9. Hodgkin's Disease

MICROVESSEL DENSITY AND THE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR (PDGF) IN CLASSICAL HODGKIN LYMPHOMA (HL)

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Introduction: The role of angiogenesis in Hodgkin lymphoma (HL) has not been investigated previously. The present study examines microvessel density and the expression of vascular endothelial growth factor (VEGF) and platelet-derived endothelial growth factor (PDGF) in lymph node biopsies of patients with HL at presentation and relapse.

Methods and results: Using immunohistochemistry, vascularisation was assessed in Hodgkin tissue from patients at presentation and relapse. The microvessel density (MVD) increased with disease progression in 6/10 cases. Expression of VEGF was observed in endothelial cells (EC) of some microvessels and also in follicular dendritic cells. The Hodgkin Reed-Sternberg (H-RS) cells as well as the inflammatory lymphocytes were negative for VEGF. Cytoplasmic or cytoplasmic and nuclear expression of PDGF by the H-RS cells was observed in 5/11 cases. The expression of PDGF increased with disease progression in 6/10 cases.

Conclusions: Hodgkin tissue shows prominent vascularisation. The increased MVD and PDGF expression with disease progression merits further investigation.

TUMOUR SUPPRESSOR GENES P53 AND RB--THE PROGNOSTIC ROLE IN HODGKIN LYMPHOMA

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Introduction: In the past several years investigations in the field of biology of Hodgkin lymphoma were very extensive. Biological markers like tumour suppressor genes may contribute to malignant transformation and may be of prognostic relevance.

The aim of the present study was the investigation of expression of tumour suppressor genes as well as, Retinoblastoma (Rb) and p53 in Hodgkin lymphoma and their clinical relevance.

Methods: In 18 months period, 40 de novo patients with confirmed diagnosis of Hodgkin lymphoma were studied. Immunohistochemical examinations using monoclonal antibodies were performed on paraffin-embedded lymph node specimens.

Results: The series included 24 males and 16 females. The median age of whole population was 31 years, ranging between 15 and 68 years. The expression of p53 correlated significantly with the histopathological localization of Hodgkin lymphoma, anemia and elevated LDH level on presentation. Expression of p53 and pRb did not correlate statistically significantly with the outcome, but immunohistochemical expression of Rb gene >20% correlate statistically significantly with overall survival vice versa expression of p53 gene.

Conclusions: Expression of tumour suppressor genes including, p53 and pRb have different level of expression in some cases of Hodgkin lymphoma. The analysis of larger number of cases and a more prolonged observation time are needed to fully assess the prognostic role of tumour suppressor genes in patients with Hodgkin lymphoma.

EFFECT OF EBV ON HUMAN TUMOUR CELLS IN CULTURE

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Introduction: We have examined expression of Epstein–Barr virus (EBV) in Hodgkin’s lymphoma (HL) and its impact on treatment and survival.
Methods: EBV genome was detected by PCR in the DNA of formalin-fixed, paraffin-embedded histological samples from 109 patients (45 females, 64 males).

Results: Virus association was confirmed in 61 cases (56%). During this retrospective study, no significant difference was found between EBV positive and negative patients as regards to gender distribution, mean age at the time of diagnosis of HL, clinical stages, general symptoms, histologic subtypes, organ manifestations, treatments employed or reaction to treatment. During the mean follow up time of 83 months (9–300 months), the overall survival rate was more favourable in negative than in positive cases though the difference was not significant (P=0.6644). As regards to event-free survival, the results were also better in EBV negative cases though the difference was again not significant (P=0.6741). On analysing the overall results of survival and event-free survival rates of EBV positive and negative groups, no significant differences were found between the two groups in relation to gender, age group (50 years old or younger or 50 years) or histologic subtypes (mixed cellularity or nodular sclerosis).

Conclusions: We have come to the conclusion that EBV association in HL does not have an effect on clinical symptoms, therapeutic results, overall and event-free survival rate that would lead to significant alterations. However, contradictory data that have been found in the literature so far necessitate further investigations involving a large number of international cases.

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THERAPEUTICAL RESPONSE ACCORDING TO EBV STATUS IN HODGKIN'S LYMPHOMA
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Introduction: Epstein–Barr virus (EBV) has been associated with many hematopoietic malignancies. The EBV-encoded latent membrane protein (LMP)-1 has been involved in neoplastic transformation in around 40% of classical Hodgkin's lymphoma (HL) cases and this association is believed to be causal. Classical HL that contain the EBV genome may differ etiologically from EBV-negative HL tumors.

Methods: We have examined 96 cases of HL newly diagnosed in Institute of Hematology Clinical Center Belgrade. We used immunohistochemistry to detect LMP-1 in the Reed–Stemberg cells. The study was conducted at the Department of Pathology from 1999 to 2003.

Results: LMP-1 was detected in 44.8% of our cases: high EBV associating is found at the peak in older adults predominantly with mixed cellularity type. The EBV association was independently affected by histologic subtype (79.16% in mixed cellularity and 35.3% in nodular sclerosis), sex (77.4% in males and 31.25% in females), and age (77.4% in patients aged 40 years and older and 35% in patients younger than 40 years of age). Most patients presented with stage II (31.32%) or III (53.12%). The patients were treated with chemotherapy alone (54 pts), and with chemotherapy + radiotherapy (38 pts). 90% EBV-associated patients had achievable therapeutic response, with 80% CR and 20% PR. In the group of EBV negative pts 65% had CR and 17% PR, but 6% had stable disease and 12% had progression of disease.

Conclusion: The results of the current study showed that the therapeutic response in HL pts correlated significantly with EBV status.

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CLINICAL IMPACT OF MORPHOMETRIC PARAMETERS AND IMMUNOPHENOTYPE IN CLASSICAL HODGKIN LYMPHOMA
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Background: The prognostic value of different morphological and immunophenotypic parameters in classical Hodgkin Lymphoma (cHL) has been widely debated in the literature, but most studies did not prove significant correlation of these factors with prognosis.

Material and methods: We have studied immunophenomological features in 200 patients (pts) with cHL treated at the hematologic department between 1995–2002. There were 45.5% males and 54.5% females, median age 29. The histology showed 57% NS, 30% MC, 8% LD and 3% LRCNL. Early stage pts (52%) were treated with 4–6 cycles CVPP or CVPPA+BD + IF-RT 36 Gy, advanced stage pts (48%)—6–8 cycles CVPP or CVPPA+BD + RT 30 Gy (only bulky orard residual disease). The 5-year disease free survival (DFS), freedom from treatment failure (FFTF) and overall survival (OS) were 65%, 61% and 95% respectively.

Results: The following immunomorphological parameters were studied: lack of CD15 expression and CD20 expression by neoplastic cells, expression of activation antigens (HLADR, CD23, CD38) by inflammatory cells, morphological atypia of Hodgkin Reed–Sterberg (HRS) cells, sheets of HRS, cohesive clusters of lacunar cells in nodular sclerosis, detection of L&H cells in cHL, mature T-lymphocyte depletion (excluding 16 pts with LD), tissue infiltration by neutrophils, eosinophils, plasma cells, histiocytes. The following parameters negatively influenced the prognosis (DFS or FFTF): presence of CD15+ atypical HRS (19% of cases; FFTF P=0.05), T-cell depletion (68% of cases, DFS P=0.01, FFTF P=0.05), tissue eosinophilia (33% of cases, DFS P=0.10). Cohesive clusters of lacunar cells showed negative trend on FFTF (P=0.07), plasma cells infiltration showed favorable trend on FFTF (P=0.06).

Conclusions: Our data confirm the prognostic significance of different immunomorphological parameters in cHL, which may be useful in determining groups of patients at high risk of treatment failure.

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VALUE OF CLASSICAL PROGNOSTIC FACTORS IN EARLY STAGES HODGKIN'S LYMPHOMA (HL): A MULTIVARIATE ANALYSIS BASED ON EORTC-GEILA SERIES
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Introduction: Several prognostic factors are available today that can be applied to define treatment strategies. The characteristics used differ from one index to the other and their prognostic value may vary between series. The present study aimed at selecting characteristics that predict for high risk of failure or death based on two trials conducted by the EORTC Lymphoma Group, the second in cooperation with the GEILA, from 1988 to 1998.

Methods: Characteristics tested were those included in the EORTC, GHSG and Canadian-E COG scoring systems for early stages and the International prognostic score (IPS) for advanced stages. Overall, 2411 patients were enrolled onto the EORTC H7 (n=833) and the EORTC-GEILA H8 (n=1578) trials. Patients were treated with radiotherapy alone or radio-chemotherapy of different intensity or duration according to the EORTC scoring system at December 2004, the median follow-up was 89 months (H7 trial: 102 months; H8 trial: 85 months).

Results: Overall, 326 patients experienced a treatment failure and 218 have died. The 5-year failure-free (FFS) and overall survival (OS) rates were 87% and 93%. With multivariate analysis, male gender (RR=1.5), WBC ≥16,000/μL (RR=2.0) and lymphocytes<600/μL (RR=1.9) significantly correlated with poor FFS; age ≥50 (RR=2.4), male gender (RR=2.0), WBC ≥16,000/μL (RR=1.8), lymphocytes<600/μL (RR=2.5) and B symptoms with elevated ESR (RR=2.3) correlated with poor OS.

Conclusion: The study confirms most of the prognostic factors that are included in the EORTC or the IPS scoring systems. Should the prognostic value of these factors validated in independent series, their use could ease international collaborative prospective trials.

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THE NATURAL HISTORY OF NODULAR LYMPHOCYTE PRE- DOMINANT HODGKIN'S LYMPHOMA (LP-HL)
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Introduction: The best management of patient with nodular LP-HL is debated. High remission rate is usually obtained, but recurrent relapses, in
most cases still responsive to further therapy, are not considered infre-
quency. A higher probability of Non Hodgkin Lymphoma (NHL) develop-
ment has been described. 

Methods: Out of 168 consecutive patients with HL, 14 (8%) had LP-HL, 
10M4/4F. Median age was 39 y (range 20–67); 13/14 were in stage I–II 
including 2 with B symptoms, 1/14 was in stage IV. Only 1 had a 
subdiaphragmatic presentation. Median follow-up was 48 months (range 
14–108). 4/14 were treated with radiotherapy only (RT), 1/14 with 
chemotherapy (CHT), 10/14 with RT plus CHT.

Results: Complete remission was observed in 13/14 cases, with 1 relapse 
after 27 months in a patient treated with RT only. The stage IV patient 
was refractory to three chemotherapy lines and died from toxicity. Interes-
tingly, 2 patients developed NHL diffuse large-cell B type and 1 patient 
developed Systemic Ankylosing Spondylitis (SA) with severe nephrotic syndrome. The histology at diagnosis of patients who developed NHL was reviewed and confirmed. Negativity of CT scan, PET and bone marrow biopsy 
excluded HL relapse in the patient developing SA. These 3 patients had 
been treated with mantle RT (2) or involved field RT (1) plus 4 ABVD 
courses.

Conclusions: We can confirm that LP-HL generally presents in early 
stage, without bulky disease, rarely involving the subdiaphragmatic nodes. 
It is characterized by an high rate of complete remission and an increased 
risk of NHL. However, with the limitation of a brief follow-up, we did not 
observed relapsing relapses.

IS THE INTERNATIONAL PROGNOSTIC SCORE (IPS) USEFUL 
PROGNOSTIC AND PREDICTIVE FACTOR IN HODGKIN 
LYMPHOMA? 
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Introduction: A study from the International Prognostic Factors Project 
for Advanced Hodgkin’s lymphoma developed International Prognostic 
Score (IPS) which defined seven adverse independent factors (male sex, 
hemoglobin <10.5 g/dl, leukocytosis, lymphocytopenia, hypoalbuminemia, 
age ≤ 60 years, stage IV). IPS value represents the number of pre-

dent negative prognostic factors. IPS value ranging from 0–2 denotes low 
risk, 3–4 intermediate risk, ≥5 high risk.

Methods: We performed a prospective review 40 de novo patients 
with confirmed diagnosis of Hodgkin’s lymphoma who were treated in 
our hospital during the last three years. Analysis was performed to 
determine prognostic value of IPS and the influence of prognostic 
facators identified by the IPS to overall survival (OS) and event free 
survival (EFS).

Results: According to IPS score, on presentation, 50% patients were in 
range 0–2 while 42.5% patients were in group of intermediate and 7.5% 
patients were in high risk group. Patients in low risk group had EFS 32.2 
months while patients with greater risk for bad response had EFS 21.8 
months. IPS value correlates significantly with survival rate before adverse 
event. Anemia and lymphopenia represent independent prognostic factors 
of survival up to adverse event as well as for overall survival.

Conclusions: We can conclude that IPS in our study has statistically sig-
nificant influence on differentiations of patients according to existing risk 
facators. In order to confirm this data larger group of patients and pro-
gressed time of observation are needed.

TREATMENT RESULT OF HODGKIN’S DISEASE IN CHINESE 
PATIENTS: A SINGLE INSTITUTION’S EXPERIENCE 
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Background: The annual incidence of Hodgkin’s disease (HD) in Chinese 
population is much lower than that of western population (around 0.5 
and 5 per 100 000 respectively). Treatment result for Chinese patients (pts) 
with HD was scarcely reported in the literature.

Purpose: To retrospectively analyze the clinical outcome of treatment in 
Chinese pts with HD in a single institution.

Materials and method: Sixty-six newly diagnosed HD pts received pri-
mary treatment in our institution between 1/1997 to 12/2002 and were 
included in the analysis. There were 37 males and 29 females with a 
median age of 36 years (range 16–89). Histological subtypes were: 
NLPHD (4 pts), LRCRH (7 pts), NS (34 pts), MC (16 pts), LD (1 pt) and 
unspecified (4 pts). Treatment plan was adapted to pt’s risk group (gp) as 
defined by clinical stage and presence of unfavorable factors. There were 
15 pts in early stage favorable gp (gp 1), 39 pts in early stage unfavorable 
groups (gp 2) and 12 pts in advanced stage gp (gp 3). Treatment plan 
for pts in gp 1 and gp 2 consisted of combined modality treatment with 4 
cycles (in gp 1) and 6 cycles (in gp 2) of chemotherapy (chemo), followed 
by involved field radiotherapy (RT) with dose ranging from 30 to 36 Gy. 
Treatment plan for gp 3 pts consisted of 6 to 8 cycles of chemo followed 
by RT to residual or initial bulky sites.

Results: Median FU time was 39 months (range 1 to 92 months). Three 
pts in gp 1 had stage IA NLPHD and were treated with mantle field RT 
alone (with dose of 40 to 44 Gy) in view of the favorable histology. All 3 
pts (100%) achieved CR and remained alive and disease free. For the 
remaining 12 pts in gp 1, all of them (100%) achieved CR and remained 
alive and disease free as well. Of the 39 pts in gp 2, 32 pts (82%) 
achieved CR while the remaining 7 pts (18%) failed to complete the 
planned treatment. Three (9%) out of the 32 pts who achieved CR had 
relapse of disease 6 to 34 months after treatment. Ten pts (25%) in gp 2 
died (2 pts died of disease progression, 4 pts died of treatment-related 
complications and 4 pts died of intercurrent disease). Fifteen pts (38%) in 
gp 2 were older than 60. The 3 yr PFS and OS for gp 2 pts was 88% and 
77%. Of the 12 pts in gp 3, 8 pts (67%) achieved CR after treatment. The 
3 yr PFS and OS for pts in gp 3 was 66% and 83%. The 3 yr PFS and OS 
for pts with age ≤ 60 yrs was 88% and 83%. On multivariate analysis, age≥60 
(P<0.001), ECOG PS ≥1 (P=0.001) and IPI score ≥2 (P=0.001) were 
significant adverse factors for OS while lymphopenia (P=0.026) was 
a significant adverse factor for PFS.

Conclusion: Risk adapted treatment strategies for Chinese pts with HD 
resulted in favorable outcome comparable to that reported in the western 
literature.

CHARACTERIZATION OF 312 ADULTS DIAGNOSED WITH 
HODGKIN’S DISEASE (HD) FROM TWO ONCOLOGICAL 
CENTERS IN ARGENTINA 
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Introduction: Incidence, clinical features, histopathology and behavior of 
HD patients present distinctive characteristics in populations arising from 
different geographical and socio-economic status. Nutritional, infec-
tious, racial and other not well known factors have been mentioned.

Methods: Between 1994 to 2004, 1668 adult patients were diagnosed 
with non-Hodgkin lymphomas in two oncological centers: Hospital 
Marie Curie and Henry Moore Institute. Diagnosis of HD was made in 
312 (18.7%).

Results: The average age was 36.7 years (range 15–83), male: 54.8%, 
female 45.2%. The distribution by decade was: ≤20 years: 11.2%, 21–30: 
80: 2.2%, 81–90: 0.9%. Clinical stages: I: 4.17%, II: 44.87%, III: 23.71%, IV: 
7.7%. Not determined: 19.45%. Seven patients (2.24%) were HIV positive. The histologi-
cal subtypes were: nodular sclerosis (NS): 40.7%, mixed cellularity (MC) 
36.33%, lymphocyte predominant Lp: 5.13%, lymphocyte depleted (LD): 
2.9%, without subtype: 14.74%. The location was: cervical: 53.2%, medi-
testinal: 47.11%, supravacular: 17.31%, retroperitoneal: 16.7%, axillary: 
14.4%, spleen: 6.4%, inguinal: 5.76%, bone marrow: 3.52%. Frequent 
associations were: cervical and mediastinal: 32.7%, supravacular and mediastinal: 
9.9%. Bulky disease was present in 7.4%. In the mediastinal location the histological subtypes were: NS: 56.4%, MC:34%, LP: 4.1% 
and LD 1.3%, 4.1% without subtype. The age was less than 25 years in 
38% of the patients, with predominance of NS in this group (82%).

Conclusions: The largest incidence was present in the decade of 20–30 
years (33.6%); we didn’t notice the double curve described in the age’s 
distribution. It remarkable the scarce number of histological subtypes like 
PL and DL and abdominal and inguinal locations.Prelavence of NS 
was associated with mediastinal locations and younger patients (less than 25 
years).
A SINGLE INSTITUTION EXPERIENCE ON HODGKIN'S LYMPHOMA (HL) TREATMENT: AN OUTCOME STUDY
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Introduction: Few data are available about the long-term outcome in unselected patients with HL treated during the last decade, when treatment intensity was reduced in order to avoid secondary complications. We present an outcome analysis of all cases treated in our center since 1 January 1994.

Methods: 168 consecutive patients (71M/87F), median age 31 yrs (range 13-79) were diagnosed and treated. 128 patients (76%) were in stage I-II, including 13 with bulky disease; 40 patients (24%) were in stage III-IV, including 4 with bulky disease. 146/168 were treated with combined therapy (CT) based on MOPP/ABV or ABVD usually 4 courses in early stages and reduced intensity and limited field radiotherapy. Median follow-up from diagnosis, was 54 months (range 5-115).

Results: 154/168 (91%) obtained complete remission (CR), 1 died of bleomycin induced lung fibrosis. A total of 21 patients (13%) required a second-line treatment for relapse, partial or no response. At December 31, 2003, 152/168 were alive with a 5-year overall survival of 91%; 145 were in CR, 7 on therapy, 3 for persistent HL, 3 for Non-Hodgkin Lymphoma and 1 for Systemic Amyloidosis; 16 patients have died, 11 from progressive HL or acute therapy, 2 from acute myeloid leukemia, 3 from unrelated causes, 4 cancers (lung 2, kidney 1, tongue 1) but no severe vascular events were recorded.

Conclusions: A very high cure rate is confirmed in this unselected HL patient population treated in a single tertiary center. Most patients are enjoying a good quality of life. Modern CT seems less toxic than in the past decade but no less effective.

CHANGING PATTERNS OF CARE IN EARLY STAGE HODGKIN'S DISEASE—SURVIVAL DEPENDS UPON INITIAL TREATMENT
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Introduction: The Scotland and Newcastle Lymphoma Group (SNLGL) has been collecting population data for more than 20 years on all new lymphomas registered within the catchment area (population 8.5 million). We used this data to look at the changes in patterns of care in early Hodgkin's during the 1990s, and to examine whether initial treatment choice influenced outcome.

Method: We extracted data for all new cases of clinical stage Ia or IIA Hodgkin's disease registered with the SNLGL population database between 1993 and 1997 (minimum follow-up of 5 years). For each case we obtained demographics, presentation, treatment and outcome. Analysis of results was performed by primary treatment: radiotherapy alone (RT), chemotherapy alone (CT) and combined modality with both chemo and radiation (CTM).

Results: 379 patients were registered in the five year period with median follow-up of 8 years. There were 174(46%) with IA disease and 205(54%) IIA; age range 5-94 years with median 34 years. Survival overall was 77% (291) with 16% (63) deaths and 7% lost to follow-up. Of the deaths 7% were recorded as lymphoma, 1% as treatment-related and 8% as non-lymphoma deaths. 177(47%) patients were treated with RT of whom 76% were stage IA, 13% had bulky disease and 25% had lymphocyte predominance disease. Relapse rate was 34% and 66% of those relapsing were salvaged by further therapy. CT alone was used in 77(23%) patients, of whom 11% had stage IA and 31% had bulky disease. 23% relapsed and 72% of those were salvaged. 112(30%) patients received CMT, of whom 55% had bulky disease and 25% had stage IA. Relapse rate was 18%, with 55% salvaged by further treatment. Overall survival was 90% for RT, 81% for CT and 86% for CMT. Formal statistical analysis of survival is pending.

Conclusion: This population data demonstrates high relapse rates for both RT and CT alone, with poor salvage in all three arms following relapse. Although this prognostic index the CMT group was worst, it had the lowest relapse rate and best survival.

MICROGLOBULIN (MCG) B-2 CONCENTRATION IN BLOOD OF PATIENTS (PTS) WITH HODGKIN’S DISEASE (HD) AFTER SEGMENTAL CHEMORADIATION THERAPY (CRT)
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Introduction: Measuring of mcg B-2 concentration in different stages of treatment allows to estimate the efficacy of cancer therapy.

Methods: The mcg B-2 concentration in the blood has been studied in 26 Stage III-IV HD pts before therapy, after 3 courses of polychemotherapy (PCT), after local-regional radiation therapy (24Gy-30 Gy), and additional 3 courses of PCT.

Results:

<table>
<thead>
<tr>
<th>Stages of therapy</th>
<th>Pts</th>
<th>mcg B-2 level</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before therapy</td>
<td>26</td>
<td>3908.2 ± 4166</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After 3 courses of PCT</td>
<td>22</td>
<td>2716.4 ± 543.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>After radiotherapy</td>
<td>16</td>
<td>2941.1 ± 525.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>After more courses of PCT</td>
<td>16</td>
<td>2641.9 ± 435.7</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Conclusion: The research has shown that the sequential CRT has decreased the mcg B-2 level that says about the efficacy of the therapy. However, during the 1st year of the disease-free survival we did not observe the complete normalization of the immune status in those pts.

CLINICAL RESULTS IN ADVANCED HODGKIN DISEASE USING DOSE-INTENSIFIED BEACOPP REGIMEN
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Introduction: BEACOPP regimen was first described by the German Hodgkin Lymphoma Group for advanced adults Hodgkin disease (HD). The objective of this presentation is to analyze outcomes of this regimen in a group of patients with advanced disease.

Patients and Methods: Fifteen patients with histologically proven HD were evaluated. Patients characteristics: Age between 18-60yrs (median age 27y); 12 patients with newly diagnosed HD in advanced Ann Arbor stages 3 and patients with relapsed disease to previous therapeutic approaches (Ann Arbor stage were as follows:IIIA with mediastinal mass(4),IIIB(1),III(3),IV(2) and relapsed disease(3)). Patients were assigned to 8 courses of increased dose BEACOPP plus G-CSF from day 8 of each course until leukocyte recovery. Treatment was administered in outpatient basis. Results: were analyzed using SPSS for windows.

Results: In the group of newly diagnosed HD, 8/12 patients reached complete remission (CR-65%) and 4/12 partial response (PR-32.5%). Ann Arbor stages in this 4 patients with PR included IIA with mediastinal mass in 3 of them. In the group with relapsed disease who received BEACOPP regimen, 1 patient achieved CR, with PR and 1 patient had no response. Neutropenia was common to all patients and only 1/14 patient presented with grade IV neutropenia. Infectious events were as follows: respiratory tract(2),local candidiasis(2) and febrile neutropenia admitted to hospital(1). Other adverse events: hyperglycemia as result of steroids. Mediastinal mass was the only unfavourable event in statistical analysis.

Conclusion: BEACOPP regimen is an appropriate approach in newly diagnosed HD patients with 65% CR. This regimen also offers 66% CR as salvage approach in relapsed to other chemotherapy strategies, considering outpatient basis and low toxicities. Mediastinal mass is associated with poor response and seems to be an independent factor to Ann Arbor stage in statistical analysis. In this group of patients with mediastinal mass, local adjuvant interventions should be considered.
A NEW BACOPP SCHEDULE IS FEASIBLE AND EFFECTIVE IN ELDERLY HODGKIN’S LYMPHOMA PATIENTS - INTERIM RESULTS OF A MULTICENTER PHASE-II TRIAL OF THE GERMAN HODGKIN STUDY GROUP (GHSG)


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Introduction: The outcome of Hodgkin’s lymphoma (HL) patients (pts) increased over the last decades. However, treatment results in HL pts >60 years are poor, particularly in patients with advanced stage disease. Large prospective studies are still lacking in this group. Based on the results for adult HL pts, we adapted the BEACOPP schedule for elderly HL pts with an increased dose of doxorubicin while omitting etoposide.

Methods: From December 2003 to February 2005, 41 HL pts with a median age of 66 (range 61–75 years) and newly diagnosed HL in unfavourable clinical stage (CS) I, II and CS III, IV were prospectively recruited to receive 6 to 8 cycles of bleomycin (10 mg/m²), doxorubicin (50 mg/m²), cyclophosphamide (650 mg/m²), vincristine (1.4 mg/m²), procarbazine (100 mg/m²), and prednisone (40 mg/m²) (BACOPP) followed by local radiotherapy to residual lymphoma. Epopetin beta (30,000 IE once weekly) was administered to avoid therapy induced anemia.

Results: Up to day 23, 11 pts (27%) were evaluable for feasibility and response of treatment. 10 pts (43%) reached complete and 13 pts (57%) partial remission, one patient died due to pneumonia after 4 cycles of chemotherapy. Another patient died several weeks after treatment due to underlying unclassified Cancers. The treatment was generally well tolerated with WHO grade 4 toxicities: 7 pts (30%) leukopenia, 4 pts (17%) thrombocytopenia, one patient (4%) with infection and one patient (4%) with pulmonary complication.

Conclusion: The first interim analysis of this new BACOPP schedule showed that this regimen is feasible in elderly HL pts. Full analysis of updated results will be presented.

MOPP/BEVCAD CHEMOTHERAPY WITH LIMITED RADIOTHERAPY IN ADVANCED HODGKIN’S LYMPHOMA: 10-YEAR RESULTS


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Background: In 1987 the MOPP/BEVCAD chemotherapy regimen was one of the first attempts at reducing late toxicity and second tumor incidence while carrying out some basic concepts of the Goldie and Coldman theory on cell growth to increase effectiveness. The new treatment program intensified and hybridized all the drugs of three previously alternating regimens (CAD, MOPP and ABV), lowered the cumulative dose of mechlorethamine and made irradiation optional, reducing it to no more than 2 sites which responded uncertainly or partially to chemotherapy.

Methods: The patients treated so far with MOPP/BEVCAD were included in one open and controlled GISL study and in two randomized trials (GISL and IBL) in which it represented one of the compared treatment arms. Staging and treatment criteria were identical in the 3 trials. Drug dosages (mg/m²) were as follows: HN2 6 i.v. d 1 (cycles 1, 3 and 5), CCNU 100 p.o. d 1 (cycles 2, 4 and 6), VDZ 3 i.v. d 1, MPH 6 p.o. d 1–3, Pred p.o. 40 d 1–14, EPI 40 i.v. d 8, VCR i.v. d 8, PECZ 100 p.o. d 8–14, VBL 6 i.v. d 15 and Bleo i.v. d 10 and 15 (9 day 28 day for 6 cycles). Radiotherapy doses ranged from 25 to 40 Gy.

Results: A total of 307 treated patients were reviewed. With a median follow-up of 110 months, 10-year overall-, disease- and failure-free survival were 78%, 81%, 79%, respectively. Two hundred and ninety patients
MAINTAINING ABVD DOSE INTENSITY (DI) WITH AN ABBREVIATED COURSE OF GC SF IN PATIENTS (PTS) WITH HODGKIN’S LYMPHOMA (HL)

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Introduction: ABVD chemotherapy (Adriamycin 25 mg/m², Bleomycin 1500IU, Vinblastine 6 mg/m², Dacarbazine 375 mg/m²) is given day 1 and 15 each 4 weeks. DI is thought important in treating HL and rather than delaying or decreasing dose in response to neutropenia (<1.0 x 10⁹/L) on treatment (RTx) days, the Wellington Cancer Centre (WCC) has adopted the practice of prescribing GC SF 300 mg daily for 5 days starting the day after chemotherapy for neutropenic pts. We undertook a review to determine the effectiveness of this approach.

Methods: Retrospective analysis was performed of HL pts treated with ABVD during 1999–2002 at the WCC. Each ABVD cycle was reviewed with respect to neutrophil counts, dose delays or modifications, GC SF prescription, and Rx complications.

Results: 41 pts were identified and 40 reviewed (1 set of notes not found). Median age was 33y (range 16–74). Four pts had stage I, 20 stage II, 7 stage III, and 9 stage IV disease. A total of 176 cycles ABVD were administered (median 4, range 2–6). Seventy-four episodes of neutropenia on Rx days were identified affecting 28 patients. Forty-seven episodes (63.5%) were prescribed GC SF; of these, 37 (78.7%) had neutrophil <1.0 x 10⁹/L at next dose and did not require GC SF, 6 (12.8%) were neutropenic, and 4 were not recorded as they had completed Rx. Six episodes of neutropenia (8%) were treated by dose delay, 4 (5.4%) by GC SF and delay, 1 by dose decrease, 1 stopped due to toxicities, and 15 (20.1%) continued Rx without modification or GC SF. Of these 15, 5 (33.3%) were neutropenic at next dose. Only 4 episodes of febrile neutropenia were noted, none in those receiving GC SF for that part-cycle. There was a total of 36 weeks of Rx delays, 10 due to neutropenia and the remainder due to toxicity, co-morbidities, or personal reasons. The relative DI administered for Adriamycin, Bleomycin, Vinblastine and Dacarbazine were 96%, 90%, 95%, and 96% respectively. If 1 week dose delays rather than GC SF had been used for neutropenia the DI would have been 91%, 85%, 90%, and 91%.

Conclusions: Five days 300 mg GC SF administered in response to Rx day neutropenia helps maintain ABVD DI in pts with HL.

SAFETY AND EFFICACY OF SINGLE FIXED DOSE PEGFILGRASTIM (NEULASTA) IN CHEMOTHERAPY REGIMENS GIVEN EVERY 14 DAYS: RESULTS OF ABVD PLUS PEGFILGRASTIM IN PATIENTS WITH HODGKIN LYMPHOMA

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Background: Although the safety and efficacy of pegfilgrastim has been established in association with chemotherapeutic regimens that are repeated every 3 weeks, its safety in patients receiving chemotherapy regimens every 2 weeks is currently under investigation. Of concern is the possibility of stem cell damage because of the long half life that may overlap with subsequent courses of chemotherapy.

Methods: ABVD chemotherapy is widely used for the treatment of patients with Hodgkin lymphoma. Up to 65% of patients receiving ABVD will require growth factor support to 1) maintain dose intensity 2) to prevent neutropenic fever and 3) to prevent delays in chemotherapy administration. All patients received a single dose of 6 mg pegfilgrastim 24 hours after ABVD therapy. CBC was performed on a weekly basis, until completion of therapy, and then was monitored every 3–4 months thereafter.

Results: 25 patients are enrolled, all of whom are evaluable for efficacy and toxicity. 22 pts completed at least 3 full cycles (6 doses of ABVD), and 13 Pts completed 6 cycles. A total of 225 doses of ABVD are administered. Day 14 ANC count below 1,000/μL occurred in only 5 doses of ABVD (2%). Non-neutropenic infection developed in 7 doses (3%) requiring delay in the subsequent dose of ABVD beyond day 14. Long-term safety is available on 11 pts who had a follow-up ANC counts beyond one year after completion of ABVD treatment. The median ANC after at least one year of follow up was 7,290/μL (range 1,350 to 7,060/μL). Five pts had a follow up beyond 18 months, and their median ANC was 2,300/μL (range 1,570 to 3,360/μL). Furthermore, PK data from 5 selected patients were performed on day 14 following the first cycle of ABVD. In all 5 patients, serum pegfilgrastim levels were below clinically significant levels.

Conclusion: Single dose per cycle of pegfilgrastim is effective in maintaining ABVD dose intensity, keeping therapy on schedule, and preventing neutropenic fever. Our preliminary long-term follow up data demonstrate the safety of pegfilgrastim administration in conjunction with standard dose chemotherapy given every 2 weeks.
ONCE WEEKLY EPOETIN BETA EFFECTIVELY MAINTAINS HB LEVELS IN ELDERLY HODGKIN’ LYMPHOMA PATIENTS TREATED WITH A NEW BACOPP SCHEDULE – INTERIM RESULTS OF A MULTICENTER PHASE-II TRIAL OF THE GERMAN HODGKIN STUDY GROUP (GHSG)

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Introduction: Therapy related anaemia severely affects quality of life of oncology patients. Moreover, low HB levels before and during cancer therapy correlates often with a lower response to treatment. In this analysis we present the first results on the efficacy of Epoetin beta in elderly Hodgkin’s lymphoma (HL) patients treated with the new BACOPP schedule, a BEACOPP schedule with an increased dose of doxorubicin while omitting etoposide.

Methods: From December 2003 to February 2005, 41 HL pts with a median age of 66 (range 61 to 75 years) and newly diagnosed HL in unfavourable clinical stage (CS) I, II and CS III, IV were prospectively recruited to receive 6 to 8 cycles of bleomycin (10 mg/m²), dactinomycin (50 mg/m²), cyclophosphamide (650 mg/m²), vincristine (1.4 mg/m²), procarbazine (100 mg/m²), and prednisone (40 mg/m²) (BACOPP) followed by local radiotherapy to residual lymphoma. 30,000 IE Epoetin beta (Neoreccorm®) once weekly was administered by Hb < 14 g/dl to maintain Hb levels between 12 and 14 g/dl throughout the therapy.

Results: Up to day, 23 pts (11 males and 12 females) were evaluable for treatment response. All pts presented with Hb <14 g/dl and hence received Epoetin beta (Neoreccorm®) therapy. Average Hb levels were 11.2 g/dl (staging), 12.2 g/dl (1st restaging) and 11.7 g/dl (2nd restaging). Conclusion: This first interim analysis on the efficacy of 30,000 IE Epoetin beta in elderly patients with HL treated with the new BACOPP schedule showed that once weekly Epoetin beta effectively corrects and maintains HB levels throughout the therapy. Average Hb levels were at all points maintained above baseline levels and the pts remained transfusion free.

PECIFILGRASM TUMOR EFFECTIVELY INCREASES THE REFRACTORY DOSE INTENSITY (RDI) OF MODIFIED CHL/VPA/VBPV REGIMEN IN PATIENTS WITH ADVANCED HODGKIN DISEASE (HD)

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Introduction: Pegfilgrastim is a new granulocyte growth factor which allows to intensify chemotherapy regimens restoring absolute neutrophils count with a single injection. We recently published our experience with CHlVPA/VBPV regimen in advanced HD, severe neutropenia was the most relevant toxicity and caused a delay or dose reduction in 57% of pts. In order to evaluate feasibility and a possible correlation between increased RDI and response rate and survival, we introduced the Pegfilgrastim in the schedule.

Methods: From January 2004, 33 pts with advanced (HD) were treated as follow: D1 vinblastine 6mg/sm; D1 – 17: PCZ 80mg/sm, CHl 6mg/sm, PDN 50mg/d; D8: ADM 30mg/sm, Bleo 7.5 IU/sm, VCR 1mg; D8 – 10: VP16 100mg/sm; D11: pegfilgrastim 6 mg sc., every three weeks for six cycles.

Results: Main haematological toxicity was grade 3–4 anemia that occurred in 42% of pts, requiring transfusional support. Severe neutropenia was recorded in 53% of pts. Grade 2 infection (HZV, UT, respiratory tract) occurred in 5 pts. Main non haematological toxicity was neurological (such as constipation or paresthesia) and occurred in 11 pts. Delay of one week for at least one cycle was necessary in 5 pts because of fever (1 pt), pyotymia (1 pt), acute HZV infection (1 pt), G3 neutropenia (1 pt), G3 neurotoxicity (1 pt). With a median follow up of 8 months, the 9 pts that completed the therapy are in CR. We evaluated the RDI of the schedule in 17 of out 33 pts that received at least 4 cycles; all drugs were delivered at 85–100% of provided dose. Comparing this data to the previously found, we increased the relative dose intensity particularly for the myelosuppressive drugs (ADM: 96% vs 90%; VP16: 95% vs 69%).

Conclusion: The introduction of Pegfilgrastim effectively allowed a better RDI of the schedule. The relationship between RDI and response rate will be discussed.
while the progression free survival is 38% (95% CI 21–65%). Multivari-
ate analysis of the different prognostic factors affecting the survival;
proved that the initial remission status and stage at relapse affect signifi-
cantly the survival with P-values, 0.040 and 0.035 respectively.
Median age was 45 yrs (16–62), median follow up was 50 months (2–120
months). Complete remission was documented in 32 pts (78%). 8 pts (23%)
relapsed in a median time of 9 months (2–52). Main characteristics of non
responders pts were: B symptoms in 9 pts, elevated LDH in 6 pts and
stage 4 in 5 pts. The 5 years overall and event free survival were 52%
and 44%. 3 (7.3%) toxic death (1 MOF and 2 septic shock) and 8 (19.5%)
Hodgkin related deaths were observed. No myelodysplastic syndrome
was observed. The five years overall survival and event free survival of
standard risk subset of pts was superior, but not statistically better to high
risk subgroup. In our analysis, high risk pts, relapsed pts and pts who
expressed an induction failure, b symptoms, stage 4 and elevated LDH
were predictive of poor survival. These results suggest that pts with high
risk refractory Hodgkin lymphoma should receive second line che-
motherapy with the intent of administering HDAC/ASCT to those with
chemosensitive disease as soon as possible. New therapeutic approaches
(doubles ASCT or reduced intensity allotransplantation) are needed to
improve the outcome of pts with poor risk Hodgkin disease.

A PHASE II STUDY OF THALIDOMIDE AND VINBLASTINE
FOR PALLIATIVE PATIENTS WITH HODGKIN’S LYMPHOMA
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Introduction: Patients (pts) with Hodgkin’s Lymphoma (HL) that has
progressed after primary therapy and subsequent autologous stem cell
transplantation (ASCT) cannot be cured with conventional treatments. We
have combined thalidomide (THAL), an agent with anti-angiogenic and
immunomodulatory properties, with vinblastine (VBL) to study this strat-
egy in pts with refractory HL to determine the objective response rate,
remission in B symptoms and toxicity.
Methods: The treatment regimen comprised of THAL 200mg orally given
daily. After two weeks, intravenous VBL was given weekly at a dose of
6mg for 6 weeks on an eight-week cycle. CT scans of the chest, abdomen
and pelvis were obtained at baseline and were repeated every 6 months
or earlier at the investigators discretion. Responses were categorized by
International Workshop Criteria and toxicity was assessed by NCI com-
on toxicity criteria.
Results: 11 pts have been enrolled, 1 progressed within 6 days of study
enrollment and was subsequently treated with alternative palliative therapy
and thus 10 pts are reported. 7 pts had relapsed after ASCT, 1 pt had a
primary refractory lymphoma to three separate lines of chemotherapy and
2 pts declined ASCT. The median number of prior chemotherapy regi-
mens was 3 (range 1–5). Of the 7 ASCT pts, 4 had primary refractory HL
and 3 had relapsed HL. The median time to progression post-ASCT was
9 months (range 2–29). The ORR to THAL+VBL was 40% with 4 PR and
2 SD pts. The median duration of response was 9.5 months (range 2–22
months). B symptoms were present at enrollment in 3 pts, stage 4 and elevated
LDH were reported in 1 pt. Neutropenia: Grade 0–1; 6 pts, grade 2;
2 pts, grade 3; 2 pts. Non-hematologic toxicities observed: neurotoxicity:
grade 0–1: 4, grade 2: 6; GI toxicity: grade 2: 1 pt, grade 0–1: 9.
Conclusions: In this small study in heavily pre-treated or chemotherap-
refractory HL, the combination of THAL and VBL demonstrated a
response rate of 40% with some durable responses. Grade II neurotoxi-
city and neutropenia were frequent. The response rate appears promising
though not necessarily superior to single agent VBL. These results suggest
that combination of chronic low dose chemotherapy combined with
less toxic immunomodulatory or anti-angiogenic drugs warrants further
study.

IMPAIRED FERTILITY IN MEN WITH NEWLY DIAGNOSED
HODGKIN’S LYMPHOMA
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Introduction: Hodgkin’s lymphoma (HL) is a tumour disease mostly
affecting the younger sections of the population. In recent years, even
more emphasis was laid on the monitoring of late adverse effect of

LONG TERM FOLLOW-UP AFTER AUTOLOGOUS STEM CELL
TRANSPLANTATION FOR CHEMOSUMITANT HODGKIN’S
LYMPHOMA
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High dose chemotherapy and autologous stem cell transplantation is still the
optimal strategy in relapsed malignant lymphoma patients (pts). However the potential role in chemosensitive disease (partial remission
<50%PR), primary refractory (early relapse) is still controversial and
optimal timing and indication is still to be assessed. The outcome of 41
pts with chemosensitive Hodgkin lymphoma who underwent second line chemotherapy with DHAP-IEV and BEAM plus ASCT were analysed. Pts
were classified as standard risk (SR) or high risk (HR) based on the following
prognostic score >2 was documented in 20 pts (48%) bulky disease in 18
patients (43%), extranodal disease in 12 (29%), B symptoms in 28 (68%) at
stage 3–4 in 26 (63,4%). First line treatment was ABVD in 10 (24,9%),
ABVD like in 20 (48,8%), MOPP in 5 (12,2%) and EVE in 6 (14,6%)

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the therapy applied. As far as the impaired fertility is concerned, the
blame is often put on the alkylating cytostatic drugs. However, as demon-
strated several times before, serious damage to fertility is found in men
with HL even before the start of the cytostatic treatment.

**Objectives:** In newly diagnosed patients with HL, andrological and
hormonal examinations were made in order to demonstrate the damage to
fertility as early as at the time of diagnosis.

**Methods:** We examined a total of 48 patients, in which andrological tests
were carried out with sperm collection (spermogram evaluations accord-
ing to the WHO classification), and hormonal examination of the FSH,
LH and testosterone levels (using the RIA method). The median of the
patients’ age was 26 years (16–36). The early stage of the HL was found
in 11 patients (23%), the intermediate stage in 14 patients (29%) and the
advanced stage was found in 23 patients (48%). About a half of the
patients (n=23) reported some of the B symptoms at the time of diagnos-
sis. The spermogram parameters were compared with a reference set of
89 healthy men interested in sperm donation, with age median of 23 years
(18–35). The *t*-test was used for statistical evaluations.

**Results:** In 96% (n=46) of the patients, insufficient sperm quality was
established at the time of the diagnosis. Severe defects of fertility such as
azoospermia and oligoasthenoteratospermia were found in 23% (n=11)
and 21% (n=10) patients respectively. In other patients, combined impair-
ments such as asthenospermia in 15% (n=7) and asthenaoteratospermia in
37% (n=18) of the patients were identified. Normal findings in the sper-
miogram were only established in two patients. In comparison with the
control set of healthy men, prospective sperm donors, statistically signifi-
cant differences were found (*P* = 0.05) in the average concentration of
sperm cells (29.0 vs 55.7 million/ml) and in the presence of progressively
motile sperm cells (12.9% vs 43.6%). No deviation from the normal refer-
ence range of serum levels of gonadotropic hormones and testosterone
could be established in anyone of the patients.

**Conclusion:** Serious impairment of fertility is met in most patients with
HL as early as at the time of diagnosis. Even though the exact mechanism
of these changes is not exactly known, some effect of the biological fac-
tors due to the basic illness is presumed (cytokines). We have not estab-
lished any correlation between hormone levels and the state of fertility.
10. CLL/Multiple Myeloma

RICHTER SYNDROME: AN HETEROGENEOUS DISEASE?
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Introduction: Richter's syndrome (RS) denotes the development of high grade non Hodgkin lymphoma (NHL) and Hodgkin disease in patients (pts) with low grade NHL or chronic lymphocytic leukemia (CLL). Three historical types were described: classical diffuse large B cell variant (DLBV), large cell variant with "Stemberg-like" cells (DLVCV) and Hodgkin disease variant (HDV).

Methods: Clinical, histological, and immunohistochemical data of 33 pts were reviewed. EBV status (LMP expression, EBER in situ hybridisation or EBV PCR), and a comparison between initial hematological diseases and RS B cell clones were performed in 29 and 11 pts respectively.

Results: 20 pts developed a DLBV. Initial disease was CLL (n = 15), prolymphocytic leukemia (PLL = 1), Waldenstrom disease (WM = 2), marginal zone lymphoma (MZL = 1) and lymphoplasmacytic lymphoma (n = 1). Median time from initial hematological disease to RS diagnosis was 54 months. EBV was associated to the tumor in 1/16 tested cases. Median survival time was 3 months. Six pts developed DLVCV (CLL = 4, PLL = 1, and MZL = 1). Median time from initial hematological disease to RS was 54 months. EBV was associated to the tumor in 3/6 tested cases. Median survival time was 6 months. Seven pts developed HDV (CLL = 6, MZL = 1). Median time to RS was 144 months. EBV was associated to the tumor in 6/7 tested cases. 4/7 pts are still alive and in complete remission. Immunoglobulin gene rearrangement of the initial disease and RS were identical in the 11 tested samples and molecular studies of other samples are ongoing.

Conclusions: In our cohort, HDV is not so rare and its prognosis seems better. The high rate of association of tumor with EBV infection in the DLBV-SC and HDV subtypes (9/13:70%) suggests an oncogenic pathway related to EBV, conversely to the DLBV group (1/16) (P = 0.02). A statistical study is ongoing comparing the three groups to find a relationship between the clinical, biological therapeutic characteristics and the histological findings.

LYMPHOCYTIC GLUTATHIONE LEVEL IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Introduction: Glutathione (GSH) has a number of biological functions. It is involved in protection against oxidative damage, detoxification and transport processes, γ-glutamyl cycle, and important determinant for sensitivitiy of tumours to chemotherapy and radiotherapy. Lymphocytes from Chronic Lymphocytic Leukemia (CLL) patients have significantly raised GSH levels compared to normal subjects, suggesting that GSH estimation may be helpful in knowing the disease activity and response to chemo/radiotherapy.

Method: In 20 patients with CLL and 20 age and sex matched healthy controls, Lymphocyte GSH (L-GSH) level was estimated before and after chemotherapy.

Results: Significant raised L-GSH was observed in CLL patients as compared to controls and levels decreased after treatment.

Conclusion: Serial estimation of GSH may be helpful in understanding the metabolic mechanism of the disease, predict onset of relapse and response to chemo/radiotherapy.

MCL-1 GENE PROMOTER INSERTIONS DO NOT CORRELATE WITH DISEASE OUTCOME, STAGE OR VH GENE MUTATION STATUS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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CYTOGENETICS AND FISH ANALYSIS IN 413 PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA
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Introduction: We aimed to examine the incidence and prognostic associations of karyotype and FISH analysis in B-CLL.

Methods: We analysed 413 sequential patients with both standard karyotype and fluorescence in-situ hybridisation (FISH) with probes for 11q22 (ATM gene), 12 (CEP12), 13q14.3 (D13S319) (near RB locus), 13q34.3 (LAMP) and 17p13.1 (p53 gene) in B-CLL at MDACC between October 2003 and January 2005.

Results: Of 413 sequential patients, 268 were previously untreated. There were 285 males and 128 females. 369 (89%) had successful cytogenetic analysis and 251 had a normal karyotype. The commonest sole abnormalities were +12 (20pts), 11q (9pts), 13q (6pts) and 20q (2pts). One abnormality was present in 52 pts, two in 13 pts and three or more in 46 pts. No patient had 17p as a sole abnormality. Deletion of 20q was a recurrent abnormality for CLL occurring in 6 patients; 5/6 at 20q11.2 locus, 2 as the sole abnormality, 2 with 11q32- and 2 as part of more complex abnormalities. All 20q- were in previously untreated patients.

FISH abnormalities were seen in 277 patients with 11q in 27%, +12 in 25% and 17p in 16%. Probes to 13q showed loss of 13q14.3 in 64% and 13q34 in 4% patients and 36% of the latter did not correlate with loss of D13S319. A normal karyotype was present in 51% of patients with abnormal FISH results.

Conclusions: Analysis of 413 patients at MD Anderson over 18 months shows abnormal karyotype in 32% and abnormal FISH in 67%. 20q- was identified as a recurrent abnormality in CLL.

CHARACTERIZATION OF P53 INACTIVATION IN B-CELL PATIENTS
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**Introduction:** Defects of the p53 gene confers the worst outcome for B-CLL patients. Dysfunction is reported to be associated with advanced stage disease, transformation to Richter’s syndrome and resistance to therapy. It is not clear however, in what particular stage of the disease the p53 inactivation really occurs.

**Methods:** We analyzed status of the p53 gene in 135 patients diagnosed with B-CLL of all stages (both treated and untreated) by using functional analysis (FASAY) and monitoring of protein expression by Western-blot. Cytogenetic data concerning the p53 (deletion 17p13) were available for two thirds of the analyzed patients.

**Results:** We detected p53 inactivation in 20 patients (14.8%), what is at the upper edge of published data (10–15%). The deletion of one and mutation of the second allele was by far the most frequent type of inactivation (80%). Protein expression correlated well with point mutations or small deletions in the gene, since all these patients expressed considerably higher amount of protein than any sample with wild-type p53. Inactivation was distributed over both early/intermediate stages (0I, II; 12 patients) and advanced stages (III,IV; 8 patients). Thirty five patients with wild-type p53 were consecutively analyzed again by Western-blot and/or FASAY within the time period ranging from 3 to 21 months (average 9 months; 20 patients being in progression of the disease) and no change in the p53 status was detected in any sample.

**Conclusion:** Inactivation of the p53 may arise already in lower stages of the B-CLL. Long follow-up will be required to see whether any particular point of the disease connected with transition to mutated p53. Supported by grants MSMT No.1K04017 and Epilda-Nucleus.

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**ANALYSIS OF SOMATIC MUTATIONS IN IGH REARRANGEMENTS IN HAIRY CELL LEUKEMIA (HCL)**

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Currently, the origin of the HCL tumour clone is believed to be marginal zone B-cell that has previously contacted the antigen. Therefore, it should have undergone somatic hypermutation (SHM). However, the low frequency of HCL has hampered the investigation of IGH rearrangements in large series of patients. In this paper we have characterized the IGH gene in 29 HCL patients in order to ascertain the frequency and characteristics of both incomplete DJH and complete VDJH rearrangements and to determine their somatic mutations status. 29 patients with unequivocal diagnosis of HCL (CD19 + CD25 + CD22 + FCm7 + CD23 – CD103 +) were included in the study. Amplification of clonal rearrangements was carried out using the Biomed-2 strategy (Leukemia 2003; 17:2257), followed by direct automated sequencing of the fragments. All 29 patients displayed a clonal rearrangement, including 26 VDJH rearrangements (90.6%) and 11 DJH rearrangements (37%). Families VH3 YVH1 were over-represented, while families VH5, VH6 and VH7 were completely absent. As far as the DH segments is concerned, DH3 family was the most common in complete rearrangements (35%) followed by DM2 and DH6 (17% each). Contrary, DHS and DH1 were the most frequently DH segments used in the incomplete rearrangement (27% each). Moreover DH1 was totally absent in the complete rearrangements, which suggest that this family is involved in negative selection process for the formation of the complete rearrangement. Finally, JH6 and JH4 segments were the most frequently used. Complete rearrangements observed in both complete and incomplete rearrangements. Sequence analysis showed that most VDJH rearrangements in HCL were mutated (62%), with an average deviation to germline sequence of 6.2%. By contrast, all of incomplete rearrangements displayed a >99% homology with the germline sequence. Although HCL seems to be a homogeneous disease, molecular analysis of IGH rearrangements of tumour cells shows an important heterogeneous pattern. Thus, incomplete rearrangements are present in part of the cases and preferential use of some IGH segments is seen. Furthermore, although most of cases seem to have a post-follicular origin, nearly of 40% may have originated in cells that have not undergone the SHM process.
cancer (site, age at diagnosis and status). Statistical differences between each group were analysed using Chi-Square tests.

Results: Of the 85 CLL included, 31% were CD38+. Fifty (59%) reported a first-degree relative with cancer, from which 7 (8%) were haematological tumours. In the table, clinical characteristics are presented according to the presence or not of a first-degree relative with haematological neoplasms.

Conclusions: Family history of haematological neoplasms, CD38 expression and a younger diagnosis of the disease seems to identify a subgroup of CLL that could have a genetic origin.

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DOUBLING TIME OF SOLUBLE CD23 IS AN ADDITIONAL MARKER IN CLL PATIENTS EXPRESSING OR NOT ZAP-70


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Background: Chronic lymphocytic leukemia (CLL) is a disease with unpredictable natural history in stage A patients (pts). Recently, it has been demonstrated that ZAP-70 and IgVH mutational status are powerful biological prognostic factors and we previously reported that the doubling time of soluble CD23 (SCD23DT) could be used as a prognostic factor for progression of disease in untreated stage A CLL. Therefore we evaluated prospectively ZAP-70 expression and IgVH mutational status in untreated CLL patients (pts) and correlated these markers with SCD23DT.

Methods: SC23 level was evaluated by commercial available enzyme-linked immunosorbent assay (ELISA). ZAP-70 expression is determined in leukemic cells by flow cytometry, and positive expression was defined as ≥20% positive cells.

Results: 67 pts with 53 stage A, 9 Stage B, 5 stage C were evaluated, the median age was 62 years. The median follow-up was 55 months. At present, results of 31 pts are available for analysis and an update will be made for June 2005. In the group of pts with positive ZAP-70 expression (n = 11), 55% (n = 6) required a treatment and 33% had a SCD23DT inferior to one year. Among ZAP-70+ and SCD23DT.

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ZAP-70 IS MORE LIKE TO DETERMINE POOR PROGNOSIS THAN IGVH MUTATIONAL STATUS

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Introduction: B-CLL patients with an unmutated immunoglobulin heavy chain gene (IgVH) status have a poorer prognosis than those with somatically mutated variable region heavy chains. Determination of mutational status is costly and difficult to perform, restricting its use in many centres. In B-CLL, cells the expression of a protein tyrosine kinase, ZAP-70, which is normally expressed by T and NK cell sub-types, strongly correlates with IgVH mutational status and patient prognosis. This study collates information on IgVH mutational status and patient prognosis.

Methods: We collected information on 34 patients with B-CLL. The BIOMED2 protocol was used to examine mutational status and IgVH gene usage. Flow analysis was carried out to examine CD38 and ZAP-70 expression and chromosomal abnormalities were determined using FISH.

Results: There was a correlation between ZAP-70 status and mutational status but 26.5% of patients were discordant for these prognostic factors. 9/34 (26.5%) patients with unmutated IgVH (UM) gene were ZAP-70+, and 17/34 (50%) patients with mutated IgVH (M) were ZAP-70-. Of those patients that were ZAP-70+ and UM, all had additional poor prognostic markers (CD38+ or bimodal expression chromosomal abnormalities, unfavourable IgVH gene usage and/or LDT≤1year). Of the 9/34 (26.5%) patients that were discordant for ZAP-70 and IgVH mutational status, all 9 were ZAP-70+ and had mutated IgVH genes. 3/9 (77.7%) had at least 2 additional poor prognostic markers and 1/9 (11%) had one other poor prognostic marker. More patients in the ZAP-70+ mutated group required treatment than in the ZAP-70- mutated group (49% versus 6/17), at an earlier time from diagnosis. The ZAP-70+ mutated patients are behaving more like the ZAP-70+ unmutated patients (6/8 required treatment).

Conclusion: ZAP-70 could be used to distinguish between good and bad prognosis patients independently of mutational status and identifies patients that are likely to require earlier treatment.

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PROGNOSTIC VALUE OF HEAVY-CHAIN GENE MUTATION STATUS AND ZAP-70 EXPRESSION IN 113 CLL PATIENTS FROM A SINGLE INSTITUTION


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Introduction: The IgVH status represents a strong prognostic marker in chronic lymphocytic leukemia (CLL). Recently, ZAP-70, the 70-kD zeta-associated protein (ZAP-70), has been reported to be overexpressed in unmutated CLL. In this study we reported our findings on the VH status, ZAP-70 and CD38 expression in our series of 113 patients.

Methods: Leukemic B cells obtained from 113 patients (pts) observed in our institution were investigated for IgVH status, ZAP-70 expression (both by western blot and flow cytometry analyses using a cut-off of 20%) and CD38 expression.

Results: In order to determine the predictive value and the degree of concordance between the three prognostic criteria, the results obtained have been related to the outcome of the disease and to the overall survival (OS). Following analysis of IgVH status, we identified a sub-group of pts with unmutated IgVH and a long survival (1 death/54 mutated pts) and another sub-group with unmutated IgVH genes and a shorter survival (12 death/42 unmutated pts) (P = 0.001). When we analyzed ZAP-70 expression, ZAP-70+ patients showed a shorter OS (10 death/42 pts) with respect to ZAP-70 negative group (3 death/27 pts) (P = 0.04). Furthermore, among unmutated patients the majority of them (78%) were ZAP-70 positive while 22% were ZAP-70 negative. In our series of pts the majority of death developed in the ZAP-70pos/IgVHneg group, while we did not find any difference in the other three groups of pts (ZAP-70neg/IgVHneg; ZAP-70neg/IgVHpos; ZAP-70pos/IgVHpos). The CD38 expression analysis didn’t show a prognostic value when we used a cut-off of 30%, but it had a prognostic value (P = 0.01) when CD38 expression was described as completely positive, bimodal or completely negative.

Conclusion: In conclusion, ZAP-70 and IgVH analysis are useful in predicting patient survival in CLL patients that permits to define subgroups of pts with different prognosis.

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SERUM INSULINLIKE GROWTH FACTOR IS NOT ELEVATED IN PATIENTS WITH EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA BUT IS STILL A PROGNOSTIC FACTOR FOR DISEASE PROGRESSION

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Using a quantitative sandwich immunoassay technique (ELISA) (QUANKITNE2, Human IgF-1 and IGFBP-3, R & D Systems), we measured the concentration of IGF-1 and its major binding protein IGF-binding protein 3 (IGF-1p3) in serum at the time of diagnosis from 77 Binet stage A CLL patients. Either IGF-1 or IGFBP-3 were significantly decreased compared to healthy age- and sex-matched controls (P ≤ 0.0001 for both; Mann-Whitney test). Serum levels of IGF-1 and IGFBP-3 paralleled each other (P ≤ 0.002); in contrast, no significant correlation was found between serum levels of IGF-1 and age (P ≥ 0.253), sex (P = 0.270), Rai clinical subgroups (P = 0.140), LDH (P = 0.950), β2-microglobulin (P = 0.388), lymphocyte count and doubling time (LDT) (P ≥ 0.233). When correlation were attempted with doubling time (LDT) (P ≥ 0.233).
circulating levels of angiogenic cytokines such as vascular endothelial growth factor (VEGF) (P = 0.971), basic fibroblast growth factor (FGF-2) (P = 0.095), angiotensin (P = 0.282) or adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) (P = 0.318), intercellular adhesion molecule-1 (ICAM-1) (P = 0.883) and platelet endothelial cell adhesion-1 similar results were found.

Serum levels of IFG-1 were further evaluated as a dichotomous variable with respect to progression-free survival (PFS), an endpoint surrogate for overall survival in early B-cell CLL. The best separation of curves was seen with the cutoff point at the 75th percentile of IFG-1 levels (t.e., 93 pg/ml). Median PFS was 63 months in the patient group with low IFG-1 (i.e., <93 pg/ml), compared to a median PFS of 40 months in the remaining patients (P = 0.002; HR, 0.311, 95% CI, 0.885–0.630). In the multi- 

variation analysis performed including variables significant at univariate analysis only Rai stage retained prognostic significance (P = 0.006). However, after removing from analysis LDT (only 6 out of 77 patients had LDT < 12 months), either IFG-1 or Rai stage entered the model at a significant level (P = 0.03 and P = 0.01, respectively).

Although IFG-1 did not correlate with markers of tumor burden or clinical status in CLL results of the present study highlight its involvement in mechanisms of disease-progression in early CLL.

ORAL FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN ELDERLY PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOBLASTIC LEUKEMIA DISORDERS

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Background: Fludarabine (FA) in treatment of patients with chronic lymphocytic leukemia CLL (B-CLL) and low grade non-Hodgkin’s lymphoma (LG-NHL) extends the interval of disease-free survival but is not clear that it may extend the overall survival. A strong synergistic effect of FA and Cyclophosphamide (CY) has been demonstrated in the treatment of this disease, although in full doses have been reported. A high incidence of severe neuropenia and infectious complications, particularly in elderly patients.

Aims: We tested efficacy and safety of the new oral formulation of FLU combined with CY at low doses in elderly out patients with relapsed/refractory B-CLL or FL.

Methods: 27 elderly patients or with poor biologic status (median age 68 years) with relapsed/refractory B-CLL or LG-NHL were treated between March 2003 and December 2004 with oral FA 30 mg/m² and oral CY 100 mg/m² both for 4 days every 4 weeks, for 4 to 6 courses. The median value of course per patient was 4. The study group consisted of 22 patients with B-CLL and 5 patients with LG-NHL.

Results: 22 (81.14%) patients were responsive to this treatment (SCR and 19 PR), the remaining five patients did not show any response (3 progressive and 2 stable disease). Hematological toxicity was acceptable; grade 2–3 neutropenia/trombocitopenia in 10/27 (37.03%) cases and a mild extra-hematological toxicity, consisting mainly in grade 1-2 infectious relapsing in 9/27 cases (33.3%), was observed. Four patients (2 CLL and 2 NHL) died: 2 because of infectious complication, 1 because of progression and the remaining 1 patients due to sudden death (not related to hematological disease or the treatment).

Conclusion: Oral combination of low dose FLU and CY is safe and equally effective as the i.v formulation, since it may induce rapid responses in about 80% of elderly patients with CL/LG-NHL. A longer follow up and a larger trial, are needed to confirm these results.

THE ADDITION OF RITUXIMAB (R) TO FLUDARABINE AND CYCLOPHOSPHAMIDE (FC) SIGNIFICANTLY IMPROVES OUTCOMES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) AND IDIOPATHIC NON-HODGKIN LYMPHOMA (NHL)


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Introduction: The addition of concurrent R to FC (FC-R) may improve outcomes in patients with indolent lymphoproliferative disorders.

Methods: 72 patients with CLL/LGL (n = 32), follicular NHL (FL; n = 22), mantle cell NHL (MCL; n = 7), marginal zone NHL (MZN; n = 2) or Waldenstrom Macroglobulinemia (WM; n = 9) were treated with a median 4 (range 1–18) cycles of FC-R (25 mg/m² d1–3, C 250 mg/m² d1–3, R 375 mg/m² d1; q28 days) between 1200 and 1204. Baseline characteristics (median (range)): age 60 years (30–89); male sex 64%; # prior therapies 2 (0–9); previously untreated 35%. Results were compared with a historical cohort of 64 patients treated with FC.

Results: 69 patients were evaluable for response. Overall (OR) and complete response (CR) rates were 84% & 43% respectively. All patients with previously untreated CLL/LGL (n = 11) or FL (n = 7) responded, with CR rates of 75% and 86%; in addition, no evidence of subclinical disease was detected using disease appropriate molecular studies (CLL/LGL, n = 7; FL, n = 3) & for positron emission tomography (FL, n = 6). For pretreated patients, CR/OR rates are: CLL/LGL 5½/11%; FL, 87½/67%; MCL, 33½/53%; MZN, 50½/50%; WM, 5½/40%. At median 16.5 months of follow-up, time to progression (TTP) and overall survival (OS) compared to FC were significantly superior in previously untreated patients (TTP, no progression vs FC median 13 months P = 0.0002; 2 year OS, 100% vs FC 74% P = 0.03), but not in pretreated patients (TTP, median 15 months vs FC 11 months P = 0.34; 2 year OS, 67% vs FC 60% P = 0.45). There was no significant increase in myelotoxicity (grade 4 neutropenia 18% vs FC 17%cycle, P = 0.75) or infections (grade 3-4 infection vs FC 6%cycle, P = 0.14). Myelodysplasia was diagnosed in five pretreated patients (with 1–3 previous therapies), 5 of whom proceeded to completion of FC-R.

Conclusions: FC-R is highly effective in patients with CLL and indolent NHL, achieving high CR rates and producing durable remissions particularly in patients with previously untreated disease.

PURINE ANTAGONISTS FOR CHRONIC LYMPHOCYTIC LEUKAEMIA: RESULTS OF A COMPREHENSIVE META-ANALYSIS

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Background: Recent trials suggest improved response rates for purine antagonists compared to alkylator-based regimens in the treatment of CLL. However, none was able to show a survival advantage, which may be due to study design or sample size.

Objectives: To determine if there is any advantage of purine antagonists compared to alkylating agents (alone or in combination) in the treatment of patients (pts) with previously untreated CLL.

Methods: Randomised controlled trials comparing purine antagonists as single agents with alkylator-based regimen in pts with previously untreated B-CLL were included. Endpoints included overall survival (OS), overall response (OR), complete remissions (CR), time to progression (TTP), treatment-related morbidity and mortality. Medical databases (Cochrane Library, MEDLINE, EMBASE), conference proceedings and internet-based trial registers were searched (1990–2005). We included full-text and abstract publications as well as unpublished data. Data extraction and quality assessment were done in duplicate, missing data was obtained from original authors.

Results: Five trials with 1838 randomised pts were included. There was a trend for improved OS after treatment with purine antagonists compared to alkylators, but statistical significance was not reached (HR 0.89 [95% CI 0.78–1.01], 5 trials, n = 1838). However, the relative risk for achieving an OR (RR 1.22 [95% CI 1.13–1.31], 5 trials, n = 1751) and CR (RR 1.94 [95% CI 1.65–2.28], 5 trials, n = 1751) was significantly improved, resulting in a longer TTP (HR 0.70 [95% CI 0.61–0.82], 5 trials, n = 1838). Incidence of grade III/IV infections was significantly higher in patients receiving treatment with purine antagonists (RR 1.83 [95% CI 1.30–2.58], 4 trials, n = 1620). There was no significant difference concerning the RR for grade III/IV neutropenia (RR 1.14 [95% CI 0.98–1.34], 4 trials, n = 1620) and therapy-related death (RR 0.94 [95% CI 0.45–1.95]). Overall incidence of hematologic anemia was low, but significantly increased in
the purine antagonist group (RR 3.36 [95% CI 1.27–8.91], 3 trials, n = 1258).

Conclusion: Despite significantly increased OR & CR rates and longer TTP, first-line treatment of B-CLL pts with single-agent purine antagonists does not result in improved OS compared to alkylator-based regimens. Furthermore, the use of purine antagonists also augments the risk for grade III/IV infections and hemolytic anemia.

Figure 1: RITUXIMAB IN COMBINATION WITH SARGRAMOSTIM (GM-CSF) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Rituximab given at standard doses to patients(pts) with CLL has been associated with overall response (OR) rate of 15–35% in previously treated pts and 10% in untreated pts. One of the explanations for this low response rate is the low level of CD20 expression present in CLL cells. Exposure to GM-CSF increases surface CD20 expression on CLL cells, in vitro, and GM-CSF enhances A-DCX, in vivo, contributing to the clearance of CLL cells after treatment with rituximab. We therefore designed this phase II study to evaluate rituximab in combination with GM-CSF in pts with CLL.

Methods: Group 1: untreated pts; Rai stage 0–II and B2M ≤3 mg/mL or symptoms or significant fatigue. Group 2: untreated pts age 70 or older with indication for treatment. Group 3: previously treated pts, Rai stage III or IV or earlier stage with evidence of active disease. All pts received 4 weekly infusions of rituximab 375 mg/m² and GM-CSF 250 mcg sc three times a week for 4 weeks.

Results: 34 pts have been enrolled and 18 are evaluable. 10 pts achieved a response with an OR rate of 66%. 3 pts achieved a nodular PR (17%) and 7 pts a PR (39%). Response by study group were: Group 1: 2 nodular PR and 5 PR; Group 2: 2 PR; Group 3: 1 nodular PR. High-risk genetic abnormalities were identified in 5 patients: del(17p) in 4, and del(11q) in 1 patient. Only 1 PR was observed among these pts. 13 pts were classified as expressing mutated and 5 as expressing unmutated IgVH genes; 9 of the 13 pts with mutated IgVH genes responded to treatment whereas only 1 of 5 with unmutated IgVH genes achieved a response. The treatment was well tolerated, 1 pt developed grade III thrombocytopenia and grade IV neutropenia. 4 pts experienced grade II local reaction at the site of GM-CSF injection and 2 pts reported grade II bone pain attributable to the treatment. One of the patients was observed in 2 pts. Correlative studies evaluating CD20 expression and soluble CD20 levels are ongoing. QOL will address changes in fatigue and other associated symptoms.

Conclusions: The combination of rituximab and GM-CSF is well tolerated and able to induce significant responses in pts with CLL. Accrual to this study is ongoing.

Figure 2: COMPARABLE EFFICIENCY OF THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA
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Traditional chemotherapy of chronic lymphocytic leukaemia (CLL), including monochemotherapy with alkylating agents and with different chemotherapy regimens, such as COP, CHOP, which includes these agents, in very rare cases induce overall responses with very short duration. With the aim of inducing the effectiveness of chemotherapy results in progressive disease, it is reasonable to find out new therapeutic modalities. In this research work we introduce the results of treatment 169 CLL patients, with chemotherapy regimens with purine analogs and monoclonal antibodies anti-CD20: FCM – fludarabine, cyclophosphamide, mitoxantrone (63 patients); RFC – rituximab, fludarabine, cyclophosphamide (15 patients); FC – fludarabine, cyclophosphamide (64 patients); F – fludarabine (27 patients). The age of patients was varied from 32 to 76 years (the average age was 58 years). 18 patients were in stage A, 98 in stage B, 53 patients were in stage C. Among all patients, 97 were previously untreated, 72 were pre-treated with 1–3 and more chemotherapy regimens (chlorambucil, COP, CHOP). Treatment results showed that in CLL chemotherapy including fludarabine has a high level of effectiveness in comparison with conventional therapeutic approaches. The most effective programmes are RFC and FCM combinations that permit to receive overall response in 100% and 94% of cases, respectively. On FC treatment the overall response was achieved in 79% patients, with F – monotherapy – in 64%. All these regimens are well tolerated with acceptable haematology toxicity, which was less expressive, if these programmes were used as a first line therapy. The RFC and FCM combinations increase the number of complete remissions significantly, especially in previously untreated patients.

Figure 3: PENTOSTATIN, CYCLOPHOSPHAMIDE AND RITUXIMAB: AN EFFECTIVE REGIMENT FOR PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA AND LYMPHOPLASMACYTIC LYMPHOMA
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Pentostatin has demonstrated significant activity as a single agent in patients with low-grade B- and T-cell lymphomas, and is less myelosuppressive than other purine analogs. We have conducted a phase II trial with the combination of pentostatin, cyclophosphamide and rituximab in 14 patients with Waldenström's Macroglobulinemia (WM) and 3 patients with lymphoplasmacytic lymphoma without monoclonal serum-IgM (LL), followed by a maintenance therapy with rituximab (375 mg/m² every 3 months) for those patients achieving complete or partial remission after 2 to 5 cycles. 9 patients were untreated, 8 had previously been treated with 1 to 3 regimens. The first 9 patients received pentostatin 4 mg/m², cyclophosphamide 600 mg/m² (PC), and the subsequent 8 patients received the same combination together with rituximab 375 mg/m² on day 1 (PC-R). Cycles were repeated every 3 weeks. An objective tumor response after PC and PC-R was confirmed in 11 out of 17 evaluable patients (64.7%), with 2 CR (11.7%) and 9 PR. In patients who have received rituximab (n = 13), either simultaneously or subsequently, the overall response rate was 76.9%. Toxicity was mild, with only infections WHO grade 2–3 and nausea/vomiting grade 2. Hematotoxicity grade 3 occurred after 9 of 49 cycles (18.3%), grade 4 after 2 cycles (4%). 10 patients were subsequently treated with rituximab every 3 mo with 2–9 cycles so far (median 4 cycles). None of them has relapsed to date with stable IgM serum levels. In 3 patients with PR after completion of chemotherapy, remission has further improved, with normalisation of the IgM level in 1 patient and achievement of a complete remission in another. Our data indicate that PC, together with rituximab, is safe and highly active in patients with WM. Maintenance therapy of WM with rituximab as single infusion every 3 months can be administered safely and may improve remission status.

Figure 4: A PHASE II STUDY OF S/C CLADRIBINE AND ORAL CYCLOPHOSPHAMIDE IN RELAPSED/REFRACTORY LYMPHOPROLIFERATIVE DISORDERS
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Introduction: The purine analogue drugs have demonstrable activity in CLL and indolent lymphomas. The activity of these drugs can be augmented by the addition of other agents with activity in these diseases. The addition of cyclophosphamide is widely used in combination with fludara- bine but few studies have looked at it in combination with Cladribine. We have investigated a low dose combination of CDA/cyclo in a phase II multicentre trial.

Patients and Methods: 22 patients with B-CLL (9) or indolent NHL (13) were treated. The median age of the group at treatment was 64 years. All had relapsed/refractory disease requiring therapy, median prior treatments 2 (range 1–5). Treatment consisted of s/c Cladribine 2 mg/m² and oral cyclophosphamide 100 mg/m² both given daily for 5 days, repeated every 28 days to a maximum of 6 cycles. Patients were re-staged after every 2
courses of therapy. If there was no response or progressive disease patients were taken off study.

Results: In the patients with CLL the response rate was 33% (2 CR, 1 nodular PR). In NHL, 1 patient with primary refractory disease was excluded on histological review with mantle cell lymphoma. Of the 12 other patients the overall response was 75% (8 CR 1 PR). The median event-free survival has not been reached. The treatment was extremely well tolerated, all responding patients received 6 cycles of therapy. 1 patient with progressive CLL died during treatment from an unrelated event (myocardial infarction). The haematological toxicity was modest. Of these patients with responding disease 2 had treatment delays and 1 required a dose reduction based on grade III haematological toxicity. 5 patients required blood and 2 platelet transfusions during the study period, all of these patients except 1 had progressive disease. There were 4 episodes of sepsis requiring antibiotics of which only 1 required admission and intravenous treatment. 1 patient developed shingles. Nausea was minimal with some patients requiring no anti-emetics at all. There was no alopecia. Of the 22 patients 3 self-administered the CDA.

Conclusions: This is a highly active regimen in relapsed indolent NHL with equivalent efficacy to previously described combinations but with considerably less toxicity. The treatment schedule is easily deliverable in the home setting and warrants further evaluation particularly in combination with Rituximab.

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EXPRESSION AND FUNCTIONAL ROLE OF CHEMOKINE RECEPTORS IN MULTIPLE MYELOMA

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Introduction: Chemokines and their receptors play a pivotal role in the regulation of B lymphocyte trafficking. Few data are available on the molecules and receptors involved in the compartmentalization and migration of malignant plasma cells (PC) recovered from patients suffering from multiple myeloma (MM).

Methods: This study was aimed at investigating the pattern of chemokine receptor expression and migration of malignant PC obtained from the bone marrow (29 MM patients) and extramedullary sites (5 MM patients), 3 myeloma cell lines and 5 normal plasma cells suspensions. To this purpose, we investigated the expression of chemokine receptors, including CCR1 to CCR3, CXCR5 to CCR7, CXCR1 to CXCR5.

Results: Flow cytometry analysis showed that the receptors mainly expressed on bone marrow malignant PC were represented by CXCR4 (70%), CCR1 (25%), CCR2 (25%), CCR5 (17%) and CXCR3 (20%) of the patients, respectively. The analysis performed on extramedullary malignant PC demonstrated that the most represented receptors in these compartments were CXCR4 (100%), CCR7 (100%), CCR2 (65%) and CXCR1 (60%). The evaluation of the migratory capability of malignant PC at resting conditions allows to identify 3 groups of patients with different basal migration (low, intermediate and high migratory capability). Since CXCR1 was the dominant chemokine receptor expressed by MM plasma cells, the migratory effect of its ligand (CXCL12) was assessed in MM patients and the data obtained showed an increase in the migration of the malignant clone. This increase was higher in samples with a lower basal migration.

Conclusions: These data suggest that malignant PC from MM patients display different chemokine receptor profiles and that these receptors are fully functional and might play a role in the spreading of the disease.

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ANALYSIS OF THE MEK/ERK MODULE AS A THERAPEUTIC TARGET IN MULTIPLE MYELOMA

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Introduction: Although the IL-6R/STAT3 pathway is known to protect Multiple Myeloma (MM) cells from apoptosis, they survive IL-6R blockade if they are cocultured with bone marrow stromal cells (BMSCs). To elucidate BMSC-stimulated and IL-6R-independent prosurvival pathways, we investigated the importance of the MEK1/2/ERK1,2 pathways, however, entailed strong induction of apoptosis even in the presence of BMSCs. This was equally true for MM cell lines and for primary MM cells. Pathway analyses revealed that BMSCs stimulate STAT3 via the IL-6R, and MAPK in parallel with IL-6R-independent mechanisms. Additionally, we identified crucial components of the MEK/ERK module in MM cells, and the survival of primary MM cells.

Results: Sole blockade of the activities of MEK1,2 or of ERK1,2 was insufficient to induce cell death. Combined disruption of the IL-6R/STAT3 and MEK1/2/ERK1,2 pathways, however, entailed strong induction of apoptosis even in the presence of BMSCs. This was equally true for MM cell lines and for primary MM cells. Pathway analyses revealed that BMSCs stimulate STAT3 via the IL-6R, and MAPK in parallel with IL-6R-independent mechanisms. Additionally, we identified crucial components of the MEK/ERK module in MM cells, and the survival of primary MM cells.

Conclusions: Our data indicate that the MEK/ERK module – in addition to its previously described role in cell proliferation – can also critically contribute to MM cell survival. BM-mediated activation of MEK/ERK and of the IL-6R/STAT3 pathway independently contribute to protect MM cells from apoptosis, and simultaneous blockade of the IL-6R/STAT3 and IL-6R-independent ERK1,2 pathways in primary MM cells results in cell death. This implicates a well-directed therapeutic strategy might
require the combined targeting of different and independently activated signal transduction pathways.

MULTIPLE MYELOMA WITH SECOND MALIGNANT NEOPLASMS
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Introduction: A small number of patients have been described with second malignant neoplasms (SMN) following treatment for multiple myeloma (MM).

Methods: From 07/86 to 12/04 seven patients: two women and five men with MM developed second solid tumor; median age 68 years (range 51–73 years), stage of MM IA- four patients, II A- three patients. Three patients were given chemotherapy (9–14 cycles of MP) alone because of advanced disease. Three patients received radical resection and followed radiotherapy (dose 20–40 Gy). One patient was given radical resection alone.

Results: Mean time interval for occurrence of SMN was 6.5 years. For the entire group, there were: two patients with breast cancer, two- with lung cancer, one- with melanoma, one- colon cancer, one- with gastric cancer. Two patients are alive and well; one- 18 years after diagnosis of MM and 2 years after diagnosis of lung cancer (stage I), second- 9 years after diagnosis of MM and 2 years after diagnosis of lung cancer (stage I). One patient is alive with progression of MM and SMN: follow-up 12 months (simultaneous MM and breast cancer stage IV). Four patients died: 2- due progression of MM, one- progression of colon cancer stage IV, one- progression of MM and gastric cancer stage IV.

Conclusions: In this small group of patients, there is no relationship between the treatment used for myeloma and the occurrence of SMN. A regular follow-up is necessary to detect these lesions earlier, to allow a possible treatment.

PHASE II STUDY OF THALIDOMIDE WITH DEXMETHASONE FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA
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Purpose: To evaluate efficacy and safety of thalidomide with dexamethasone for patients with relapsed/refractory multiple myeloma (MM).

Methods: We conducted a phase II study of thalidomide with dexamethasone. Eligible criteria were as follows: relapsed/refractory MM, stage II/III, 20 to 79 years old, PS 0 to 3, with adequate organ functions, and written informed consent. Primary endpoint was response rate and secondary endpoints were adverse events and event free survival. Thalidomide was initially started with 200 mg/day and escalated to maximal tolerated dose up to 800 mg/day. 40 mg/body of dexamethasone was given orally for four days and repeated every three weeks.

Results: Seventeen patients were registered between 2002 and 2005. Age 45 to 71 years, male/female: 8/9, relapse after autoPBSCT: 6, lgG/A:B/J/PD: 5/8/1/3, stage II/III: 1/6. Responses were CR in 1, PR in 6, MR in 2, NC in 2, PD in 4, and NE in 2. Overall response rate (CR + PR + MR) was 52.9%. Time to treatment failure were 0 to 26 mo (median 3.9 mo). Major adverse events were constipation, malaise, insomnia, peripheral neuropathy, and neutropenia. Grade 3 or more non-hematological toxicities were skin rash (1), bleeding from colon diverticulum (1), nasal bleeding (1), and renal failure (1). Deep vein thrombosis (DVT) was not observed. No TRD was observed in this study.

Conclusion: Thalidomide with dexamethasone treatment was effective for relapsed/refractory MM. Several non-hematological toxicities were sometimes observed and needed cautions. DVT might be infrequent in Japanese patients with MM treated with thalidomide with dexamethasone.
**VACCINATION WITH SULFHYDRYL-BASED IDIOTYPE-CARRIER PROTEIN CONJUGATES ELICIT THERAPEUTIC ANTI-TUMOR IMMUNITY SUPERIOR TO GLUTARALDEHYDE CONJUGATES IN THE A20 MURINE LYMOPHMA MODEL**

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Therapeutic vaccination of lymphoma patients with tumor-specific Ig (idiotype, i.e. IgG or IgM) covalently coupled to the highly immunogenic foreign carrier protein keyhole limpet hemocyanin (Id-KLH) has shown promise in phase I/II clinical trials, and phase III trials are underway. Id proteins are traditionally coupled to KLH using glutaraldehyde (glut), which primarily crosslinks proteins via lysine and cysteine residues, with secondary reactions at tyrosine and histidine. This extensive crosslinking could inhibit proteosomal processing or destroy critical immunogenic epitopes. In contrast, maleimide (mal) crosslinks proteins only via cysteine sulhydryl groups, thus limiting the potential for destruction of epitopes. The murine B cell lymphoma A20 is reportedly unresponsive to vaccination with glut/Id-KLH. We hypothesized that the epitopes of some Id proteins, including A20, might be damaged by glut, but preserved using the gentler sulfhydryl-based maleimide conjugation method. Groups of BALB/c mice were inoculated subcutaneously with A20 tumor cells, and 4 days later vaccinated with 3 weekly glut/Id-KLH or mal/Id-KLH plus GM-CSF injections. As previously reported, tumor eradication was uncommon in glut/Id-KLH-treated mice (16–25% in 3 experiments), with no statistical advantage over control-treated mice (P = 0.05). In contrast, tumor was eradicated in 33–58% of mice vaccinated with mal/Id-KLH, with tumor protection highly significant compared to control mice (P = 0.0001) and glut/Id-KLH-vaccinated mice (P = 0.0084). Vaccination with both glut/Id-KLH and mal/Id-KLH did not improve survival over mal/Id-KLH alone (both 58%). Thus, the method of Id conjugation to KLH can substantially affect the level of tumor protection elicited by Id-KLH vaccination. This may explain why some patients fail to respond to traditional glut/Id-KLH vaccinations. Ongoing studies are determining the roles of antibody versus T cell effector mechanisms in this and other murine lymphoma models. These findings have important implications for the ongoing development of therapeutic vaccines for lymphoma targeting Id.

**PRECLINICAL ANTITUMOR ACTIVITY OF FARNESSYL-TRANSFERASE INHIBITOR R115777 IN MANTLE CELL LYMPHOMA**

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**Introduction:** It has been recently demonstrated by gene expression profiling that mantle cell lymphoma (MCL) exhibits specific molecular signatures, particularly an overexpression of the transcript of the α subunit gene of farnesyltransferase (Frase), a key maturation enzyme of several oncogenic proteins. We investigated the effect of Frase inhibition in MCL.

**Methods:** qRT-PCR for Frase subunits were performed on 43 MCL tumours and MCL cell line (NCEB). Analysis of proliferation, viability, and apoptosis of 5 MCL cell lines (Granta, NCEB, REC, UPN1 and VAR) were realized in presence of R115777 alone or associated with vincristine, doxorubicin and bortezomib by cell counts, MTT and annexin assays, respectively. R115777 activity was analyzed in vivo in MCL xenografted nude mice with UPN1 MCL cell line.

**Results:** α and β Frase subunits were overexpressed in MCL compared to normal B-lymphocytes (1.55 and 2.35 respectively). In vitro, R115777 inhibited growth of MCL cell lines with IC50s ranged from 2 to 15μM. Fifty percent decrease of cell viability was obtained between 0.08 and 17μM of R115777, with an induction of apoptosis in 51 to 77% of the cells. In addition, R115777 potentiated significantly the effect of vincristine, doxorubicin and bortezomib with an increase of cytotoxic activity to 30%, 46% and 42%, respectively. We observed in vivo a cytostatic activity at 500 mg/kg/d given orally twice a day during 8 days.

**Conclusion:** We demonstrated that the Frase inhibition could specifically induce in vitro the inhibition of MCL proliferation and a marked apoptosis of all MCL cell lines, and had in vivo a cytostatic activity.

**REVERSION OF CHEMORESISTANCE IN AGGRESSIVE LYMPHOMA BY QUININE: A PHASE II TRIAL**

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**Introduction:** Multidrug resistance has been one of the most studied mechanisms of cancer cell resistance to chemotherapy. Evidence of its involvement in hematological malignancies is known since many years. One well-designed phase I trial showed that about 20 to 25% of patients strictly resistant to EPOCH schedule could become responsive again by the addition of Dexamethasone (J Clin Oncol 1995; 13:1995–2004). We decided to perform a phase II trial to evaluate whether quinine could reverse resistance to chemotherapy in aggressive lymphoma.

**Methods:** Patients included had all received at least two lines of chemotherapy including CEP (cyclophosphamide 750mg/m², etoposide 60mg/m², vincristine 1.4mg/m² IV day 1 and prednisone 40mg/m² orally daily to day 7) and were demonstrated as strictly refractory to CEP (i.e. neither no change nor progression). Q-CEP was administered as follows: quinine 30mg/kg/day for two days from day 0 to day 1 as a continuous infusion, CEP at the beginning of day 1.

**Results:** 15 patients have been included with the following characteristics: median age: 52 years (32–64), 66% stage III-IV, 60% elevated LDH. The median number of previous regimens was 3 (2–5). 13 patients had diffuse large B cell lymphoma including 3 with previous history of follicular lymphoma. Two had mantle cell lymphoma. Seven patients were included directly since they were initially refractory to CEP while eight were shown refractory after two further cycles of CEP. Etoposide pharmacokinetics was not influenced by quinine. Mean dose intensity of etoposide was 94%. Toxicity was mild. 4 responses were observed at the end of treatment including 2 complete and 2 partial responses (26%, IC 95%: 8–55%). Only the 2 CR patients are still alive with no evidence of disease at 35+ and 61+ months.

**Conclusion:** These results add further arguments to confirm that MDR1 plays a significant role in resistance to chemotherapy in lymphoma. Quinine allowed unexpected long term remission in 2 responding patients.

**PIXANTRONE (BBR 2778) IN NHL: AN ACTIVE ANTHRACENEDIONE ANALOGUE WITH REDUCED CARDIOTOXIC POTENTIAL**

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**Background:** Exposure to anthracyclines and anthrancenediones is associated with irreversible and progressive deterioration of cardiac function and congestive heart failure (CHF). Doxorubicin as part of

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**Table 1.**

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Regimen N</th>
<th>Clinical Symptoms</th>
<th>LVEF&lt;sub&gt;≥&lt;/sub&gt;20%</th>
<th>LVEF&lt;sub&gt;≥&lt;/sub&gt;10–20%</th>
<th>ORR&lt;sub&gt;≥&lt;/sub&gt;20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:05: rel.</td>
<td>P-SHAP</td>
<td>19</td>
<td>0</td>
<td>71</td>
<td>61%</td>
</tr>
<tr>
<td>1:06: rel.</td>
<td>FFDR</td>
<td>27</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>1:07: rel.</td>
<td>CPO</td>
<td>49</td>
<td>2</td>
<td>7</td>
<td>79%</td>
</tr>
<tr>
<td>1:01: rel.</td>
<td>Single agent</td>
<td>33</td>
<td>2</td>
<td>6</td>
<td>27%</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>8 (7%)</td>
<td>3 (3%)</td>
<td>26 (24%)</td>
<td></td>
</tr>
</tbody>
</table>

1Left ventricular ejection fraction;
2Grade2 LV failure in 2 pts heavily pretreated,1 pt with h/o CHF;
3DM and CAD in 1 pt;
4Mediastinal mass in 1 pt.
the CHOP-regimen in first-line therapy induces cardiac events in 20% of pts, which was associated with LVE dysfunction in 8.9% of patients (Liman et al., Ann Onc 14:277–81, 2003). Pxicantrene, a novel anthraene-dione analogue, was developed to reduce treatment related cardiotoxicity while retaining efficacy.

Preclinical studies: The cumulative cardiotoxic potential of pxicantrene was evaluated in doxorubicin-pretreated and doxorubicin-naive mice, confirming the significantly lower cardiotoxic potential of pxicantrene when compared to equitoxic doses of doxorubicin and mitoxantrone. Moreover, exposure to pxicantrene did not worsen preexisting cardiomyopathy in doxorubicin-pretreated mice.

Clinical studies: Cardiac toxicities observed in Phase I/II studies are summarized below, all pts were pre-exposed to anthracyclines.

Conclusions: Preclinical data show that pxicantrene has a reduced cardiotoxic potential compared to doxorubicin or mitoxantrone. The limited occurrence of cardiac dysfunction observed in clinical studies suggests that pxicantrene may be suitable for patients pre-exposed to anthracyclines.

**584**

BENDAMUSTINE IN COMBINATION WITH THALIDOMIDE AND PREDNISONE IN PATIENTS WITH REFRACTORY OR RELAPSED MULTIPLE MYELOMA. PRELIMINARY RESULTS OF A PHASE I CLINICAL TRIAL

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Thalidomide is remarkably active as single agent in advanced relapsed or refractory multiple Myeloma (MM), but with a significant morbidity due to side-effects such as polyneuropathy. We questioned whether lower doses of thalidomide in combination with weekly doses of bendamustine and prednisolone had an effective regimen with fewer side-effects especially in relation to neurotoxicity. The treatment consists of a fixed dose of bendamustine (60 mg/kg) i.v. daily, 1, 8, and 15 and prednisolone (100 mg) p.o. day 1, 8, and 15. At the same time, thalidomide was given in patients cohorts with escalating doses, starting with 50 mg to a maximum of 200 mg daily. 4 patients were enrolled at each dose level. If 1 dose limiting toxicity (DLT) occurs, then additional 2 patients would be enrolled at that dose level. Cycles were repeated every 28 days for a minimum of 2 and a maximum of 10 cycles until a maximum response was achieved, a DLT or a disease progression were observed. 9 patients (4 in the first dose level with 50mg thalidomide, 4 in the second dose level with 100mg thalidomide and 1 patient in the third dose level with 200mg) are enrolled until now.

**Results:** All patients completed 2 cycles of BPT-treatment (3 completed 6 cycles, 4 completed 4 cycles, 1 completed 3 cycles and 1 completed 2 cycles) and were hence evaluable. Response was assessed using EBMT criteria modified to include near complete remission (nCR) and very good partial remission (VGPR). 7 of 9 patients responded after at least 2 cycles of chemotherapy with 1 nCR, 2 VGPR and 4 PR. 2 patients had stable disease.

No DLT was observed in the first 2 doses level. None of the 9 patients developed dose-limiting haematotoxicity as defined by an ANC < 1.0 x 10^9/L for > 7 days or an ANC < 0.5 x 10^9/L for > 3 days or platelet count < 25 x 10^9/L. Neutropenia was reported in 3 patients (WHO grade 2) but no thrombocytopenia was observed. No grade 3 or 4 non haematological toxicity was encountered and no dose modification was required. BPT with a dose of 50 or 100mg thalidomide daily is well tolerated in patients with relapsed or refractory MM.

**585**

GALIXIMAB PHARMACOKINETICS DETERMINED IN PATIENTS WITH RELAPSED OR REFRACTORY FOLLICULAR NHL

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**Introduction:** Galiximab (G) is a PRIMATIZED monoclonal antibody that binds to the CD20 antigen. CD20 may play a role in the regulation of normal and malignant B cells and is expressed on a variety of NHLs, including follicular and other subtypes. The efficacy and safety of G in combination with rituximab (R) was explored in a phase I/2 study for the treatment of relapsed or refractory follicular NHL with pharmacokinetic (PK) determinations made as part of the study.

**Methods:** Cohorts of 3 patients each received 4 weekly doses of 125, 250, 375 or 500 mg/m^2 of G along with 375 mg/m^2 R. Additional patients were enrolled in the highest dose group (500 mg/m^2 G + 375 mg/m^2 R). Blood samples were collected for determination of G and R concentrations from all patients during all dosing regimens. Population PK was used to analyze the data, with various demographic features used to determine their influence upon the PK.

**Results:** A two-compartment model best described G PK. Linearity was demonstrated across all doses. A summary of the median PK parameters is given in the table. Testing various demographic features of the population showed body surface area (BSA) and sex correlated with clearance (Cl) and volume of distribution of the peripheral compartment (V2).

**Table 1.**

<table>
<thead>
<tr>
<th>G Dose (mg/m^2)</th>
<th>Cl (mL/day/m^2)</th>
<th>V1 (L/m^2)</th>
<th>V2 (L/m^2)</th>
<th>Half-life (days)</th>
<th>Cmax (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>90.4</td>
<td>0.0070</td>
<td>2.39</td>
<td>20.8</td>
<td>161</td>
</tr>
<tr>
<td>250</td>
<td>75.9</td>
<td>0.0055</td>
<td>2.34</td>
<td>22.7</td>
<td>430</td>
</tr>
<tr>
<td>375</td>
<td>49.4</td>
<td>0.0071</td>
<td>2.14</td>
<td>27.9</td>
<td>633</td>
</tr>
<tr>
<td>500</td>
<td>71.0</td>
<td>0.0066</td>
<td>2.12</td>
<td>22.4</td>
<td>804</td>
</tr>
</tbody>
</table>

**Conclusions:** G accumulates with weekly dosing. PK over this dose range is linear. BSA and sex influence the variability of PK parameters.

**586**

LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF ELDERLY PATIENTS OR PRETREATED PATIENTS WITH LYMPHOMA

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**Introduction:** Myocet (liposomal doxorubicin) has an improved pharmacokinetic profile with less myelosuppression and GI toxicity and has a reduced risk of cardiotoxicity at dose level equivalent to standard formulations of doxorubicin.

**Methods:** We replaced the conventional doxorubicin with Myocet for the treatment of elderly patients (pts), or pts with cardiac dysfunction or pts with previous treatment with doxorubicin. From June 2003, we have treated with COMP 10 pts with aggressive NHL and with MBVD 2 pts with Hodgkin's lymphoma, replacing doxorubicin with Myocet (50mg/m2 in COMP and 25 mg/m2 in MBVD). The median age was 70 years (range 54–75). Two pts were stage I, 4 stage II, 1 stage III and 5 stage IV. According to IPI score, for NHL only, 3 pts were low risk, 4 low-intermediate, 2 intermediate-high and 1 high risk. Three pts were pretreated, 6 pts showed cardiomyopathy (1 ischemic, 3 hypertensive and 2 hypokynestic). The median left ventricular ejection fraction (LVEF) at diagnosis was 59% (range 42%-75%). All pts but one had no change in LVEF.

**Results:** At the moment 9 out 12 patients are evaluable for response: 6 pts obtained a complete remission (67%) one a partial remission and two pts stopped therapy for toxicity. One of these two pts presented a myocardial dysfunction resolved with medical therapy and he is alive with disease, the other pts died of stroke. After 55 cycles we have observed only these two adverse events. One pts died of disease and after a median observation period of 10 months (range 2–21) the overall survival was 77%. No significant hematological or extrahematological toxicity was recorded.

**Conclusions:** Liposomal doxorubicin did not increase toxicity and it was feasible and active in a subset of lymphoma pts with very negative clinical characteristics at diagnosis.
SPHINGOSOMAL VINCRISTINE IN CHOP ± RITUXIMAB: A PROMISING NEW TREATMENT FOR PATIENTS WITH HIGH RISK UNTREATED AGGRESSIVE NON-HODGKIN'S LYMPHOMA (NHL)

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Introduction: Spingosomal vinristine (SV) is a novel formulation of vinristine encapsulated in sphingomyelin liposomes or 'spingosomes'. SV was well tolerated with 45% ORR in multiply relapsed aggressive NHL (ASH Abst. 412, 1999). The addition of rituximab to CHOP improves response in aggressive B-cell lymphomas in the elderly (Coiffier et al., NEJM 2002;346: 235-42). Based on these data, a phase II study of RCHOP, substituting SV for free vinristine, was undertaken in patients with previously untreated aggressive NHL (excluding rituximab in T-cell lymphoma).

Methods: Patients were treated with standard dose CHOP that included SV 2.0 mg/m² without dose stepping ± rituximab 375 mg/m², given every 21 days for 6 to 8 courses (ASH Abst. 338, 2002).

Results: Of 73 patients enrolled in the study, 68 were evaluable for response. Median age was 63 range (22-80). Patients received a median of 6 study treatments (range 1-8). ORR was 93% (63/68 pts) with 62 pts achieving CR (91%), and 1 PR (2%). 3 pts had PD (4%) and 2 were not assessed for response (3%). Responses according to IPI score were as follows: patients with IPI 0-2 (n = 44) – ORR was 93% (41), CR was 77% (34), CRu was 16% (6), and PR was 2% (1). For patients with IPI ≥ 3 (n = 24); ORR was 92% (22), CR was 88% (21), CRu was 4% (1), and PR was 0% (0).

The median PFS and OS have not been reached at a median follow up of 29.5 months. Overall survival probability was 94% at 27 months (1 death in the group with IPI 0-2 and 2 deaths in the group with IPI ≥ 3. The probability of being progression free was 86% (5 relapses and 1 death, reason unknown) for pts with IPI 0-2 and 77% (6 relapses + 2 deaths) for pts with IPI ≥ 3. Neutropathy was generally mild (Gr. 1-2). Hematological toxicities were as follows: 64% Gr. 3-4 neutropenia, 6% Gr. 3 anaemia, and 14% Gr. 3-4 thrombocytopenia.

Conclusions: CHOP plus rituximab regimen with spingosomae vinristine substituted for free vinristine demonstrated promising activity with durable responses similar in both groups of patients with IPI score 0-2 and IPI ≥ 3. The treatment was well tolerated with only mild neutropoietic.

SPC2996 – A NEW BCL-2 INHIBITOR

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Introduction: The antisense compound SPC2996 is a Bcl-2 mRNA antagonist. SPC2996 is an antisense oligonucleotide with Locked Nucleic Acids (LNA). The LNA modification of antisense oligonucleotides causes a significant increase in the affinity towards complementary mRNA and in addition gives these compounds a high biostability as compared to phosphorothioates.

Methods: A number of pre-clinical studies were conducted in cancer models. Cellular assays for specific down regulation of Bcl-2 mRNA and protein were performed. Apoptosis was studied by monitoring activated caspases and extracellular phosphatidyl serine expression. Human cancer cells were xenotransplanted on atumic mice. Preclinical toxicology was performed in rodents and cynomolgus monkeys.

Results: Cellular responses on Bcl-2 mRNA and protein levels were seen at concentrations as low as 1 nM SPC2996. These effects led to strong apoptosis induction, which resulted in anti-proliferative activity. Treatment with SPC2996 in a number of animal models of cancer led to anti-tumour activity. Tumour weight and volume were reduced with approximately 40%.

Conclusion: SPC2996 is the first member of a new generation of potent and specific mRNA antagonists and shows Bcl-2 down regulation and anti-tumour activity. Toxicology studies have been finalised and the first phase I/II dose escalating study in 42 CLL patients will commence in several countries in the EU in April 2005.

ANTIPROLIFERATIVE ACTIVITY OF NOVEL AUREOYLIC ACID ANTIBIOTIC ANALOGUES IN HUMAN LYMPHOMA CELL LINES

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Introduction: Non Hodgkin’s lymphomas are aggressive malignancies and new treatment modalities must be established to increase patient survival. Deregulated gene expression due to chromosomal translocations and gene amplification contributes to the pathogenesis of the majority of human lymphomas. Selective transcriptional inhibitors may be useful in the treatment of these diseases. Aureolic acid antibiotics, like mithramycin (Mtm), are interesting lead compounds for new drug development in light of their ability to bind to GC-rich sequences in DNA and inhibit preferentially transcription of Sp1-regulated genes.

Methods: New Mtm analogues have been generated by genetic manipulations of the Mtm biosynthetic pathway in the producing microorganism S. Argilaeus. The transcriptional inhibitory activity of the compounds was evaluated using luciferase reporter assays and RT-PCR. The antiproliferative activity of selected Mtm analogues (SDK and SK) in human lymphoma (Raji, Karpas 422, Jeko-1) and leukemia (HL-60) cell lines was evaluated using a colorimetric assay.

Results: Our screen identified two compounds, SDK and SK, with improved activity compared to Mtm. Mtm, SK and SDK inhibited growth of all of the cell lines. SDK had the greatest anti-transcriptional activity and growth inhibitory activity. SDK and SK downregulated multiple genes involved in cell proliferation, apoptosis and angiogenesis with high degree of selectivity toward Sp1-regulated genes.

Conclusions: These studies indicate that aureolic acid antibiotic derivatives are active against human lymphoma cells. In addition to their anti-proliferative activity, the ability of these compounds to modulate expression of multiples cancer related genes may open new avenues for the treatment of lymphomas.
MOTEXAFIN GADOLINIUM (MgDOTA) CLINICAL RESULTS WITH A NOVEL TUMOR-SELECTIVE AGENT THAT INDUCES OXIDATIVE STRESS IN LEUKEMIA AND LYMPHOMA

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Background: MgDOTA is a synthetic expanded porphyrin that selectively accumulates in tumor cells and oxidizes intracellular metabolites to generate reactive oxygen species. Using gene expression profiling, we found that various, hitherto unappreciated genes are upregulated in response to MgDOTA, including genes encoding metallothionein, heat shock proteins and heme oxygenase. Furthermore, MgDOTA induces apoptosis in lymphoid and myeloid cells in vitro and in vivo.

Methods: In a pilot phase II, we treated 13 patients (pts) with CLL/SLL with MgDOTA administered at 5 mg/kg/day IV for 5 days q 3 weeks. A phase III trial involved 6 patients with NHL to receive combination Zevalin and MgDOTA. Patients receive MgDOTA days 1 to 4 and days 8 to 11 with standard Zevalin given day 8.

Results: 13 CLL/SLL pts had a median age of 66 yrs (range 54–80) and 4 median prior therapies (range 2–9); 12 pts had fludarabine-refractory disease. 4 pts received grade 2 drug-related non-hematologic grade 3 adverse event (AE). No grade 4 hematologic AE was seen. Evidence of tumor activity was seen in 3 pts including decrease in WBC, nodes and/or splenomegaly. One responding pt during cycle 2 of MgDOTA developed bowel perforation and was found to have massive tumor necrosis of large cell transformation at the perforation site. For the NHL MgDOTA/Zevalin trial, there are 3 evaluable follicular NHL pts with mean age of 58 yrs, each with 3 prior therapies, and 1 resistant to rituximab. No grade 3/4 non-hematologic AE or grade 4 hematologic AE has been seen. All 3 pts obtained complete remission by 1 month. Pt 1 had a local scalp relapse, but systemic disease remains in remission at 15 months. Pts 2 and 3 achieved prolonged remission at 6 and 7 months. MRI of the CNS showed no progression from pt 1 and pt 2’s MgDOTA infusion demonstrated a decrease in MR signal intensity indicating lymphoma-selective uptake of MgDOTA.

Conclusion: MgDOTA is an anti-tumor agent with a novel mechanism of action that appears to have single-agent activity with superiority in heavily pre-treated CLL/SLL, and has induced prompt complete remissions in combination with Zevalin in NHL. These results provide the rationale for ongoing and planned clinical trials studying MgDOTA as a single-agent and in combination with chemotherapy, antibody and radioimmunoconjugate therapy for treatment of hematologic malignancies.

ACTIVE COMBINATION THERAPY OF BORTEZOMIB WITH RITUXIMAB OR ZEVALIN IN A DLBCL MODEL

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Chemotherapy-resistant diffuse large B-cell lymphoma (DLBCL) pose an unresolved clinical problem and most patients die of their disease within months. The goal of this study was to test novel, non-chemotherapy containing regimens for lymphomas, by combining the proteasome inhibitor Bortezomib with Rituximab or Zevalin. Using the t(14;18) DLBCL cell line SUDHL-16 in MTT cell proliferative assays, a clear additive anti-proliferative effect of Bortezomib (0.5 to 3.0 x 10^-9 M) and Rituximab (0.1-0.5 µg/ml) was observed. Apoptosis was investigated as a possible mechanism of this combination effect using TUNEL-assays, flow cytometry staining for Annexin V, and western blotting for the involvement of caspase 3 activation by analyzing PARP degradation. Bortezomib and Rituximab together increased PARP degradation at 3hrs and induced apoptosis. Bortezomib treatment did not alter the CD20 expression levels of SUDHL-16 cells. The combination was evaluated in vivo in a SUDHL-16 xenograft model. 1 Mio SUDHL-16 cells injected s.c. in the flank resulted in local tumor growth within 4 weeks. When tumors became palpable, animals were stratified into 4 different treatment groups of 10 mice each. Mice received i.v. injections of either diltuent, single agent Rituximab, single agent Bortezomib, or the combination of both.

Rituximab was injected i.v. at a dose of 1 mg/mouse once a week. Bortezomib was given i.v. at a dose of 0.01 mg/mouse twice per week. This dose was well tolerated whereas in preliminary tests a higher dose of 0.03 mg/mouse was toxic. All treatment schedules delayed tumor growth compared with controls. After 2 weeks of treatment, Bortezomib reduced tumor progression by 38%, Rituximab by 71%, and the combination treatment by 88%. The tumors of the control and treatment groups did not differ by microscopic morphological appearance. Tumor-derived cells were tested in MTT assays and showed an equal sensitivity to Bortezomib in vitro, excluding the selection of a Bortezomib-resistant phenotype arising within the treatment period. In addition, we combined Bortezomib with radio-labeled anti-CD20 (Zevalin). The combination of Bortezomib and Zevalin was more active than single agent treatments. In summary, we have shown that the combinations of Bortezomib with Rituximab or Zevalin are active and at least additive treatment regimens in a DLBCL model. These data support clinical trials using these combinations; and we speculate that these regimens may be active in lymphomas (for example follicular lymphoma) which are sensitive to CD20 targeted therapies.

BORTEZOMIB (VELCADE) FOR THE TREATMENT OF RELAPSED CLASSICAL HODGKIN LYMPHOMA

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Background: The proteasome inhibitor Bortezomib has demonstrated clinical activity in patients with multiple myeloma and different types of non-Hodgkin's lymphoma. Its activity in patients with Hodgkin lymphoma (HL) is unknown. We have recently reported that bortezomib had a significant activity against HL-derived cell lines in vitro (Ghadge et al, Clin Cancer Res 2004). Based on this encouraging preclinical results, we initiated a pilot study of single agent bortezomib in patients with relapsed classical HL.

Methods: Eligibility: (1) relapsed classical HD with a measurable disease (2) At least 2 prior treatment regimens, (3) Patients with prior autologous stem cell transplant (ASCT) are eligible (4) platelet counts > 50,000/µl and ANC counts of > 1,500/µl, (5) no HIV infection, or CNS involvement with HD, (6) bilirubin < 2 mg/dl and creatinine < 2.5 mg/dl. Patients were treated with 1.3 mg/m2 bortezomib intravenously on days 1, 4, 8, 11 of 21-day cycles in an outpatient setting. After 3 cycles of bortezomib therapy patients were evaluated for treatment response. If there was no evidence of disease progression after 3 cycles of therapy, patients were allowed to receive a maximum of 6 cycles.

Results: 13 patients are enrolled (8 men and 5 women), with a median age of 31 years (range 21 to 74 years). All patients were heavily pre-treated, with a median number of 5 prior treatment regimens (range 2 to 7 regimens), and 12 patients have previously failed ASCT. 12 patients received at least one dose of bortezomib and are evaluable for treatment toxicity. Treatment was reasonably well tolerated with the majority of toxic effects were grade 1 and 2. Two patients had grade 3 dyspnea and one patient had grade 3 neutropenic fever. Prophylactic antibiotics was the most common hematologic toxicity, which frequently caused delayed in therapy. Nadir platelet count below 30,000/µl was observed in 4/12 patients during the first cycle. One patient achieved a partial remission while receiving concurrent low dose steroids and one had a minimal response with improvement in PET scan activity.

Conclusions: Our preliminary data demonstrate encouraging clinical activity of bortezomib in this heavily pretreated patients with classical HL. To better assess the activity of bortezomib, our next study will be Bortezomib plus ESHAP for patients in first relapse, with first cycle of bortezomib given alone.

THE IMPACT OF POST-THERAPY CLEARANCE OF IODINE-131 TOSITUMOMAB ON DISEASE-FREE SURVIVAL AND TOXICITY

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Introduction: The dose limiting toxicity of BEXXAR® (Tositumomab and Yttrium-90 Tositumomab, USA) is hematological. As the inter-patient differences have been observed in the in vivo clearance of 131I labelled anti-CD20, a pre-treatment dosimetry phase is required to determine individual residence times to be used in prescribing treatment.
The aim of this study was to examine the impact of any clearance related deviations from the prescribed dose on outcome and toxicity.

Methods: Twenty-nine patients with non-Hodgkin lymphoma received 185MBq (5mCi) $^{131}$I tositumomab for dosimetry during 3 multi-centre clinical trials. The therapeutic activity required for a total body dose of 750mGy (24 patients) or 650mGy (platelets<150,000/mm$^3$; 5 patients), was calculated using the measured residence time and body mass. Total body clearance was monitored during the week after treatment and used to compare the residence time with that from the dosimetric phase.

Results: In all cases, the total body clearance followed a single exponential ($t = 0.98$). A wide range of residence times (TRES) was observed (56.8 to 120.5 h, a range of 63.7 hours), with up to 17% intra-patient variation between dosimetric and therapeutic TRES measurements. Using the post-therapy TRES and actual administered activity, total body doses were calculated to be within 15% of the prescribed value in 25/29 patients. There was no correlation between the calculated dose received and incidence of grade 3/4 thrombocytopenia/neutropenia or disease free survival.

Conclusions: The inter-patient differences in clearance rates were much greater than any intra-patient variation observed between dosimetric and treatment administrations of BEXXAR. These results emphasise the value of pre-treatment dosimetry in constraining the radiation dose delivered. The intra-patient differences observed may be due to several factors, but had no impact on the immediate haematological toxicity or disease free survival in this group.

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REDUCED COURSE RASBURICASE REMAINS EFFECTIVE IN THE PROPHYLAXIS OF TUMOUR LYYSIS SYNDROME IN AT RISK PATIENTS WITH HIGH GRADE NON HODGKINS LYMPHOMA

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Rasburicase (Fasturtex, Sanofi-Aventis) is recombinant urate oxidase, licensed for the prevention and treatment of tumour lysis (TL) syndrome. It is conventionally given at 0.2 mg/kg for 5 to 7 days. We carried out a single centre retrospective audit of Rasburicase use in patients with NHL at high risk of TL (bulky, rapidly proliferating, treatment sensitive tumours, especially those with existing metabolic disturbance or renal insufficiency). 33 patients (median age 60.8 yrs - range 14.5-77.9y) 3 Lymphoblastic lymphoma, 19 Diffuse large B cell NHL (DLBIC), 5 high grade T cell NHL, 3 mantle cell NHL, 2 Anaplastic NHL, 1 Burkitts NHL, were included. There were 22 new diagnoses, 10 relapsed patients and 1 treatment refractory patient. Median pre-treatment uric acid was 0.45 mmol/l (range 0.21-0.89) with 12 values greater than sex adjusted upper limits of normal. Median LDH was 1056 IU/l (range 342-5557). Pre existing renal impairment (serum creatinine greater than age adjusted upper limits) was present in 8 patients. All patients received disease appropriate intra venous chemotherapy regimens of curative intent. Rasburicase was given at 0.2 mg/kg on the day of treatment initiation (1 day prior in 7 patients). Patients received hydration but no allopurinol or urinary alkalinisation. The duration of treatment was 1 day for 9 patients, 2 days in 15 patients and 3 days for the remaining 19 patients. 1 patient developed mild hypertension immediately following infusion, otherwise there were no side effects. No patient had a delay in receiving chemotherapy. As defined by the Cairo Bishop Grading Classification, 1 male patient (DLBIC NHL, stage IVB, LDH 4000, urate 0.8, renal insufficiency) developed grade II TL which responded to treatment (three days Rasburicase in total) and did not require dialysis. The remaining 32 patients did not develop TL. There was a rise in phosphate above normal limits in 10 patients and a fall in calcium below normal limits in 4 patients neither of which required therapeutic intervention. In 6 of 8 patients with pre-existing renal impairment there was an improvement in serum creatinine. The remaining patients whose renal function deteriorated both died of early sepsis with no evidence of TL. In summary: in those patients judged to be at risk, Rasburicase remains effective in the prevention of TL when used over a shorter duration than conventionally recognized.
Results: CR was achieved in 17/29 patients (59%). One child is still on treatment. 12 PR children were irradiated. Site of irradiation was the mediastinum in 22/29 patients (median 6 months; range 3–18 months). Five patients relapsed: 2 after CT alone, and 3 after CT and RT (median time 12 mos). For the two children who relapsed after CT only, the site of relapse occurred in areas previously uninvolved in a child and in children involved at diagnosis in the other. For the 3 pts who relapsed after CT + RT, the relapses occurred in previously involved areas, two within the radiation field, and one out. 4/5 relapsing patients are in 2R due to second line treatment (3 including autologous stem-cell transplantation) and one is alive with progressive disease. Late morbidity is still of major concern, and patients are carefully monitored.

Conclusions: Preliminary results suggest that a significant number of children with HD might be cured with CT alone. RT consolidation may produce the best results in selected patients and the challenge is to select the patients who require RT.

LONG-TERM OUTCOME OF THE AIEOP LN92-92 PROTOCOL FOR THE TREATMENT OF PEDIATRIC ANAPLASTIC LARGE CELL LYMPHOMAS

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Background: Different therapeutic approaches have been used for the treatment of anaplastic large cell lymphomas (ALCL). Given the need for a homogeneous approach to such an infrequent disease, the Italian Association of Pediatric Hematology and Oncology (AIEOP) in 1992 initiated an AIEOP national trial based on a leukemia-like treatment.

Objectives: To enrol all newly diagnosed ALCL patient aged 0–15 years in a national protocol for the diagnosis and treatment of ALCL, with the aim of improving the outcome of patients who were previously treated according to single institution policies and to implement central review of the diagnosis.

Patients and methods: From January 1993 to October 1997, 34 eligible patients were prospectively treated in the AIEOP LN92-92 protocol (18 M/16 F; median age 12 years). Therapy was based on a modified LSA2-L2 protocol with a total duration of 24 months for any stage of disease. CNS prophylaxis (i.e. triplets) was delivered to all of the patients. RT was administered only if residual tumor persisted after the Consolidation phase. Patients were staged according to the St. Jude’s classification system.

Results: 10 patients had localized stage disease; 17 were stage III and 7 stage IV. Nine patients had LDH serum levels >300 IU/L. Mediastinum was involved in 47% of the cases; lung in 6%; isolated nodes in 24%; skin or subcutaneous nodes in 12%; CR was obtained in 88% of the patients; in 3 after RT for a residual mass. OS at 10 years was 85% and EFS 65% with a median follow-up of 8.4 years. There were 3 disease progressions and 8 relapses; 3 of them were rescued by second-line therapy. One child died of sepsis during the Induction phase. No other toxic deaths were observed. Centralized review of the diagnosis was accomplished in 85% of the cases.

Conclusion: the AIEOP LN92-92 protocol was well tolerated. Long term outcome was comparable to more aggressive, yet shortest, chemotherapy regimen.
TREATMENT OF HODGKIN DISEASE WITH UNFAVORABLE PROGNOSIS IN CHILDREN IN KRYGYZSTAN

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Introduction: To achieve improvement in the treatment and survival of children with Hodgkin disease with unfavorable prognostic factors of risk with B2,3A,3B,3B and 4 stages of disease effective protocols of treatment were introduced. Usage of these protocols helped achieve long-term survival rate of children with Hodgkin disease.

Methods: There were 56 children with Hodgkin disease 28b,3A,3Bb and 4 stages of disease. Unfavorable prognostic factors include: puberty, age, male sex, affection of mediastinal lymph node above 8 cm, genelicized affection of all peripheral lymph groups, skin affection, presence of intoxication symptoms and "biological" activity. All patients received treatment according to protocols OPEA, BEACOP, IEP, OPEA that included 6 cycles of induction chemotherapy, local-and-regional radiotherapy only on the affected areas. The treatment was held from 1996 till 2004.

Results: On the background of induction chemotherapy the complete remission was achieved in 53 patients (94.6%). 46 among 53 patients had been under medical observation and examination for 5 years, 5 nonrelapse survival rate is 95.6% and 5-year survival rate among these patients is 96%. Treatment results for children with unfavorable factors in prognosis were improved by 60% in comparison with methods and treatment protocols used before.

Conclusions: Introduction of intensive chemotherapy protocols for the period of induction therapy increased the survival rate among children with Hodgkin disease with unfavorable factors in prognosis where 5-year survival rate is 96% which is 50% higher than prior to the use of effective protocols.

PEDIATRIC HODGKIN DISEASE OF PRESCHOOL AGE IN TURKEY

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Introduction: It is known that Hodgkin’s disease (HD) in children of preschool age is rare in Western countries. However, this lymphoma may occur in very young children in Turkey. The purpose of this study is to review HD cases of preschool age (under 6 years) in Pediatric Oncology centers in Turkey and to analyze clinico-epidemiologic characteristics and some labora-

tory findings of HD cases of preschool age observed in our center.

Methods: HD in 5 major Pediatric Oncology centers were reviewed. The epidemiological, clinical and histologic characteristics of 54 biopsy proven HD in children under 6 years of age from our center were retrospectively analyzed. The association of EBV with HD was studied by “acrolog” (51 cases) and “immunohistochemical” methods (LMPI) in 34 patients. T-cell immunity and trace elements, mainly zinc and copper were also studied in some cases.

Results and Conclusion: There were 1142 HD cases in five Pediatric Oncology Centers and 382 of them were under six year of age (33.4%). Analysis of 54 HD of preschool age group in this study represented 27% of 250 biopsy proven HD cases observed in our center. The majority of the patients (76%) were male and 79.1% of them had advanced clinical stages (st III and IV) associated with high percentage of MC histologic subtype (60%). EBV positivity was found to be 100% and 60% respectively. T-cell humoral deficiency and chronic Zn deficiency were additional findings found in these patients. Further studies on environmental factors are needed and future use of vaccine against EBV may be considered for prevention of early EBV infection.
13. Miscellaneous

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GEMCITABINE, DEXAMETHASONE AND CISPLATIN (GDP) SALVAGE CHEMOTHERAPY IS A DOMINANT ECONOMIC REFRACTORINESS WHEN COMPARED TO MINI-BEAM PRE-AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR RELAPSED OR REFRACTORY HODGKIN’S LYMPHOMA
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Background: ASCT is the standard of care for relapsed or refractory Hodgkin’s Lymphoma (HL) but the optimal salvage chemotherapy (CT) is not known. We have previously demonstrated that GDP CT results in a superior progression free survival post-ASCT when compared to mini-BEAM (MB) CT in a retrospective analysis of a median follow-up of 1.8 years (ASCT vs mini-BEAM 905). A cost analysis was performed to determine if GDP was a more cost-effective salvage CT than MB.

Methods: A decision analysis tree summarizing clinical outcomes for 2 years following salvage CT for HL was built using TreeAge Pro 4.0 (TreeAge Software Inc. MA). The decision node compared salvage CT with outpatient GDP vs inpatient MB. Both arms included the costs of salvage CT (including drug costs, drug administration and hospital admission), second line salvage CT, PSL mobilization (including costs of apheresis, mobilizing CT and G-CSF), subsequent ASCT and palliative chemotherapy. Radiation therapy costs were not included. Costs were calculated in Canadian dollars and were obtained from clinical data at Princess Margaret Hospital. Treatment outcome probabilities were obtained from local clinical experience. A utility analysis could not be performed due to a lack of measured utility data in pts with relapsed HL.

Results: Baseline analysis indicated that salvage CT with GDP was a dominant strategy compared to MB, with a higher proportion of patients successfully transplanted (70.9% vs. 29.6%) and lower average costs (CS 78,200 vs. CS 82,900). One-way sensitivity analysis varying the cost of MB indicated that both strategies had equivalent costs at a MB cost of CS 5,000 (baseline cost CS 9,900). Varying the probability of remission after GDP CT prior to ASCT did not influence costs for the GDP strategy.

Discussion: GDP is a dominant strategy, being both more effective and less costly than MB. The lower costs are mainly due to avoidance of hospitalization and lower PSL mobilization costs. Our analysis suggests these results are robust and are not influenced by the effectiveness of GDP compared to MB.

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ARABIC-VERSION EORTC-QLQ C30 (+30): A PILOT STUDY AMONG ADULT AEGYPTIAN NHL PATIENTS
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Purpose: A pilot cross-cultural study to test the validity and reliability of the Arabic-version EORTC-QLQ C30 (+30) questionnaire as a tool to evaluate health-related quality of life (HRQOL) in reproducible sample of adult Egyptian cancer patients.

Methods: 100 Egyptian NHL patients (18–60 y) attending NEMROCK and Beny Swel cancer centers in Egypt, 50 male and 50 females, during therapy with 6 cycles of CHOP regimen or within 3–6 months follow up. The EORTC Arabic-version questionnaire and a user acceptability checklist were completed by the patients in outpatient clinic, then during the same visit an observational checklist was completed by the coordinator physician in addition to the sociodemographic data (age, gender, marital status, residence, education and employment).

Results: Mean age was 41.5 y, 53% of patients were married, 37% unemployed vs 20% university graduate, 47% unemployed, 18% trade workers and 32% specialized employees. Women reported better functioning while men had more symptoms (P<0.05). University graduates and specialized employees had higher scores of global QOL (P=0.032). Patients under therapy reported less emotional problems vs those under follow up (P=0.001). The M version was able to express the effect of cancer and treatment on QOL of 85.5% of cases, however 64% found that Q n r. 7 and Q n r. 23 being the most confusing with the words (activities and irritable) being wrongly translated in arabic, 94% needed the scale in arabic, 71% of patients needed >30 minutes to complete the questionnaire with 56% being reluctant or overloaded.

Conclusion: The findings in our pilot study suggested that Arabic-version EORTC-QLQ questionnaire can work efficiently among Egyptian cancer patients with reliability and validity. We recommend its use in Egypt as well as other Arabic-speaking countries.

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FATIGUE INTERFERENCE WITH QUALITY OF LIFE (QOL) IN ADVANCED NON-HODGKIN’S LYMPHOMAS (NHL)
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Introduction: Fatigue is one of the most distressing symptoms in patients with lymphoid malignancies. The effect of fatigue severity level on a patient’s QOL in new NHL patients is under-reported. Understanding of fatigue interference with a patient’s QOL provides the basis for better management of NHL patients. The goal of research was to study fatigue prevalence and severity in new patients with advanced NHL and to investigate QOL impairment depending on fatigue severity level.

Patients and methods: 122 new NHL patients—60 aggressive NHL, and 62 indolent NHL (stages IIIB-IV, male/female 60/62; mean age 60.03) were enrolled in the study. SF-36 and Brief Fatigue Inventory were used for QOL assessment. QOL impairment was evaluated on the basis of integral QOL index. The following grades of QOL impairment as compared to a population norm (PN) were used: mild (25% decrease from a PN), moderate (25–50% decrease), severe (50–75% decrease) and critical (>75% decrease).

Results: Fatigue was observed in 77% of patients: 91.8% aggressive NHL; 62.9% indolent NHL. 36.5% of patients (65% aggressive NHL, and 9.7% indolent NHL) experienced severe (7–10) fatigue, and 21.3% (25 aggressive NHL, and 17.7% indolent NHL) – moderate (4–6) fatigue. Severe fatigue resulted in critical QOL impairment in the majority of patients. The half of the patients with moderate fatigue also had critical QOL impairment. The majority of patients with mild fatigue had no QOL impairment (87%). Patients with no fatigue (6.3% aggressive NHL and 37.1% indolent NHL) also exhibited no QOL impairment (89.3%).

Conclusion: The majority of new patients with advanced NHL experience severe, and moderate fatigue which in most cases results in critical QOL impairment as compared to a population norm. Assessment of fatigue severity and grading of QOL impairment is worthwhile to provide an adequate management of NHL patients.

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QUANTIFICATION ANALYSIS OF IRREGULARITY IN CONTOURS OF LYMPHOID CELLS FOR PATIENTS WITH HODGKIN’S DISEASE AND NON-HODGKIN’S LYMPHOMAS
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Introduction: Cancer is often characterized as a chaotic, poorly regulated growth. Malignant lymphomas are characterized by the neoplastic proliferation of lymphatic cells carrying clonal chromosome abnormalities. The genomic chaos may mask a unifying chromosome abnormality. Large-cell lymphoma has insertion of genomic material into the anaplastic large-cell lymphoma locus indicated by irregularity in contour (spatial chaos). Atypical change of lymphatic cell shape and their structure is accompanied by increase of spatial chaos. The objective of the paper was analysis of irregularity in contours of lymphoid cells for patients with malignant lymphomas.

Materials and Methods: We have studied 30 patients with lymphadenitis, Hodgkin’s and non-Hodgkin’s lymphomas. All groups consisted of male and women with middle age of 32 ± 2 for. 2. For this studies digital microscope Axioskop 40 (ZEISS, Germany) has been used. Quantification analysis of

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irregularity contours of lymphoid cells has used the irregularity parameter. The principle and-titative estimation of the parameter irregularity is based on a method of pseudo-phase space. It has allowed distinguishing statistically randomness in divergence of shapes of cells.

Results: The increase of irregularity parameters for malignant lymphomas in researched groups was next: Hodgkin’s disease (lymphatic cells) → non-Hodgkin’s lymphoma with small B-cell → Hodgkin’s disease (Hodgkin’s and Sternberg cell) → non-Hodgkin’s lymphoma with B-cell lymphoma with irregular nucleus contours → lymphadenitis → non-Hodgkin’s large B-cell lymphoma. The contours of non-Hodgkin’s large B-cell lymphoma had the largest irregularity. It was 98.4% (t < 0.05) larger than in patients’ non-Hodgkin’s B lymphoma small cells. As it is well known the patients with B-cell lymphomas has the worst survival prognosis for non-Hodgkin’s lymphoma.

Conclusion: The obtained results of study of spatial chaos in contours of lymphoid cells have been focused on the identification of probable mechanisms of chaos in ethiology of cancers and have also proved to be useful clinical markers for computer diagnostics and prognosis of malignant lymphomas.

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THERAPEUTIC INHIBITION OF HEAT SHOCK PROTEIN-90 FUNCTION IN HODGKIN LYMPHOMA

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Background: HSP-90 chaperones several cellular proteins that regulate tumor cell survival and proliferation, making it a good target for cancer therapy. HSP-90 client proteins include AKT and ERK survival proteins. Inhibition of HSP-90 has been reported to induce cell death in several tumor types. However, the role of HSP-90 in regulating Hodgkin lymphoma (HL) cell survival is currently unknown.

Methods: We examined HSP-90 expression in primary and cultured HL cells by Western blot and Immunohistochemistry. The effect of HSP-90 small molecule inhibitor was examined by the MTS cell proliferation assay, Annexin V binding assay, and Western blot.

Results: HL cell lines and primary HRS cells expressed high levels of HSP-90 compared to reactive lymphocytes. 17-AAG inhibited HL cell proliferation and induced apoptosis in a dose and time dependent manner.

Conclusion: Induction of apoptosis was associated with activation of the intrinsic pathway as evident by caspase 9 cleavage, and was partially reversed by the pan-caspase inhibitor ZVAD-fmk. Furthermore, 17-AAG depleted HL cells of AKT and ERK survival proteins, and decreased cellular contents of cyclin D. HSP-90 and HSP70 levels were upregulated by CD30L and CD40L in HL cells.

Conclusions: HSP-90 is overexpressed in HL cells. Inhibition of HSP-90 function by 17-AAG induced apoptosis by depleting survival proteins and activation of caspase-9. Our results suggest that 17-AAG may have a therapeutic value in HL. To examine the possibility, we are currently conducting a phase II study of 17-AAG in patients with relapsed HL.

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THYROID AUTO-ANTIBODIES ARE COMMON IN PATIENTS WITH WALDENSTROM’S MACROGLOBULINEMIA

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We and others have observed an increased incidence of thyroid abnormalities in patients with Waldenström’s macroglobulinemia (WM) along with their first degree relatives. Auto-antibodies have frequently been reported in WM patients, though the presence of anti-thyroid antibodies remains to be defined. As such, we sought to elucidate the presence of thyroid auto-antibodies in WM patients. We therefore evaluated 97 patients with the consensus panel definition of WM for the presence of anti-thyroglobulin (TgAb), and thyroperoxidase antibodies (TPOAb). The median age for these patients was 63 (range 42 to 84); 27 (28%) of these patients were previously untreated, and 22 (23%) patients had a familial history of WM or a related B-cell disorder. A two-site commercial chemiluminescence immunoassay was used for the measurement of TgAb and TPOAb in human serum. Fourteen of 97 (14%) patients demonstrated thyroid auto-antibodies to either Tg, TPO or both. Six and 13 patients had TgAbs and TPOAb, respectively. Five of 6 patients with TgAbs were also TPOAb positive. Interestingly, 10 (54%) of the patients with thyroid autoantibodies had no clinical history of thyroid abnormalities. Among those patients with TgAbs, half had a history of thyroid dysfunction including one patient who had a history of thyroid carcinoma. No differences in thyroid autoantibody presence was observed when age, sex or treatment status was evaluated. Only one of the 14 patients with anti-thyroid antibodies evaluated had a familial history of a B-cell disorder. Thyroglobulin immunonegativity used to distinguish heavy chain constant domains failed to demonstrate that the anti-Tg antibodies were components of the monoclonal gammopathy for two patients with TgAbs. These studies demonstrated that these studies demonstrate that anti-thyroglobulin and anti-thyroperoxidase antibodies are commonly present in patients with WM but not part of the monoclonal gammopathy. Ongoing studies are elaborating the basic mechanisms for these findings.

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A COMPARISON OF IN-HOUSE AND BIOMED-2 PRIMERS IN THE POLYMERASE CHAIN REACTION FUNCTION OF T-CELL RECEPTOR Jγ GENE REARRANGEMENTS IN PARAFFIN EMBEDDED MATERIAL IN ROUTINE DIAGNOSTIC PRACTICE

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Introduction: To compare the use of in-house primers with commercially available Biomed-2 primers (Invitrogen) in the assessment of T-cell receptor (TCRγ) chain gene rearrangements by polymerase chain reaction (PCR) in paraffin embedded material in routine diagnostic practice.

Methods: DNA extracted from paraffin blocks of 32 cases of T-cell lymphoma and a control group of 24 reactive cases was amplified by PCR using in-house TCRγ primers (Oswel). The reaction conditions were as described by McCarthy K.P et al with slight modification. 18 T-cell lymphoma and 21 reactive cases were also analysed using Biomed-2 primers for TCRγ. All PCR products were evaluated by a genetic analysis system (Beckman-Coulter CEQ8000).

Results: Of 32 cases of T-cell lymphoma tested using in-house primers, 16 (50%) were monoclonal by PCR, 10 (31%) were polyclonal and no amplification product was detected in 6 (19%). 18 T-cell lymphomas were analysed using Biomed-2 primers. A monoclonal reaction was identified in 13 (72%) polyclonal in 2 (11%) and 3 cases (17%) did not amplify. Using Biomed primers, T-cell monoclonality was detected in 11 cases of T-cell lymphoma in which in-house primers resulted in a polyclonal reaction. The 2 polyclonal cases in the Biomed analysis were prior treatment status.

Conclusions: Biomed-2 TCRγ primers are superior to in-house primers in detecting T cell monoclonality by PCR in paraffin embedded routine diagnostic material. In our study, 3 false positive reactions were detected with the Biomed method illustrating the need for careful interpretation of PCR results in conjunction with clinical and other pathological features.

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IFOSFAMIDE, ETOPOSIDE AND G-CSF AS EFFECTIVE MOBILIZING REGIMEN IN LYMPHOMA PATIENTS IN ORDER TO ACHIEVE A HIGH NUMBER OF PROGENITOR CELLS FOR PURGING IN VITRO OR POOR MOBILIZERS

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Introduction: In heavily pre-treated patients and known poor mobilizers alternative approaches including combination of chemotherapy and G-CSF need to be considered. We present our results using the combination of ifosfamide, etoposide and G-CSF in this setting.
Patients and methods: At present, we have treated 26 patients (median age 43) with the iFSFamide + etoposide + G-CSF combination. The diagnoses are as follows: follicular lymphoma (4), diffuse large B-cell lymphoma (4), T-cell lymphoma (3), Hodgkin's disease (1) and solid tumor (1). Nineteen patients were in CR whereas 7 patients were not in remission at time of mobilization. Thirteen patients had received more than 3 lines of therapy, 16 patients had received prior therapy with fludarabine and 6 patients had failed the first mobilization. The mobilization regimen consists of iFSFamide (10/ 4176 total dose, by continuous infusion for 3 days) and etoposide (900 mg/m² total dose) plus G-CSF (beginning on day 5 and continuing until the completion of leukapheresis).

Results: The median interval between chemotherapy and apheresis was 19 days (range 13–26) and the median of days with G-CSF was 15 (range 10–23). The median number of apheresis was 1 (range 1–3) to collect a median of 6.3 × 10⁹/kg CD34+ cells (0.64%); only 1 patient needed 3 apheresis to collect more than 3 × 10⁷/kg CD34+ cells and 2 patients failed to achieve more than 3 × 10⁷/kg CD34+ cells. The major complication was febrile neutropenia related to the central venous line and all patients responded to empirical antibiotic therapy and no toxic deaths were observed.

Conclusion: The administration of iFSFamide and etoposide is an effective mobilization regimen associated to a high number of progenitor cells collected with a low number of apheresis, even in heavily pre-treated patients and poor mobilizers.

RISK FACTORS FOR THROMBOEMBOLIC EVENTS WITH NEORECORMMON® (EPOETIN BETA): A META-ANALYSIS OF CONTROLLED CLINICAL TRIALS
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Introduction: Patients treated with recombinant erythropoietin can experience a slightly higher frequency of thromboembolic events (TEE). The reason for this is unclear and a meta-analysis was performed to assess relationships between haemoglobin (Hb)-related parameters and risk of TEE.

Methods: Data were pooled from nine randomised, controlled (placebo or standard care) trials of epoetin beta in patients with cancer. All TEEs were assessed during treatment and for a further 28 days. Univariate Cox regression analyses were performed to assess if Hb-related parameters were correlated with thromboembolic risk. All randomised patients were included in the analysis. Patients receiving epoetin beta were eligible if they received ≥1 dose.

Results: In total, 1413 patients were included (epoetin beta, n=800; control, n=613). Both groups were similar demographically, each having a mean baseline Hb of 9.9 g/dL. During treatment, maximum Hb achieved was greater in the epoetin beta (12.5 g/dL) than the control (11.5 g/dL) group. In the epoetin group, no significant change in relative risk of TEE was found for the majority of Hb-related measures. An inverse association between increased Hb-AUC (mean 1.02 ± 1.5) or Hb increase up to Week 4 (mean 0.84 ± 3.4), and incidence of TEE, reflected a significantly reduced relative risk of TEE for both parameters (0.73, P=0.0164 and 0.72, P=0.0325, respectively). Notably, in line with European Organisation for Research and Treatment of Cancer (EORTC) guidelines, treatment at a baseline Hb of <11 g/dL or to a Hb level up to 13 g/dL was not significantly correlated with increased TEE.

Conclusions: Epoetin beta therapy is not associated with a significantly increased thromboembolic risk with regard to Hb-related parameters, suggesting epoetin beta can be used according to EORTC guidelines.

CBP/PAG EXPRESSION IN LYMPHOMA CELLS
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Introduction: The Cbp/PAG membrane phosphoprotein is a ubiquitous Src kinase regulator typically associated with sphingolipid and cholesterol-enriched plasma membrane rafts. Studies with murine T lymphocytes have shown that Cbp/PAG functions as an anti- oncogene by setting the threshold for T-cell activation. The role of Cbp/PAG in B lymphocytes is certainly different and the contribution of Cbp/PAG to the continuing proliferation and resistance to apoptosis of human lymphoma cells remains to be investigated.

Methods: For immuno-histochemistry, the PAG02 and MEM255 mabs were used. The sub-cellular localisation of Cbp/PAG was determined using sucrose gradient centrifugation.

Materials: 97 cases of malignant lymphomas (with the corresponding cell lines) were stained, including 24 diffuse large B cell lymphomas (DLBCL), 25 low grade B cell lymphomas, 8 mantle cell lymphomas, 23 cases of classical Hodgkin's, of which 11 cases with LKCHL lymphoma and 11 cases of lymphocytic predominance Hodgkin's lymphoma (NLPHL). 7/NK, ALK and peripheral T-cell lymphomas were included for comparison.

Results: All B-NHL express Cbp/PAG, with variable numbers of positive cells and staining intensities. Low grade B-NHLs showed the majority of Cbp/PAG-positive cases. Mantle cell lymphoma showed intermediate number and intensities, between low grade B-NHLs and DLBCL. In DLBCL, most cells were moderately to strongly stained and the percentage of positive cells was higher than in low grade B-NHL. HD- and RS cells were positive, showing a variable staining intensity from case to case. 5 cases of multiple myeloma and 7 multiple myeloma cell lines were all strongly Cbp/PAG positive. All B-NHL cell lines contained Cbp/PAG, 80% of which was found in the raft fractions.

Conclusions: Cbp/PAG is expressed in all T and B NHL cells, irrespective of the cell of origin. Blastic cells in high grade lymphomas reproduce bly express more Cbp/PAG. In contrast HD cell lines and HD-cells may express or fail to express Cbp/PAG. Thus, actively proliferating cells (blasts) tend to be strongly Cbp/PAG positive. This suggests that Cbp/PAG is involved in the actively proliferating phenotype of B NHL in contrast to the high expression in resting murine T cells.

EXPRESSION OF P-53/MUT, BCL-2 IN NON-HODGKINS LYMPHOMA PATIENTS
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Non-Hodgkin's lymphomas (NHL) are characterised by differences in types of progression, responses to chemotherapy (CT) and survival. Despite the presence of several prognostic markers, prognosis is difficult in early stage patients to predict the course of the disease.

The aim of the study was to evaluate and estimate the prognostic significance of p-53/mut, bcl-2 expression levels during the course of the disease and to compare it with effects of CT.

85 patients were evaluated, median age was 58 years (age range 34–78).

Results: Biological markers of malignant cells of peripheral blood and bone marrow were investigated immunocytochemically with monoclonal antibodies ("DAKO"). Our data showed the link between the expression levels of these markers and the stage of the disease. The highest levels of these parameters were associated with stages III and IV of NHL. There was a strong link between expression levels of these markers and responses to therapy. Poor response to therapy was associated with highest primary levels of the markers. Incidence of remissions in the group of patients with high bcl-2 (50%) expression levels was 24%, in the group with low expression - 76%. During courses of CT, expression of p-53 and bcl-2 markers decreased. Extent of this decrease correlated with the CT regimen. The most significant decrease was revealed in patients treated with fludarabine, cyclophospham and mitoxantron (VAD) in comparison with FC and leukeran, and CHOP. Patients with primary high bcl-2
and p-53 expression levels after the courses of FCM had higher incidence of complete remissions (56%), than those after standard CT programs. Our findings showed that p-53mut, bcl-2 markers may be used as an additional prognostic factors to predict the course of the disease and response to therapy.

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SCREENING OF ENHANCERS AND INHIBITORS OF COMPLEMENT-MEDIATED CYTOTOXICITY (CDC) WITH RITUXIMAB: SMALL BASIC PEPTIDE, LACTOFERRICIN (LFcIN) ENHANCES CDC ACTIVITY WITH RITUXIMAB

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Introduction: Rituximab has improved outcome for non-Hodgkin’s lymphoma, but approximately 20% of patients do not show good response to the treatment of rituximab. Our purpose is to search for enhancers and inhibitors of CDC with rituximab and to improve the effect of rituximab much more.

Methods: B cell malignant cell lines, Raji, SKW6.4, ARH-77, and Daubi were cultured with or without lactoferrin hydrolytic products (LFH), LFcIN, proparganimum, protamine sulfate, heparin sulfate, and other milk-derived hydrolytic products. CDC assay with rituximab was performed by FACscan and microreader after staining with propidium iodide (PI) and Calcein-AM, respectively.

Results: Raji, SKW6.4, and ARH-77 cells did not show good susceptibility to CDC with rituximab in spite of high expression of CD20. However, Raji, SKW6.4, and ARH-77 cells showed high expression of LFH and its content LFcIN resulted in the significant increase in susceptibility to CDC with rituximab, whereas proparganimum, protamine sulfate, and other milk-derived hydrolytic products did not affect susceptibility to CDC with rituximab. On the other hand, heparin decreased susceptibility to CDC with rituximab.

Conclusions: The major effect of LFH on CDC was LFcIN itself, and it can effectively make susceptibility to CDC increased. The small basic peptide, LFcIN may be useful in improving outcome after the treatment of rituximab. Now we are investigating the mechanism of action for LFcIN in CDC activity.

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MEASUREMENT OF RHAMM/CD168 SPECIFIC RESPONSES IN B-CELL PATIENTS VACCINATED WITH ALLOGENIC DENDRITIC CELLS PULSED WITH TUMOR LYSESATES OR APOPTOTIC BODIES

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Recent developments have sparked new hope for more efficient cancer immunotherapies: the characterization of a large number of tumor-associated antigens (TAAAs), the identification of existing in vitro cellular immune responses to these TAAAs in patients and an increasing understanding of the complex interactions between antigen presenting cells and effector cells (T lymphocytes). Recently we identified the receptor for hyaluronic acid mediated motility (RHAMM/CD168) using the SEREX technique as a TAA and showed tumor-specific humoral immune responses and non-restricted mRNA expression in AML and CML patients. Expression of RHAMM/CD168 was found also in CLL cells and specific CTL response against RHAMM/CD168 could be generated in vitro in CLL patients. The aim of the present study was the assessment of RHAMM/CD168 in B-CLL cells by RT-PCR and measurement of RHAMM-specific responses by ELISPOT in nine B-CLL patients vaccinated with allogeneic dendritic cells (DC) pulsed with tumor lysates or apoptotic bodies. RHAMM/CD168 specific responses were detected in six of nine CLL patients. Four of these six patients tested also positive for HLA-A2. For these four patients, MLPC and subsequently ELISPOT assays were performed to assess the frequency of RHAMM/CD168 specific lymphocytes. In one of these patients enhanced CTL responses against the RHAMM/CD168 peptide R3 were observed after four DC vaccinations. In this patient, a decrease of WBC after DC vaccination could be noted. The disease remained stable till present. No specific CTL response against RHAMM/CD168 could be detected in the other three HLA-A2-positive patients. In one patient neither CTL responses against IMA nor against R3 could be observed. The disease remained stable till present in one patient, in the other ones a progressive increase of the WBC was observed during the immunotherapy with DCs and thereafter. Specific CTLs against RHAMM/CD168 were only detected in one of four RHAMM/HLA-A2 double-positive patients. In this patient transient decrease of WBC after DC vaccines was the most profound. In this patient RHAMM/CD168 might be one of the antigens responsible for induction of CTL.

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THE TISSUE TARGETED CYTOTOXIC PRODRUG, AQA4, PROLONGS SURVIVAL IN LEUKEMIA MODELS

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Introduction: AQA4 (baxoxantrone) is a tissue targeted cytotoxic prodrug that is bioreduced to AQ4, a highly potent DNA topoisomerase II inhibitor. AQ4 has in vitro activity against lymphoma and leukemia cells (i.e. P388, L1210, Namalwa, etc.), accumulates in the spleen, and causes lymphoid tissue atrophy in animals (mice, rats, and monkeys) [Cleland et al., Blood 104, 685A, 2004].

Methods: To further evaluate observed in vitro activity of AQA4, survival was assessed in L1210 and P388 murine leukemia models with varying AQA4 dose regimens along with mitoxantrone and vehicle control groups. Ten DBA/2 mice per group were injected ip with tumor cells (day 0) and treatment was initiated the next day (day 1).

Results: In the P388 model, 60 mg/kg AQA4 q0dx3 (180 mg/kg; 540 mg/m2) provided a 236% survival increase (25.1 ± 4.3 days vs 11.1 ± 0.3 days, vehicle). 2 mg/kg mitoxantrone qdx3 produced a comparable survival benefit (24.2 ± 13.7 days). In the L1210 model, 90 mg/kg AQA4 q0dx3 yielded a 755% survival increase (42.0 ± 17.2 days vs 8.1 ± 1.2 days, vehicle) with 5/10 long-term survivors. Survival increases were comparable to 2 mg/kg qdx3 mitoxantrone (525%; 38.0 ± 21.3 days). Reproducibility of survival benefit was also demonstrated in a second set of studies. A comparison of dose regimens suggested that high initial exposure to AQA4 fractionated over a short time (1-3 days) was more effective than extended dosing intervals (e.g. q4d) in these models. The mechanism of AQA4 activity in these cell lines is currently under investigation.

Conclusions: Significant in vivo activity of AQA4 was observed in standard models of leukemia and doses were well tolerated. Human safety data from an ongoing Phase 1 study indicate that single doses up to 447 mg/m2 are well tolerated [Benghit et al., J. Clin. Oncol. 22 (145), 2001, 2005; Benghit et al., ASCO 2005]. Therefore, it may be possible to achieve efficacy in lymphoma and leukemia patients at safe doses of AQA4.

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ANTI-CARDIOLIPIN ANTIBODIES IN NON-HODGKIN LYMPHOMA PHAOMAS

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Malignant lymphomas are frequently associated with abnormal autoantibodies and occasionally autoimmune diseases. The effector arm of the immune system is affected as well as the self non-self recognition which may lead to the malignant disease. The recent advances achieved with targeted immunotherapy highlight the importance of the self immune system, if appropriate aid is delivered by means of targeting the tumor cells. Several autoantibodies are found at an increased frequency in lymphomas. Amongst them antiphospholipid antibodies bear special role as they are associated with increased chance of venous thromboembolism during chemotheraphy. However, their appearance may coincide with increased apoptosis, as the normally hidden negative charged phospholipids become visible during apoptosis. We measured anti-cardiolipin IgG and IgM auto-antibodies with ELISA method in 31 lymphoma patients (15 male and 16 female).
female patients, mean age at diagnosis: 53.4 (20–80). The pre-chemotherapy value of both IgG (5.72 μg/mL) and IgM (5.58 μg/mL) in the patients who achieved a complete response with conventional chemotherapy. Their initial IgG isotype was lower than the rest of the patients (n = 12), (6.28 μg/mL vs. 7.32 μg/mL, not significant). This difference is also present in the case of IgM isotype autoantibodies (4.84 μg/mL vs. 6.38 μg/mL, P < 0.05). Both autoantibodies decreased by chemotherapy treatment, reaching a close to normal value by the end of therapy. Our results highlight that the increased amount of anti-cardiolipin antibodies are present, and the may indicate that a spontaneously increased apoptosis is present even before lymphoma treatment.

DEVELOPMENT OF SEVERE INFECTIONS IN NON-HODGKIN LYMPHOMA PATIENTS TREATED BY POLYCHEmOTHERAPY

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Background: Severe, life-threatening infections might develop on the ground of intravenous immunodeficiency states caused by cytostatic treatment, which results especially high therapeutic costs.

Our aim was to determine how many non-Hodgkin lymphoma (NHL) patients were admitted to our department due to any kind of serious infections in the period of 1999–2003.

Results: We treated 110 NHL patients in this four-year period (59 males, 51 females, mean age: 56.4 years). Altogether they received 683 cycles of polychemotherapy treatment. Hospitalizations due to infections were needed in 37 cases which mean 5.4% of all treatments. Of them, severe generalized infections were diagnosed by 24 patients (65%), when colony stimulating factor therapy had to be administered because of severe neutropenia. We lost 4 patients, but the causes of their death were not just infections but also progressive lymphoma. In 13 cases (35%) we faced mild localized infections. Bacteria could be cultured from blood samples in 13 cases, we found S. aureus as the most frequent pathogen. We used meropenem, ciprofloxacine or the combination of ceftriaxone and amikacine as the first-line therapy of severe generalized infections.

Conclusion: Recently, with the administration of modern antibiotics and colony stimulating factors, the mortality of infections caused by polychemotherapy has decreased to a minimal level in our department.

POLYMORPHISMS IN FCGRII A ARE GENETICALLY LINKED TO FCGRII A, AND SUGGEST A PRIMARY PREDICTIVE ROLE FOR FCGR II A POLYMORPHISMS IN PREDICTING RITUXIMAB RESPONSES

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Polymorphisms in position 158 and 48 for FCGRIIA131 and 158 for FCGRIIA receptors may influence clinical responses to rituximab in patients with indolent NHL. In recent work, we demonstrated that the expression of at least one Valine (V) at FcγRIIIA-158 and Leucine/Histidine (L/H) at FCGRIIA-48 was associated with higher response rates to rituximab, though prior FM for FCGRIIA-158 over 48 polymorphisms for determining rituximab response was observed on the basis of genetic linkage (J Clin Oncol 23, 2005). As part of these studies, we sought to determine if polymorphisms in FcγRIIA were linked to FCGRIIA since higher response rates have been observed in patients who express Histidine/ Histidine (H/H) in FCGRIIA-131. As such, we performed a sequence analysis of the entire coding region of FcγRIIA and FCGRIIA in 40 healthy donors. Two common polymorphisms were again observed for FCGRIIA (at positions 48 and 158). For FCGRIIA-48: Leucine/Leu-
cline (L/L); Leucine/Arginine (L/R); and Leucine/Leucine (L/L) and for FCGRIIA-158: Phenylalanine/Phenylalanine (F/F); Phenylalanine/Valine (F/V); and Valine/Valine (V/V). Two common polymorphisms were also observed for FCGRIIA (at positions 27 and 131). For FCGRIIA-27:

Glutamine/ Glutamine (Q/Q); Glutamine/ Tryp- tophan (Q/W); and Tryp- tophan/Tryp- tophan (W/W) and for FCGRIIA-131: Arginine/Arginine (R/R); Histidine/Arginine (H/R); and Histidine/Histidine (H/H). A linkage between polymorphisms in FCGRIIA-131 and FCGRIIA-158 was detected as 10/11 (91%) subjects with FCGRIIA-131/H/H expressed at least one Valine at position FCGRIIA-158, whereas only 1/11 (9%) subjects with FCGRIIA-158/F/F expressed FCGRIIA-131/H/H (P < 0.009). We also observed linkage between FCGRIIA-27 and FCGRIIA-158; all subjects who expressed at least one tryptophan at FCGRIIA-27 also expressed at least one Valine at FCGRIIA, whereas all subjects who were FCGRIIA-27/Q also were FCGRIIA-48/L and FCGRIIA-158/F/F (P < 0.0007).

These studies demonstrate linkage between polymorphisms at FCGRIIA and FCGRIIA, and suggest that polymorphisms in FCGRIIA-158 may primarily serve to predict rituximab response.

PRIMARY GASTROINTESTINAL NON-HODGKIN'S LYMPHOMAS: A RETROSPECTIVE REVIEW

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Introduction: The aim of this study was to analyze clinical presentation, prognosis and outcome in patients with PGIL.

Methods: The study presents 16 years of experience (1988-2004) in 82 patients with histological proved PGIL.

Results: 36 m and 46 w; median age 51yrs. The site of presentation of PGIL was in the stomach (63%) followed by small intestine (PSIL) (21%), colorectal: PCRL (11%), liver (3.7%)and pancreas with 1.2%. The Ann Arbor stage I=43%;II=37%; III=16%; IV=0%. PS was 0 or 1 in 86%. Abdomen pain (60%), weight loss (35%), NV (32%), fever (27%), loose stool (13%) and anaemia (10%). WHO classification, 60% were large B-cell. 21.5% were marginal zone B-cell lymphomas of MALT, 0.6% were MCT, 2.4% were Burkitt’s, T-cell lymphomas, 7.3% unspecified and Enteropathy-type, Anaplastic large cell 1.2% each.CHIOP were the most commonly regimen (80%). A CR was noted in 93%, 41%, and 62% in PGL, PSIL and PCRL respectively. Overall two-year survival was 73%, site wise 86% in PGL, 47% in PSIL and 80% in PCRL.

Conclusion: This is first Southeast Asia study of PGIL. Our data corresponds western indicating increased incidence of PGIL, better results, gastric preservation, and frequent use of surgery surgery for diagnosis or relief of symptoms in small bowel or colonic lymphoma. We have reported higher prevalence of DLBC_NHL as compared to western data where MALT-lymphoma is most frequent.

GRADING OF FOLLICULAR LYMPHOMA IN RELATION TO ITS CD20+ BONE MARROW INFILTRATION

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Introduction: Grading of follicular lymphomas /FL/ correlates with prognostic and influences the therapy: G1 and G2 FL represent an indolent disease, FL with G3 are biologically more aggressive and potentially curable. In the study we compared the grade of FL identified in diagnostic, staging and restaging biopsies of patients in a 5-years material.

Methods: Biopsy examination of all the cases included phenotypical and histological analysis according to the WHO classification.

Results: Analysis of 273 biopsies of 161 patients showed that: a/ 111 cases were classified as FL G1 or G2 resp., staging bone marrow biopsies /BM/ of 70/111 cases showed infiltration in 62 cases, features of high grade FL transformation in 4 and in no infiltration in 4 cases; b/ 91 cases represented FL G3a with residual G1 or G2 component. Femoral BMB of 57 examined cases were negative, in 1 case a low grade and in 1 case a high grade FL infiltration was found; c/ 41 cases were classified as "de novo" FL G3b, 30/34 examined BMB were negative and 4/34 BMB were positive due to low grade FL infiltration.

Conclusions: Our data show that majority of low grade FL is disseminated at the time of diagnosis and that some of them (less than 6%) show BM involvement with a blastic transformation. In some of these cases the disease is less advanced without BM infiltration (approx. 6% of all).
In contrast, approx. 3/4 of the cases being considered as primary high grade FL G3b do not infiltrate the BM, however, approx. 1/4 of them show by BM involvement a previously unrecognized low grade FL component. The presented data might contribute to the therapy management of FL and to our understanding of the biology of FL.

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PERIPHERAL T CELL LYMPHOMAS (PTLCS) TREATED WITH PROMACE-CYTARABIN: ANALYSIS OF PROGNOSTIC FACTORS AND SURVIVAL WITH A LONG TERM FOLLOW UP

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Introduzione: PTLCS represent 10% to 15% of all Lymphomas. They have post-thymic lymphocytes orgini and mature T immunophenotype, are aggressive, with low chemosensitivity. There isn’t any standard chemotherapy regimen specific for PTLCS. We performed a retrospective analysis of a subset of PTLCS reviewed according to the Revised European-American Lymphoma (REAL) Classification and treated with ProMAC-E-Cytaraboin (PC), evaluating the impact of prognostic factors and of treatment on overall survival (OS).

Methods: We analysed 36 patients (pts) with PTLCS, between Jan 1991 to Jan 2000. 19/36 PTLCS unspecified, 18 large T cell non Hodgkin lymphoma (nHL), 2 NK/T cell nHL. PTs characteristics examined were: median age (49 yrs), performance status, stage, constitutional symptoms, Bulky disease, LDH, h2m, Bone Marrow involvement, extranodal involvement, IPI. Treatment regimen was PC (Cyclophosphamide 650 mg/m2, Doxorubicin 25 mg/m2, Cytosine 1.4 mg/m2, Methotrexate 120 mg/m2, on day 8, and Prednisone 60 mg/m2 for 14 days). Median of cycle administration was 6.

Results: 20/36 cycles of chemotherapy were administered. Grade III-IV neutropenia was observed in 28% of cycles. One treatment related death occurred for septic shock. Complete Remissions (CR) were 18/36 (50%), Partial Remissions (PR) 10/36 (28%), Relapses 10/18 (55%). Median Event Free Survival was 26 months (7-48m), 5 years overall survival rate was 45% with a median follow up of 32 months (4-169). Response inversely correlated with: stage-3,4, cutaneous involvement and FS=2.

Conclusions: PC is effective and well tolerated in this poor prognosis subset, but to improve CR rate and OS, new therapeutic strategies, specific for PTLCS, should be investigated in multicentric clinical trials.

RESULTS OF TREATMENT OF HIGH GRADE NON HODGKIN'S LYMPHOMA WITH MCP 842

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Aim: To study the outcome of treatment of marrow negative LL with MCP 842.

Material and Methods: Twenty two patients with LL whose bone marrow was not involved were treated by the authors during the period 1996–2001 with MCP 842. The clinical and treatment details were collected and analysed.

Results: There were 16 males and 6 females. The median age was 22 years (range 15–32). The common presentations were lymphadenopathy, dyspepsia and SVC obstruction. B symptom was present in 8 cases. The mean duration of symptom was 8 weeks. CXR showed massive mediastinal mass in 9. All patients received MCP 842 protocol. Eight patients occurred, 4 mediastinal, 3 in the bone marrow, and 1 had both mediastinal and marrow disease. Fourteen patients are alive in remission. Survival range from 6 months–99 months with the median of 36 months.

Conclusion: MCP 842 is an effective treatment protocol for high grade NHL.

THE ABSOLUTE LYMPHOCYTE COUNT (ALC) IN PERIPHERAL BLOOD BEFORE TREATMENT FOR INDOLENT LYMPHOMA COULD BE A PREDICTOR FACTOR FOR EARLY OVERALL SURVIVAL: A RETROSPECTIVE STUDY FROM MEXICAN TRIAL OMB 01

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Background: Rituximab is one of the most effective single-agent treatments for non-Hodgkin. Several studies suggest that ALC could be a predictor factor for response using rituximab as single therapy but the relationship between ALC before treatment and overall survival (OS) has not been investigated. The purpose of this study was to investigate that issue.

Patients and methods: Trials sites. Patients were enrolled between 2000 year and 2004 year in 13 institutions from Mexico. Evaluation: Classical staging work-up was performed before and after treatment. Treatment: Three arms: Rituximab 375 mg/m2 weekly per 6 doses(R), CNO+R (Cyclophosphamide 750 mg/m2, mitoxantrone 10 mg/m2, vincristine 1.4 mg/m2 on day 1, prednisone 100 mg on 1-5 days, and rituximab 375 mg/m2 every 21 days) and CNO with B. End points: Overall response rate (ORR), OS and event free survival (EFS) depending of ALC (Cut-off 1000 µL/L). Lymphoma classification: according REAL/WHO classification.

Outcome: It was evaluated 126 patients. The Median Follow-up 14 months (range 1–46), median age was 61 years (range 25–86), male 47.6%, Stage III-IV 72.2%, Extranodal infiltration ≥ 2 sites 65.1%, ALC peripheral blood ≥1000µL/L 87.5%, hemoglobin level ≥100g/L 89.7%, Follicular Lymphoma International Prognostic Index (FLIPI) intermedia- te & high risk 61%. Follicular lymphoma (FL) 58% and non-FL 42%. ORR was 88.1%. ALC was predictive for early OS (ALC ≥1000µL/L 90% vs. <1000µL/L 75% P=0.033, at 18 months, respectively) EFS at 18 months was not different (≥1000µL/L, 78% vs. <1000µL/L, 83% P=0.35). There were no differences among three groups of treatment according to ALC.

Conclusion: The ALC could be a predictor factor for early OS but not for ORR or EFS in patients with indolent lymphoma.
COMBINATION CHEMOTHERAPY WITH GEMCITABINE AND VINORELINSE IN THE TREATMENT OF RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: A PHASE II TRIAL BY THE HELLENIC COOPERATIVE ONCOLOGY GROUP (HECOG).

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Introduction: To investigate the efficacy and toxicity of the combination of gemcitabine and vinorelbine in patients with relapsed or refractory Diffuse Large B-cell Lymphoma (DLBL).

Methods: Twenty two patients with relapsed or refractory DLBL were treated with gemcitabine 1000mg/m2 and vinorelbine 30mg/m2 on days 1 and 8 every 3 weeks for a maximum of 6 cycles. Fourteen patients were considered chemo-sensitive while 8 patients were considered chemoresistant to the last previous treatment respectively.

Results: All 22 patients were assessable for response. Three patients (14%) achieved complete remission and 8 patients (36%) had a partial remission of their disease for an overall response rate of 50%. With a median follow up of 44 months the median time to progression (TTP) for all patients was 8.1 months while the median overall survival (OS) was 12.9 months. Toxicity except was minimal and all patients were treated in an outpatient basis.

Conclusions: The combination of gemcitabine and vinorelbine is an effective and well tolerated regimen for patients with relapsed of refractory DLBL.

MULTIFOCAL EXTRANODAL NON-HODGKIN’S LYMPHOMA: A CLINICOPATHOLOGIC STUDY OF 37 CASES IN GREECE A HELLENIC CO-OPERATIVE ONCOLOGY GROUP STUDY

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Introduction: The purpose of this retrospective study was to illustrate the clinicopathological features of patients presenting with multifocal extranodal non-Hodgkin’s lymphoma (NHL).

Methods: Among 810 patients with NHL, 37 cases (4.2%) were found to have multiple extranodal involvement (two or more sites). There were 24 males and 13 females with a median age of 63 years. The majority of such cases (n=20) had gastric or intestinal (GI) involvement with or without other extranodal sites. Lung with another extranodal site was relatively common in the present series.

Results: The stratification of the 37 cases according to International Prognostic Index (IPI) showed that 89% of the patients belonged to the high risk groups. Diffuse large cell lymphoma (DLBCL) accounted for 62% and MALT for 27% of all cases. After induction treatment with anthracycline-based regimens, complete remission (CR) was achieved in 21 patients (57%), partial remission (PR) in 6 (16%) and no response (NR) in 7 (19%), while 3 (8%) patients were non evaluable.

Conclusions: In conclusion multifocal extranodal NHL are a heterogeneous group of diseases. The majority of them arise in various sites of GI tract. DLBCL was the most frequent histological subtype followed by MALT lymphoma. Risk groups as defined by the IPI were predictive of survival.