NHL, 16 CLL and 3 HD) were included in this national trial. Before transplantation, 25% were in CR1 or CR2 (good prognosis group). Median age was 54 (13–70) y.o. With a follow up of 36 months, the OS was 79%, 60%, 71% and 25% for NHL, MM, CLL and HD respectively. EFS was 35%, 26%, 50% and 0% respectively. For pts in CR1 or CR2, the OS was 74% vs 47% for the poor prognostic group. Our serie is too small to draw conclusion in subgroup of NHL. TRM is 20% for the whole group but 40% above 60 years due to an increased risk of infection when treated for GVHD. T-cell chimerism at day 30 was improved by reducing ATG (70 vs 50%). Reducing ATG increases the incidence of GVHD but not the severity of GVHD. OS was not significantly improved by reducing ATG but there is a trend for a better survival and a better EFS (lower relapse rate) with 2 days of ATG.

CONCLUSION: We confirm the feasibility and low TRM in RIC transplant for pts with lymphoproliferative disorders in CR1 or CR2. However, relapses remain a concern in MM and HD. In our small series, pts above 60 y.o. have a higher risk of GVHD and life threatening infections complications.

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ADOPTIVE IMMUNOTHERAPY WITH AUTOLOGOUS CD25- DEPLETED AND CD25/D28-COSTIMULATED T-CELLS (ACTC) ENHANCES LYMPHOCYTE RECOVERY AFTER FLUDARABINE (FL)-CYTOKIN (CY) CHEMOTHERAPY IN PATIENTS (PTS) WITH LOW-GRD FOLLICULAR LYMPHOMA (FL)


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Introduction: FL-based chemotherapy combinations are highly effective in pts with FL but cause severe immunosuppression due to depletion of normal CD4 T-cells. CD4 T-cell counts may not return to normal for two years following flu therapy. Aside from increasing the risk of serious infections, this toxicity may limit the ability of the immune system to eliminate minimal disease. Adoptive immunotherapy using ACTC may enhance immune reconstitution and improve disease control.

Methods: We initiated a phase I study in pts with purine analog-naive relapsed/refractory FL (grade 1–2). After leukapheresis, pts are treated with 4 cycles of flu (25 mg/m²) days 1–3 and cy (250 mg/m2) days 1–3. Four weeks after last chemotherapy, responding patients receive escalating doses of ACTC prepared ex vivo from antitogenous T-cells depleted of regulatory CD4+/CD25+ cells, then expanded and activated using anti-CD3 and anti-CD28.

Results: Six pts have been treated to date. Median age is 51 y (range 31–65). Median number of prior therapies is 2 (range 1–3). Overall, 2 pts achieved a CR and 2 achieved a PR. Two pts were withdrawn from the study because of hematologic toxicity. Four responders have received ACTC at a dose of 5×10⁷ CD3+ cells. There have been no adverse events related to T-cell infusions. Median follow-up after ACTC infusion is 4 months (range 1–12 months). CD4+/CD8+ counts were significantly improved at 1 month and at last follow-up after T-cell infusion (medians 106/139 at baseline, 230/168 at 1 month, 282/259 last flu, P = 0.02 for trend). The median CD4+/CD8+ ratio increased from 0.7 to 1 at one month and to 1.1 at last flu, P = 0.06. All pts were anergic to candida antigen by skin testing (DTH) before flu-cy chemotherapy. One patient developed a positive DTH response to candida antigen 60 days after ACTC infusion.

Conclusions: ACTC results in significant CD4+ and CD8+ lymphocyte recovery in previously treated pts receiving flu-cy. This lymphocyte recovery compares very favorably with historical controls. T-cell dose escalation is ongoing.

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PHASE 2 STUDY OF CHOP-GR THERAPY FOR ADVANCED-STAGE FOLLICULAR LYMPHOMA

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Introduction: Recently CHOP plus rituximab (CHOP-R) has been widely used for patients with follicular lymphoma. Expecting an enhancement of antibody-dependent cellular cytotoxicity, which is one of the main mechanisms of action of rituximab (RTX), by granulocyte-colony stimulating factor (G-CSF), we investigated the safety and efficacy of a fixed scheme of combination chemotherapy with CHOP, RTX and G-CSF (CHOP-GR) for patients with follicular lymphoma in advanced stage in a phase 2 clinical trial.

Patients and Methods: Twenty-one untreated patients with follicular lymphoma grade 1 or grade 2 in advanced stage received 2 courses of initial CHOP chemotherapy followed by 4 courses of CHOP-GR; G-CSF sc on day 11–14 and RTX (375 mg/m²) iv on day 15 in a course of CHOP chemotherapy.

Results: The overall response rate was 76% (16 of 21 patients). Twelve patients experienced a complete response or complete response unconfirmed (57%) and 4 had a partial response (19%). Two patients, 1 with no response and 1 with progressive disease died of lymphoma. One patient refused to continue the therapy during initial CHOP phase because of
gastrointestinal toxicity. The remaining 2 were re-diagnosed as not to meet the histological criteria. These 3 patients were classified as nonresponders. Four percent of FL patients had a relapse. Median time to progression had not been reached after a median observation time of 23 months. Fifteen patients (79%) continued in remission during this median follow-up period. The most frequent adverse events during CHOP-GR therapy were leukopenia (100%), neutropenia (100%), alopecia (94%), nausea/vomiting (79%), anemia (53%) and sensory disturbance (53%). In a subset of 11 patients, bcl-2 translocation in peripheral blood or marrow by polymerase chain reaction (PCR) was positive in 4 patients. Three of these patients had complete remissions and converted to PCR negativity after the therapy.

Conclusion: Our intentional fixed scheme of administration of G-CSF preceding RTX in combination with CHOP chemotherapy is safe and effective therapy for follicular lymphoma in short-term observation.

MAINTENANCE INTERFERON (IFN) DOES NOT INCREASE PROGRESSION FREE SURVIVAL (PFS) IN PATIENTS WITH INDOLENT NON HODGKIN Lymphoma (NHL) WHO RECEIVED IFN IN THE INDUCTION TREATMENT. RESULTS FROM A RANDOMIZED TRIAL ON 165 PATIENTS

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Introduction: IFN induces a significant improvement on PFS and survival in patients with low-grade NHL. However, the best IFN schedule is unknown as benefits have been observed when IFN is administered with the induction chemotherapy or, as maintenance treatment or, in both treatment phases.

Objective: To assess the role of 1 year maintenance IFN in patients treated with CVP plus IFN for 3 months.

Patients and Methods: Until October 2002, 165 patients were recruited. Patients received CVP (median 6 cycles) plus IFN, 3 MU/m² three times a week for 3 months. Complete and partial responders were randomized to receive IFN, 3 MU/m² three times a week for 1 year vs observation. With a median FU of 4.8 years (0.3–11.7 years) of surviving patients, response, PFS and overall survival (OS) were analysed.

Results: Median age was 63 years (28–79 years), 67% with follicular (FL), 18% small lymphocyte and 15% marginal zone lymphoma. Most patients (83%) had advance stage and 73% bone marrow involvement. Thirty-two percent of FL patients had FLIPI 2.3. Dose-intensity for Cytarabine and IFN in the induction phase was 77% and 72%. Ten patients withdrew the study due to grade 3–4 toxicity (6%).

Response: 87% of patients achieved response, 82% complete. One hundred and twelve out of 143 responders were randomized to receive 1 year of IFN (n = 56) vs observation (n = 56). Patient characteristics were similar in both groups. Median IFN dose was 66% (8%-113%). Sixteen patients (14%) were dropped, 7 due to IFN toxicity (6%). PFS: At 11 years, PFS of responders was 55% without differences between the 2 groups (46% for maintenance IFN vs 59% for the observation arm, P = 0.59). OS at 11 years is 81%, with 8% of deaths due to lymphoma.

Conclusion: No additional increase on PFS was observed with maintenance IFN in responders to CVP and short term IFN. Very good response rates and long lasting remissions were achieved with 3 months IFN treatment and tolerance was significantly better.

PHASE II STUDY OF CONSENSUS INTERFERON-ALPHA (YMD643) IN RECURRENT OR REFRACTORY LOW-GRADE NHL

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Introduction: To evaluate the tolerability and efficacy of YMD643, a consensus interferon with a high activity per protein content, in patients (pts) with recurrent or refractory LG NHL, we conducted a phase I/II study.

Method: YMD643 12 mIU was subcutaneously administered daily for 2 weeks (w) and thereafter 3 times/w for 22w in Cohort 1, and 12 or 18 mIU daily for 4w and thereafter 3 times/w for 20w in Cohort 2 and Cohort 3, respectively. Tolerability, pharmacokinetics and efficacy were evaluated.

Results: Twelve pts were enrolled in the phase I portion. Two of six pts in Cohort 2 and 3 of 3 pts in Cohort 3 developed dose-limiting toxicities (DLTs), including fever. In 3 pts in Cohort 1, no DLTs were identified. Based on these findings, Cohort 1 was recommended for the phase II portion, and further 11 pts were enrolled. Antitumor effect based on WHO efficacy criteria revealed partial response (PR) in 3/14 pts (overall response rate [ORR], 21%) in Cohort 1. For all 23 enrolled pts in all Cohorts, PR was observed in 8 pts (ORR, 17%). The serum concentration of YMD643 increased following the course of administration.

Conclusion: YMD643 showed objective responses in a fraction of patients with recurrent or refractory LGNHL; however, its limited efficacy would not deserve further investigations.

CHOP WITH RITUXIMAB-TREATMENT OPTION IN CD20(+)

NHL-OUR EXPERIENCE

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Introduction: In this study we have assessed the efficacy and toxicity of rituximab added to standard CHOP regimen in patients with CD20(+) NHL. Rituximab is a humanized monoclonal antibody that targets the CD20 antigen found on the surface of most (>90%) B-cell lymphomas, acts in mixed way: mainly by complement dependent cytotoxicity and antibody dependent cellular toxicity, induction of apoptosis, and as a chemosensitizer.

Methods: 33 patients (pts) with diagnosed NHL with immunophenotype CD20(+) have been treated with rituximab added to standard CHOP or COP regimens. Rituximab was administered in dose 375 mg/m² intravenously 24 hours before chemotherapy.

Patient characteristics: median age 54 years (range 20–73), 20 female/13 male, 18 patients had diagnosis of diffuse large B-cell lymphoma, 8 follicular, 1-BALT, 1 MALT type, 3 small lymphocytic lymphoma, 2 patients had no established phenotype. IPI score: 0 for 9pts, 1 for 12pts, 2 for 8pts and 3 for 4pts.

Results: Response rate (RR) was 82% (27/33) with 23/33 (70%) complete responses (CR) and 4 partial responses (PR). Six patients did not respond to treatment, all of them died. Median duration of follow up is 28 months (range 14–38). To date 19/23 in CR, 4 patients have relapsed and 2 of them died. In the group of patients who progressed/relapsed median time to progression (TTP) was 8 months. Median overall survival (OS) has not been achieved yet. 71% of patients has survived two years. Treatment was well tolerated, we did not observe grade 3 or 4 toxicity, the most commonly reported adverse event was alopecia caused by chemotherapy.

Conclusions: Rituximab in combination with standard CHOP regimen is safe and well tolerated treatment option. The higher RR rate(82%) compared to standard regimen (50–60%) indicates its efficacy and confirms the results of GELA study.

RITUXIMAB AND CHOP TREATMENT IN RELAPSED LOW-GRADE NON-HODGKINS Lymphoma

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Introduction: Different studies demonstrated that Mabthera as single agent or in combination with chemotherapy may improve clinical outcome in patients with low-grade NHL. Our purpose was to assess the results of Mabthera added to CHOCH versus CHOCH alone in patients with relapsed low-grade NHL.
RITUXIMAB MAINTENANCE THERAPY IN CD20+ B-CELL NON-HODGKIN-LYMPHOMA—FIRST INTERIM RESULTS OF A PROSPECTIVE RANDOMISED PHASE II STUDY

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Clinical and pharmacokinetic data suggest that the effect of rituximab could be improved by prolonged exposure to the drug. To test for this hypothesis we performed a prospective randomized trial of rituximab maintenance therapy in patients with CD20+ B-cell Non-Hodgkin’s Lymphoma. After completion of standard treatment patients were randomized to either observation or maintenance therapy with rituximab (375 mg/m²) every 3 months for 2 years. Patients with aggressive lymphoma were enrolled if they had achieved a complete response (CR) after initial treatment. Patients with aggressive lymphoma with residual tumor mass were enrolled if PET showed signs of tumor activity. Patients with indolent lymphoma qualified for the study if at least a partial response (PR) was achieved. So far 52 patients (pts) with CD20+ B-cell Non-Hodgkin-Lymphoma were enrolled in this trial. Histological subtypes included diffuse large cell lymphoma (24 pts), follicular lymphoma (11 pts), mantle cell lymphoma (8 pts), primary mediastinal lymphoma (5 pts), marginal zone lymphoma (1 pt), primary intestinal lymphoma (1 pt) and unclassified B-cell lymphoma (1 pt). No severe adverse events were observed during rituximab maintenance therapy. To date, all patients in the rituximab maintenance treatment arm are in continuous clinical remission. Importantly, in the observation arm 3 patients (2 diffuse large cell lymphoma, one mantle cell lymphoma) have relapsed. We conclude that rituximab maintenance therapy is feasible, safe and well tolerated in patients with CD20+ B-cell Non-Hodgkin-Lymphoma and might protect against tumor relapse. Patient recruitment for this study is ongoing.

FLUDARABINE COMBINED WITH ETOPOSIDE IN THE TREATMENT OF REFRAC'TORY LOW-GRADE LYMPHOMAS

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Background: The purine nucleoside analogues represent a novel group of cytotoxic agents with high activity in low grade lymphoid malignancies. Combined use of purine analogues with other cytotoxic agents may be more effective than monotherapy. Pre-clinical experiments indicate, that cytotoxic agents including etoposide (VP16) act additively or synergistically with FA. Aim: The aim of the study was to evaluate efficacy and toxicity of combination of FA and etoposide in relapsed and refractory low-grade non-Hodgkin lymphoma. Methods: Adult patients with relapsed or refractory low grade non-Hodgkin’s Lymphoma (NLG-L) or B-cell chronic lymphocytic leukemia (CLL) entered the study. The treatment consisted of FA given at a dose of 25 mg/m² daily for 5 consecutive days, and VP16 at a dose 100 mg i.v. for 5 days. Results: Twenty-five patients were treated according to the regimen. Median age of the patients was 55 years (range 47–76). Female/male ratio was 8/17. Six patients were diagnosed with LG-NHL, 19 with CLL. Ten patients were in relapse and fifteen patients were refractory to prior chemotherapy. All patients received minimum 2 or more cycles of chemotherapy previously (median 5, range 3–12). Thirteen of the patients received FA before. Overall response rate was 62% (24%) including complete response in 2 patient (8%) with CLL and partial response in 4 patients (16%); 3: CLL, 1: LG-NHL. Stabilisation of the disease occurred in 4 patients (40%) and 10 patients (40%) did not respond. The are 2 patients with mantle cell lymphoma but neither responded. Two patients (2-CLL, 1- LG-NHL) receiving 2-CDa previously, achieved a PR. Myelosuppression was the major toxicity of the regimen. Thrombocytopenia grade IV occurred in 8% (2%) and neutropenia at the same grade in 12 (4%) patients. Infectious occurred in 8 (32%) patients, including pneumonia in 5 patients, sepsis in 2, herpes zoster in 1. 10 patients died (28%) in disease progression 2 (8%) for related toxicity of treatment and 1 for unrelated condition with hematological disease or therapy.

RADIUM-225 PHOSPHATE (FAPM) IN RELAPSED INDOLENT B-CELL NON-HODGKIN Lymphoma

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Introduction: We conducted a multicenter phase II study to evaluate the efficacy and safety of oral FAMP for patients (pts) with relapsed indolent B-NHL with the overall response rate (ORR) as primary endpoint. Methods: Eligibility criteria were with the following: previously treated, indolent B-NHL [mantle cell lymphoma (MCL) pts were enrolled and analyzed separately from other indolent B-NHL pts]. measurable lesion: ages from 20 to 74; PS 0–2. Pts previously treated by purine analogs were excluded. Three to six courses of oral FAMP (40 mg/m²/day for 5 consecutive days) were given every 4 weeks. Secondary endpoints were complete remission rate (CR), median time to treatment failure (TTF), and toxicity. Results: Fifty-two pts (with 6 MCL) were enrolled. ORR of non-MCL group and MCL group was 65% with 30% CR and 17% without CR, respectively. The median TTF of non-MCL and MCL was 262 days and 184.5 days, respectively. Major toxicities by NCI-CTC were grade 4 neutropenia (37%) and leukenopaenia (21%), with no grade 4 thrombocytopenia. Transient grade 3 non-hematologic toxicities were upper respiratory tract inflammation in 3 pts, elevated gamma-GTP, diarrhea and infection in 2 pts each. Febrile neutropenia requiring admission occurred in only 1 pt. No deaths were observed during the study. Conclusion: Oral FAMP is a very active agent with acceptable toxicity for indolent B-NHL. It is very convenient in the outpatient setting.

RADIOIMMUNOTHERAPY WITH IODINE-131 ANTI-CD20 CHIMERIC MONOCLONAL ANTIBODY (RITUXIMAB) FOR RELAPSED OR REFRACTORY INDOLENT NON-HODGKIN’S LYMPHOMA: RESULTS OF AN AUSTRALIAN PHASE II TRIAL

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This Australian phase II study evaluated radioimmunotherapy of non-Hodgkin’s Lymphoma (NHL using radiolabelled 131Irituximab, a chimera anti-CD20 monoclonal antibody (Mabthera®). Eligibility incl. relapse/ refractory indolent NHL, no histological transformation, confirmed CD20 expression on biopsy within 12 months of rituximab exposure. Adequate haemopietic function (ANC ≥5.5×10⁹/L, plt≥100×10⁹/L) & no prior stem cell transplantation. 110 patients were enrolled from 2000–04: median of 3 prior therapies (range 1–8). Median age 63 yrs (35–84), 60% male, 68% stage IV. Histology: follicular (74%), small lymphocytic/marginal zone (16%) & mantle cell (8%). Rituiximab 375 mg/m² was followed by 152I-rituximab tracer for dosimetric imaging: 7–14 days later a therapeutic dose of 123I-rituximab prescribed to limit whole body radiation absorbed dose to 0.75 Gy, was administered after a second dose of Rituiximab. Haematological toxicity was mild. Median nadir values (time to nadir) were Hb 118/L.
A PHASE I/II TRIAL OF PIXANTRONE COMBINED WITH FLUORADARABINE (FL), DEXAMETHASONE (DEX), AND RITUXIMAB (FDP-R) FOR TREATMENT OF PATIENTS WITH RECURRENT INDOLENT B-CELL NON-HODGKIN'S LYMPHOMA (NH)

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Introduction: Fludarabine-mitoxantrone-Dex-Rituximab is an active regimen for indolent B-cell lymphoma. Pixantrone is a novelaza-anthracycline that has no delayed cardiotoxicity in animal models, and has single agent activity in NHL.

Methods: R: 33 mg/m2; dl, FL: 25 mg/m2; d1–3, Dex: 20 mg/d PO, d1–5, and pixantrone starting at 80 mg/m2 d1, increased to RD of 120 mg/m2 d1. Cycles were every 28 days. Twenty-five patients, median age 63 (range 32–78), 52% male, 16 follicular, 3 SLL, 2 lymphomas/myelomas, 3 marginal zone, 1 large B-cell (not evaluable). Median of prior treatments 1 (range 1–4). 3 patients had prior Ritux, and 1 prior auto stem cell transplant.

Results: Twenty-two patients evaluable for response, received a median of 5.5 cycles (range 2–8). ORR was 95%, CR/CRu in 77% and PR in 18%. At two years, overall survival is 85%, median FFS 25 months (range 3–29 months), 1 patient died of disease, 9% alive with disease, and 86% are alive NED. Five patients went for transplant. Toxicity was mainly hematological with grade 3 and 4 neutropenia in 76% patients and 20% thrombocytopenia. Two patients with prior anthracycline exposure and cardiac history developed cardiac toxicity grade 2.

Conclusion: FPD-R is a well tolerated regimen with a high major response rate of 95%. Toxicity is mainly hematological, and probably mainly associated with fludarabine.

ASSOCIATION OF LIPOSOMAL PEGYLATED DOXORUBICINE (CAELYX®), FLUDARABINE AND CYCLOPHOSPHAMIDE AS FIRST LINE TREATMENT IN DISSEMINATED SMALL B-CELL LYMPHOMA IN ADULT PATIENTS

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Background: In a search for the best regimen to treat disseminated small B-cell lymphoma we designed a new schedule based on fludarabine and cyclophosphamide combination with addition of pegylated doxorubicine thus expecting to add efficacy without increasing toxicity.

Methods: In a 2-step multicentric phase II trial (Simon design), patients newly diagnosed with small B-cell lymphoma were treated by cyclophosphamide 300 mg/m2 d1–5, fludarabine 25 mg/m2 d1–3 and pegylated doxorubicine 30 mg/m2 d1 IV every 28 days. Patients received 4 to 6 cycles depending on response after 2 cycles. The primary objective was efficacy (complete response or unconfirmed complete response (CR/CRu)) at the end of treatment.

Results: Among 41 patients (pts) registered, histologies were as follows: follicular – 17 pts, marginal zone -12 pts, lymphoblastic – 6 pts, mantle cell – 5 pts, unspecified small B-cell –1 pt. Median age was 56.1 years (34–70). 83% of the pts presented with stage IV disease. 12 and 5 pts classified in intermediate and high categories of the FLIPI. Among 39 evaluable patients, 22 achieved CR/CRu, 56.4% (39.6–72.2%) for an overall response rate of 94.9%. Among 188 cycles administered, main toxicities were: febrile neutropenia (11.7%), grade 3–4 leucopenia (59%), anemia (13.9%) and thrombocytopenia (10.7%). Leucopenia persisted after the end of treatment in 8 patients; most resolved within 8 months. Non-hematologic toxicities were alopecia (31.4%), vomiting (30.4%) and mucositis (11.2%). Two opportunistic infections were reported: cryptococcosis and pneumocystosis. No cardiac toxicity was observed. 12 patients stopped treatment early because of toxicity. One toxic death secondary to febrile neutropenia occurred.

Conclusions: CFC combination shows high CR rate. It compares favorably to other fludarabine containing regimen and deserves further investigation. However toxicity profile can lead to restrict it only to high risk patients.