6. Extranodal Lymphomas

Primary Extranodal Adult Non Hodgkin Lymphomas (PENHL)
In Egypt:
Clinicopathological Profile Of 249 Patients
Alexandria Univ Hospitals, Egypt.

Introduction: There are great differences in the incidence of extranodal NHL and little is known about the actual incidence in developing countries. The aim of this work is to investigate whether adult PENHL in Egypt is a distinct clinicopathologic entity with a different course.

Methods: This is a retrospective review and analysis of the data concerning 249 PENHL, recurrence at the 5 main cancer centers in Egypt between 1996-1999.

Results: 249 patients were included - 53% males & 47% females with a median age of 45 years. DCL was the most common pathology subtype encountered in 47% of cases followed by mixed 38% & LC in 15%, MALT type in 9%, MP in 6% while 5% had low grade lymphoma. The head & neck (38%) was the most frequent site of involvement, followed by the GIT (25%), skin (12%) and bone (11%). PS-2 was reported in 20% of patients. B symptoms in 14% and at least 40% of cases had stage I. 45% stage II, while 10% had advanced diseases stage III/IV. Therefore, 30% ranked in the low-IPI group, 57.5% in the intermediate group, and 12.5% in the high IPI.

ChOP was the main treatment in 65.5% of cases, 35% received additional radiotherapy. As median follow up of 24 months (range 4-50), 158 patients were evaluable for response with 63% CR rate. The correlation between CR and various prognostic features revealed a statistical significance in favor of one anatomic site involvement vs more than one site (P=0.021) & therapy using the ChOP regimen (P=0.012). No significant difference was correlated to: age (<60 years), stage (I/II/III/IV), PS (<2), B symptoms, pathological subtypes, IPI scores (P>0.05).

Conclusion: The results of our series suggest different & distinct clinical features for PENHL. With a higher incidence of head & neck involvement, an equal distribution of disease in the stomach & small intestine. CHOP appears to play an important role in achieving CR. Ongoing further analysis will clarify the impact radiotherapy on the therapeutic outcome, and assess the survival data with special emphasis to multi-variant analysis of the prognostic factors.

DETECTION OF TT VIRUS (TTV) NUCLEIC ACID IN PRIMARY CNS LYMPHOMA (PCNSL)
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Introduction. PCNSL may be, at least in part, an infectious disease, and may be initiated by viral antigen within CNS.

Materials. (1) Prospectively acquired surgical brain specimens (PCNSL: 50; CNS disease controls: 31; Cancer controls: 50); (2) Snap-frozen and maintained at -70°C; (3) 15 specimens randomly selected: 10 CD20+, B-cell PCNSL (4 newly diagnosed "typical", 3 newly diagnosed "atypical", 3 recurrent); 4 gliomas; 1 metastatic brain tumor.

Methods. (1) PCR DNA detection with validated clinical protocols, all incorporating rigorous contamination control procedures; (2) 8 candidate antigens: JCV, HSV, CMV, EBV, TTV, HHV-6, HHV-8, and pHw1 (Whipple's disease agent).

Results. One of four patients with typical PCNSL had evidence of TTV nucleic acid integration into tumor DNA. None of the other specimens displayed antigen presence. We then randomly selected 15 additional patients with newly diagnosed typical B-cell PCNSL and these were subjected to the in silico PCR analysis. Two of these were positive for TTV. No other target DNA was detected.

Conclusion. 3 of 19 "typical" newly diagnosed B-cell PCNSL, or 16%, displayed evidence for TTV nucleic acid integration into DNA extracted from PCNSL specimens. We cannot determine whether the presence of TTV DNA is an effect of tumourgenesis or its cause. Our work is the first to explore specific antigen other than EBV and the herpesviruses. Further work will be required to determine if the TTV DNA is integrated and/or transcriptionally active.

CLINICAL ASPECTS OF PRIMARY BRAIN LYMPHOMA
Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

Aim: We present clinical characteristics of 44 patients treated between 1992-2001. Based on retrospective analysis of clinical data: treatment methods, outcome, side effects and follow-up are presented.

Patients and methods: 44 patients (19 female and 25 male, age 16-83 (range 56); histology: low grade lymphoma 5 patients, high grade and Burkitt lymphoma — 39 patients. 1 woman was treated on high grade lymphoma in 34 hfd of pregnancy. Both are alive, woman with complete remission and child is in good health without late complications. Radiotherapy alone - 9 patients, chemotherapy alone - 13 patients and combined therapy(chemotherapy regimens: CHOP, CHOP+MTX i.th., MEVA-2D, MEVA-2D+HD MTX, CODOX+IVAC and radiotherapy-total dose 20-50 Gy) - 22 patients.

Results: 15 / 44 patients are alive (OS 2-72 months-range 31); 12/15 patients with complete remission (after combined treatment) and 3 / Patient with partial remission. 29/44 patients died (OS 1-52 months - range 11).

Conclusions: The older patients have poor prognostic. Combined treatment is better than chemo- or radiotherapy alone. Chemotherapy MEVA-3D (ADM 40 mg/m² day, CTD 600 mg/m² day 1, CTD 200 mg/m² days 2-4, dexamethasone 40 mg days 1-4, MTX 25 mg/m² day 6, MTX 12.5 mg/m² day 7 or MEVA-3D+HD MTX or CODOX is better than CHOP or CHOP+MTX i.th. Total dose of radiotherapy should not be lower than 40 Gy.
Withdrawn

Treatment outcomes and complications in 42 patients with primary orbital lymphomas (stage IAE) after radiotherapy
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Purpose: The goal of this study was to evaluate outcomes and complications for primary orbital lymphoma (stage IAE) after radiotherapy.

Materials and Methods: Between 1990 and 2000, 42 patients, median age: 58 years (range: 33-85), with primary orbital lymphoma (stage IAE) were treated with radiation or combined therapy. Thirty patients had Marginal Zone B-cell lymphomas, 4 had other low-grade lymphomas according to the Working Formulation, and 8 had intermediate-grade lymphomas. All patients received radiotherapy, and 16 underwent chemotherapy. The total radiation doses, ranged from 30 Gy to 60 Gy (median: 38) with daily 1.8-2.0 Gy fractions. Follow-up periods ranged from 0.3 years to 13.4 years (median: 4.3).

Results: For all patients, 5-year overall survival, cause-specific survival and local control rates were 81%, 83% and 100%, respectively. For the 30 patients with Marginal Zone B-cell lymphomas, 5-year cause-specific survival and local control rates were 87%, 100%, respectively. Severe acute complications (Grade 2-3: NC1-CTC) were detected in 11 patients, and late complications in 10 patients. All severe late complications (Grade 2-3: LENT) occurred in the patients treated with 40 Gy or more. Relapses after treatment were seen in eight patients, six of them with relapses in the area of head and neck (median interval to relapse: 55 months), and two with systemic relapses (median: 23 months).

Conclusions: Excellent local control was achieved in all patients treated with radiation alone or combined therapy. High does radiotherapy (240 Gy) led to severe late complications, so that radiation doses of less than 40 Gy should be used for primary orbital lymphomas.

The Development of Targeted Chemotherapy for CNS Lymphoma – Twin-Track Pilot Studies of the IDARAM Regimen
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Introduction: More effective chemotherapy for non-Hodgkin's lymphoma involving the CNS is required. We have developed a CNS-targeted regimen, IDARAM, based on the pharmacokinetically properties of the individual drugs in the combination.

Patients and therapy: A total of 23 patients (age range 20 to 70, median 61 years; 12 females, 11 males) were entered and fully staged (with re-biopsy where applicable). Of these, 17 patients had secondary CNS lymphoma (of which 2 had previous testicular and 2 breast lymphomas) and 6 had primary CNS lymphoma (PCNSL). There were 16 cases of parenchymal tumour, 3 cases of diffuse meningeal disease and 2 cases of cranial nerve palsy. The regimen comprised: idarubicin 10mg/m² i.v. d1,2; decasamethasone 100mg 12hr infusion d1,2,3; cytarabine arabinoside (ARA-C) 1.0g/m² 1hr infusion d1,2; methotrexate 2.0g/m² 6hr infusion d3 (with folinic acid rescue); in addition, intrathecal therapy was given: cytarabine arabinoside 70mg plus methotrexate 12mg d1,8 (repeated weekly in patients with meningeal disease to 3 weeks post-clearance of abnormal cells in CSF). G-CSF (leucogranistim) was given to all patients from d7 with the intention of repeating courses at 21 day intervals to a minimum of 2 courses. Cranial radiotherapy (to 40 Gy) was given to all responding patients. All but 4 patients received two courses; 3 patients received only 1 course because of disease progression and 1 patient had 4 courses (to good partial remission prior to high dose therapy + PBSC). The 6 PCNSL patients, a complete remission (CR) was achieved in 5, post-IDARAM, with 1 patient dying early of progressive disease. In the 17 secondary cases, a CR was achieved in 8 patients post-IDARAM and probable CR in 2 (awaiting confirmation); a CNS CR but with evidence of disease progression at another site was seen in 1 patient, and 6 had refractory, non-responsive disease. With follow up of 2-42 months (median 15.5m) 4 of the 6 PCNSL group are alive in CR, and with follow-up of 2-40 months (median 12.8m) 9 of the 17 secondary group are alive. All deaths have been disease-related.

Conclusion: IDARAM chemotherapy has demonstrable efficacy in the treatment of both primary and secondary CNS lymphoma of various disease expression.

Primary CNS Lymphoma (PCNSL), Progressive Multifocal Leuкоencephalopathy (PML), and JC Virus (JCV); Coincidental Intersection or Interaction?

Introduction. PCNSL may be, at least in part, an infectious disease, and may be cooperatively promoted by, viral oncogenesis within CNS.

Methods and Materials. (1.) Diagnostic tissue was obtained by stereotactic brain biopsy in two and by autopsy in one. (2.) PCNSL was confirmed by monoclonality for CD20 surface immunoglobulin. (3.) PML was established by in situ hybridization (ISH) for JCV.

Results. Three patients with lymphomatous malignancy each curiously developed co-localized PCNSL and PML. There were three unique findings. Firstly, lymphoma cells were located only in those areas where there was PML demonstrable by JCV ISH. Secondly, PML clinically preceded PCNSL. Thirdly, the pathologic process appeared to progress centrifugally with the late development of contrast enhancement. The case histories, imaging, pathologic and molecular biologic features of each case will be presented.

Conclusion. The presence of JCV may have served as mitogenic or antigenic stimulus for tumor cells. Further work will be required to determine if the JCV DNA was present in tumor cells and/or transcriptionally active. This data provides further evidence in support of the "inside-out" model of PCNSL tumorigenesis.
TioThera, BCNU, VM-26, Dexamethasone, and High-dose Methotrexate (TioCaTeMeD) As Therapy for Primary CNS Lymphoma (PCNSL). Andrew Gubkin, Daniel Strollokavski, Alexander Fivnik.

In 1999-2001 we followed 20 pts with PCNSL: 9 m, 11 f, median age 45 yrs. Diagnosis was confirmed histologically in all cases (STBR or tumour resection.). The basic drug was high - dose methotrexate (MTX). We began to use our own polychemotherapy (CT) with drugs that penetrate blood brain barrier in effective dosage. This novel regimen included: TioThera 40 mg/m² 1-st d IV, BCNU 60 mg/m² 1,2 ds IV, VM-26 30 mg/m² 1-5ds IV, Dexamethasone 20 mg 1-7ds IV, MTX 5-8 g/m² 1-5th d IV 6 hour infusion with Leucovorin rescue (TioCaTeMeD). Such three courses and 2 additional injections of the same dose of MTX each in a month completed the schedule. 16 pts were treated with TioCaTeMeD as the first line therapy. They showed the response rate 80% (CR-60%, PR-20%). 4 pts died-all of them had tumour resection beforehand- 3 of them after the first course (haemorrhage in the site of the operation during thrombocytopenia, grade 3) and one because of tumour progression. 7 pts are alive and disease free. From 8 pts without tumour resection all are alive and in 7 pts CR was achieved. One pt experienced early relapse. One pt with PR received radiotherapy and remains alive and stable with radiographic abnormality 14 mos. WHO grade 3 myelosuppression was the main toxicity on TioCaTeMeD (in 70% courses), median time neutrophil recovery was 5 days. Conclusion: TioCaTeMeD is feasible and effective regimen as the first line treatment of PCNSL though follow up is too short. Pts after surgery have an adverse prognosis.

Mabtera (Mab) in the treatment of primary CNS lymphomas (PCNSL). Alexander Fivnik, Andrej Gubkin, Tatjana Nikolaeva.

We treated 16 pts with PCNSL with TioCaTeMeD courses (TioThera 40 mg/m² 1d IV, BCNU 60 mg/m² 1,2d IV, VM-26 30 mg/m² 1-5d IV, Dexamethasone 20 mg 1-7d IV, Methotrexate 5-8 g/m² 15d IV for 6 hour with Leucovorin rescue), and 8 pts with another courses. In 5 pts the remission was not achieved so we used Mab as far as it declared to penetrate blood-brain barrier. In all cases the diagnosis was confirmed by standard and immuno-histology. Tumour in all 5 pts were CD 20+.
Mab was used as monotherapy 375 mg / m² once a week for times as IV injections. In one pts serum and liquor concentrations of Mab were estimated as follows:

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<td>Serum</td>
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<td>Liquor</td>
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Conclusion: Mab penetrates blood-brain barrier but is ineffective in pts with relapsed or refractory PCNSL to previous chemotherapy.

NEUROTOXICITY OF COMBINED TREATMENT OF PRIMARY CNS NHL: ANALYSIS OF 150 PATIENTS TREATED BY THE GOELEMS LCP 88 TRIAL.
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With high-dose Methotrexate chemotherapy and radiotherapy, survival medians of pts with PCNSL increase and now are between 2 and 3 years with a lot of long-term survivors. However we observe more and more neurological troubles (D) incidence and prognostic factors of which are not well recognized.

GOELEMS LCP 88 trial associated 3 courses of the MBVP regimen (6 HD-MTX 3 g/m², 6 LP and a 40 Gy WBRT). In July 2000 (*) and with a median follow-up time about 6.2 years, survival median time of our 150 pts treated between 1988 and 1998 is 26 months and 7.5-year survival rate is 25±4%. Among the 95 pts (63%) in CR, median survival time is 64 months and 7.5-year survival rate 42±6%. Among pts in CR, 93 were assessable for neurotoxicity. We noted 101 neurological: 24 A of memory, 24 of character, 22 of gait, 10 A of behaviour, 7 urinary incontinences, 3 A of language... 95% of these neurological A occurred during the 5 first years of survey but sometimes were very early, just after cerebral irradiation. These A occurred among 35 pts (23%), and the 7.5-year actuarial risk is 40±24%. Multivariate analysis retains 4 prognostic factors:

- Age ≥ 60 yrs: p=0.001
- Epilepsy at diagnosis: p=0.003
- More than 45 Gy on tumoral site: p=0.04
- Post-therapeutic PS ≥ 2: p=0.0003

Prognostic profile is very poor with once time out of two an evolution to lethal encephalopathy that presents the 2nd cause of deaths among pts in CR: 17 deaths against 28 due to relapse and 4 non-linked deaths.

This neurotoxicity minimizes results of potential curative treatment of PCNSL. EFS curve including events due to NHL and neurological A, shows among our 93 pts in CR a median EFS time about 3 years and a 7.5-year EFS rate of 34±5%.

Age is a major prognostic factor:
- Among the 50 "aged" pts, median is 25 months and 6-year EFS rate 15%. These results should be compared with results of conventional radiotherapy alone.
- Among the 43 younger pts, median is 7.5 years with a real hope of cure PCNSL, without neurological A. We postulate that intensification of chemotherapy with an ABMT in order to reduce brain irradiation in pts in CR represents a real challenge. (*) Results will be updated for presentation.

PROGNOSIS OF ORBITAL AND OCULAR ADENAL NON-HODGKIN'S LYMPHOMAS.
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Introduction. The prognostic factors of orbital and ocular adenal non - Hodgkin's lymphomas (NHL) are not well known. The aim of the work is to analyze prognosis of orbital and ocular adenal non – Hodgkin's lymphomas (NHL).

Methods. We analyzed the prognosis of NHL in 71 patients (pts) aged from 16 to 80 years. The state to female ratio was 1:2. Orbital NHL was diagnosed in 42 pts, concomitant NHL to 32 and ocular NHL in 7 pts. By the moment of orbital manifestation 51 pts had stage II and 20 had stage IV (10 pts had more than one extranodal lesion). Morphological subtypes were according to WHO Classification of Malignant Lymphomas (1999). B-cell's NHL were diagnosed in all cases. Morphological subtypes of the lymphomas were as follows: 29 MALT lymphomas, 45 low grade lymphomas exclucing MALT lymphomas (B-llular and reticula cell NHL), 7 high grade lymphomas (diffus large cell and Burkitt's like NHL). The follow up period ranged from 1 year to 17 years (median 4 years).

Results. International prognostic index in majority (77.5%) pts was 0-1. Nobody had Performance Status of 2-4, twofold or more intense of serum LDH or bone marrow involvement. Therefore we only analyze such factors of unfavorable prognosis as age greater than 60 years, clinical stages III or IV of the disease and presence of more than one extranodal lesion. Development of the disease towards extranodal NHL reluctance and mortality rate showed no correlation either age of the pts (X² = 0.14; p=0.71) or quantity of the extranodal lesions (X² = 0.73; p=0.44). Mortality rate and frequency of extranodal NHL relapses depended on the initial clinical stage of NHL (X² = 2.45; p=0.12). Furthermore, the morphological subtype of NHL had statistically significant effect on the mortality and recurrence rate (X²=14.76; p=0.0006). None of the 29 patients with MALT lymphomas died from the disease during the observation period (more than 10 years). The first extranodal manifestations were noted only in 2 of the 29 pts in the long-term follow up (7 years).

Conclusions. The mortality and recurrence rate depends on the tumour morphological subtype and the initial stages of NHL. MALT lymphomas prevailed over other subtypes as primary orbit and ocular adenal lymphomas have the most favorable prognosis.
CLINICAL ASPECTS OF PRIMARY THYROID LYMPHOMA
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Background: This paper presents clinical characteristics of 23 patients treated between 1988-2001. Based on retrospective analysis of clinical data: treatment methods, outcome, side effects and follow-up are also presented.

Patients and methods: 23 patients/ 19 female and 4 male /; age: 25-84 range 58 /, low grade lymphoma - 10 patients, high grade - 12 patients; clinical stage: IA-E 13, IIE-9, IV-A 1, Radiotherapy -1, chemotherapy -11, chemotherapy -10, one observed.

Radiotherapy using Cobalt units-two opposite fields-10 patients, one field-2. Total dose 30,6-41,4 Gy. Various single and combined chemotherapy schedules were used. Most received CHOP.

Results: 14 /23 patients are alive; 12 /14 (9 - 160 months) complete remission, 2/14 partial remission was observed from 2 to 3 months; 2/23 patients were lost in follow-up (after 5 and 7 months) ;7/23 patients died ( after 4 - 35 months ) -2/7 patients without evidence of lymphoma: 1 - circulation failure after 55 months after treatment, 1 - melanoma malignum progression after 29 months.

Conclusion: The majority of relapse occurred two years after treatment of older patients with poor prognostics factors. Combined treatment is better than chemo or radiotherapy alone. Total dose 36 Gy appers not to be enough and we observed relapse in the some radiotherapy field. Toxicity was low and acceptable. Relapses after 3 years indicate that 5 courses of CHOP are not enough.

PRIMARY EXTRANODAL NON-HODGKIN'S LYMPHOMA (NH) OF THE TISSUE OF THE ORAL CAVITY: A RETROSPECTIVE ANALYSIS OF 107 PATIENTS (PTS) TREATED WITH RADIOTHERAPY (RT) OR COMBINED CHEMOTHERAPY (CT) AND RADIOTHERAPY
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Introduction: NHL arising in the head and neck district are the second most frequent presentation of localised extranodal lymphomas. Treatment options usually include both exclusive RT as well as combined modality approach, using an anthracycline-based CT followed by involved fields (IF) radiotherapy. In order to clarify the role of radiotherapy in this particular subset of extranodal lymphomas, we retrospectively analysed clinical characteristics and treatment outcome in a series of pts with primary extranodal NHL of the head and neck.

Patients and methods: From 1985 to 2000, 107 pts with a new diagnosis of head and neck extranodal NHL were sequentially treated at our Institution. Median age at diagnosis was 65 yrs (17-86). First site of localization was Waldeyer's ring in 82 pts, and other head and neck sites in 19 pts (17.5%). 50 pts had stage I and 57 stage II disease. 86 pts (80%) had a high grade histology and 21 pts (20%) had a low grade. 53 pts (49,5%) had only extranodal disease (E) and 54 (50,4%) had both nodal and extranodal localisations (N+E). International Prognostic Index (IPI) was: Low, 76 pts (71%), Low-Intermediate, 26 pts (24,2%), Intermediate-High, 5 pts (4,7%). Treatment consisted of radical RT in 59 pts (55,5%) -with E and 25 pts with E+N disease - and in combined anthracycline-containing CT and IF-RT in 48 pts (44,5%-19 and E 29 E+N disease.

Results: CR- and PR-rate were respectively 86,4% and 13,5% after RT alone; 54% and 35% after CT and 87,5% and 8,3% after RT in the RT+CT group. After a median follow-up of 43,4 months (2,9-20,91), the 5-year actuarial overall survival rate (OS), disease specific survival (DSS) and disease free survival were respectively 60,71%, 67,5% and 61,2%.

Conclusion: Those preliminary data confirm the role of radiotherapy as effective and safe therapeutic approach for both local disease control and curative intention in limited stage Waldeyer's ring NHL patients. Subset uni- and multivariate analysis will be presented for the main clinical and treatment variables.
THE PREDICTING OF THE WHO CLASSIFICATION AND PHENOTYPING IN THE BEHAVIOR OF NASOPHARYNGEAL LYMPHOMAS


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Introduction: The recently described world health organization (WHO) classification of malignant lymphomas promises to be the most clinically relevant classification to date. In this classification there is a great emphasis on phenotyping of the lymphomas with correlation with morphology, genotype and clinical data. We attempted to test this classification on cases of primary nasopharyngeal lymphoma.

Methods: We identified cases of primary malignant lymphoma of the nasopharynx. The cases were re-classified and phenotyped according to the WHO classification.

Results: Forty three cases were identified between 1981 and 1998. There were 20 females and 13 males. The patients ages ranged from 11 to 81 years (median 55 years). Diffuse large B cell lymphoma (DLBCL) constituted the most commonly diagnosed type (n=18; 41%) followed by T/ Natural killer (T/NK) cell lymphoma (n=16; 37%). The next entity was follicle center cell lymphoma (n=5; 11%). Other entities were mantle cell, extramedullary marginal zone B cell lymphoma and Burkitt lymphoma (n=2). There were 27 cases (63%) that showed a B-cell phenotype and 16 cases (19%) that showed a T/NK phenotype. B cell lymphomas showed a 5 year overall survival (OS) of 62% whereas T/NK lymphomas showed a 2.9% OS (p=0.02). B cell lymphomas showed an event free survival of 60% whereas this 5 year survival was 20% for T/NK cell lymphoma (p=0.03). Other prognostic indicators that were significant were performance status, serum LDH levels, bulky disease and combined radiation therapy and chemotherapy versus chemotherapy alone.

Conclusion: In primary nasopharyngeal lymphomas the WHO classification with phenotyping, in conjunction with other established clinical indicators, is very significant in predicting the behavior of these neoplasms. T/NK cell lymphomas have a significantly more aggressive behavior than B cell neoplasms.

ORAL CAVITY LYMPHOMA IN IMMUNOCOMPETENT AND HIV-POSITIVE PATIENTS: A SINGLE INSTITUTION'S EXPERIENCE

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Introduction: Involvement of the oral cavity in Non Hodgkin Lymphoma (NHL) is a rare event in both immunocompetent and immunodeficient patients (pts). A new entity, Plasmablastic Lymphoma (PBL), has recently been described in the oral cavity of HIV+ pts. The differences between these groups of NHL, however, have not been clarified yet.

Methods: Since 1995, 545 immunocompetent NHL and 123 HIV+ NHL pts were admitted to our Institution. They were retrospectively reviewed and cases with primary involvement of oral cavity were selected.

Results: Eighteen cases of primary oral lymphomas were identified. Among immunocompetent pts the frequency of oral involvement at diagnosis was 1.66% (9/545). Three of 9 (33.3%) had T immunophenotype (angiocentric lymphoma), the other 6 (66.6%) showed B immunophenotype (folllicular (2), MALT (1), Diffuse Large Cell (DLC) (1), immunoblastic (1) and Burkitt (1)). The clinical stage at diagnosis was IEA (6), IIIA (1), IVA (2). Two pts were lost at follow-up; one died after 31 mo of relapsing T angiocentric lymphoma after a RXT-induced CR of 24 mo duration. Six were treated with anticyclin-containing combination chemotherapy/radiotherapy. CR was obtained in 4 cases. Two pts with T angiocentric lymphoma failed to respond. One patient relapsed after 28 mo. After a median follow-up of 31 mo (range 28-48), all pts are alive, four with no signs of disease. Among HIV+ pts oral presentation at diagnosis represented 7.3% (9/123) of cases, significantly more than in immunocompetent pts (Fisher’s exact test: P=0.002). Eight pts showed a B-phenotype and one a “null” phenotype. Three cases were B, DCL, 2 Burkitt or Burkitt-like, 1 immunoblastic and 1 anaplastic null.

Conclusion: a) involvement of the oral cavity at diagnosis is more frequent in HIV+ lymphomas; b) T angiocentric NHL occurs typically among immunocompetent pts and seems to be unfavourable; c) aggressive B cell histology and worst prognosis are characteristic of HIV- d) PBL seems to be an histologic entity confined to the HIV+; it is susceptible to a chemotherapeutic approach with better responses than other oral cavity HIV+ NHL.

CONVENTIONAL CYTOGENETIC ANALYSIS IN PRIMARY MEDIASTINAL B CELL LYMPHOMA.

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Primary Mediastinal B Cell Lymphoma (PMBL) is a recently recognized entity characterized at the histologic level by the presence of case, medium sized to large B cells, and of a variable degree of fibrosis. Patients are usually young females, and the disease is usually localized at the time of initial diagnosis. Data regarding the cytogenetic pattern of these tumors mainly results from Comparative Genome Hybridization (CGH), and the most striking reported feature consists in a gain of the short arm of chromosome 9. Over a 10 years period, from 1992 to 2001, 177 patients were studied in our institution, who had a diffuse large cell lymphoma (DLCL), and neither a 14:18 nor a 8:14 or variant translocation upon cytogenetic analysis. 58 had a mediastinal localization of the disease, and the diagnosis of PMBL was retained in 15 patients. 5 males, 10 females; mean age: 32.9 (range 24-66). Patients had Ann Arbor stage I disease in 4 cases, II in 2 cases, IE in 8 cases and III in 1 case. LDH level was elevated in 13 cases. All patients received anthracycline based regimen. Four patients died, 3 from a progressive disease and the fourth from radiotherapy related toxicity. Good quality metaphases could be obtained in 138 of 162 DLC (non PMBL) patients, and an abnormal karyotype was identified in 13. A gain of chromosome 9 or 16p or a structural abnormality of chromosome 9p was present in 20 of these patients. The repartition of these abnormalities was different among patients with (1840, 45%) or without (1293, 13%) mediastinal localization. In PMBL patients, 12 of 15 could be analyzed and 10 had clone abnormalities. Karyotypes were hyperdiploid in all patients but one. 9 had a gain of either the whole chromosome 9 or chromosome 9 short arm and both short arms of chromosomes 5 were present in 3 patients and in 1 patient, chromosome 5 was tandem 5p13 and 5q12 respectively, so that all patients with a PMBL diagnosis showed numerical and/or structural alterations of chromosome 9p. Chromosome 8 was frequently involved (7/10), followed by chromosome 3 (5/10) and chromosome 12 (3/10). These results are in agreement with previously published CGH data obtained in PMBL patients. Moreover, conventional cytogenetics allows the analysis of structural chromosomal defects present in these patients.
PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMBL): 43 CLINICAL STAGE 1 AND 2 PATIENTS TREATED WITH COMBINED MODALITY THERAPY (CMT) AT THE PRINCESS MARGARET HOSPITAL.
Princess Margaret Hospital, University of Toronto, Toronto, Canada.
Introduction: PMBL has been recognized as a distinct disease entity. The standard treatment for stage I/II patients (pts) is CMT. We have reviewed the outcome of stage I/II PMBL pts who received CMT. The objective of this study is to examine factors predictive for survival and local control and long term pattern of failure.
Methods: From 1985-1998, 43 pts with stage I/II PMBL received doxorubicin-containing CMT. The median age was 40 yrs (range 18-66), with slight female predominance (59%). Ann Arbor stage IA = 25%, IIB = 26%, II = 10%, III = 27%. Tumour bulk was > 10 cm in 38%. Chemotherapy (CT) consisted of CHOP (78%), 4/4-ACOP-D (10%), and other regimens (12%) for > 6 cycles (50%). All pts received involved field radiotherapy (RT) to a median dose of 35 Gy. The variables tested for prognostic significance were: age (> 60 yrs), stage (I vs. II), LDH (> normal), Karnofsky performance status (KPS < 70), gender, extent of disease, bulk, symptoms, bulky (> 10 cm) and the modified International Prognostic Index (miPI).
Results: The median follow-up was 5.2 years (range 2.1-14.6). The complete response (CR) rate was 60%. Overall survival at 5 years (OS) and disease-free survival (DFS) in all pts was 65% and 64% respectively. Distinct relapse rate (DRR) was 30% and local relapse rate (LRR) 19%. There were no failures after 2.5 years. The most significant prognostic factor for OS was KPS (HR=1.9, p=0.004), stage (HR=2.1, p=0.012), age (HR=1.9 p=0.17), and LDH (HR=2.6, p=0.19) did not achieve statistical significance. Using the miPI (age, stage, KPS, and LDH), the OS for miPI ≤ 1 was 87% and miPI >1 was 80% (HR=0.9, p=0.008). There were no differences in the rate of CR/MVCOP-B. Twelve of 20 pts who relapsed/failed initial treatment received aggressive salvage CT with DHA/mipom-Beam. Only 5 pts had a response (PR) for ATBMT, but died of disease within 1 year of ABMT.
Conclusions: Standard therapy for stage I/II PMBL achieves long term cure in approx 2/3 of patients. Initial treatment failure or relapse occurs early and is rarely salvageable. Late relapses (beyond 2.5 yrs) were not observed. Poor prognostic patients can be identified using the miPI (>1) and may benefit from more intensive approaches with MVACOP-B or proceed directly to high dose CT and ABMT. There were no statistically significant predictors of local relapse in our series of patients treated with CMT.
THE PROFILE OF PRIMARY GASTRO-INTESTINAL L YMPHOMA IN EGYPTIAN PATIENTS

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Introduction: About forty percent of all non-Hodgkin's lymphomas arise at extra-nodal sites with the gastro-intestinal tract as the commonest extra-nodal site. Primary gastro-intestinal lymphomas arise from the gastro-intestinal tract and its contiguous lymph nodes without the involvement of peripheral lymph node, liver, spleen, marrow or peripheral blood.

Methods: A total of 87 cases of primary gastro-intestinal lymphomas were obtained from the Pathology Department at the National Cancer Institute, Cairo University from the year 1998-2000. Clinical records were reviewed regarding the topographic and demographic data. Histopathologic sections were revised according to the WHO-REAL classification. Immunophenotyping was done for all cases.

Results: Primary gastro-intestinal lymphoma constituted 7% of total gastro-intestinal malignancies, 15.5% of total extranodal lymphomas and 6.2% of all non-Hodgkin's lymphoma cases. The mean age was 31.4 years with a range of 3-75 years. The male to female ratio was 1.8. Lymphomas of small intestine were the commonest (40.2%), stomach was second in frequency (40%) while coloectral was the least common site (21.8%). Reection specimens were 24 cases with involvement of regional lymph nodes in 13 (54.2%) cases. Three different histologic subtypes were identified: a) diffuse large cell lymphoma was the commonest (44.8%); b) Burkitt's lymphoma was second common (33.4%) and c) Mucosal Associated Lymphoid Tissue (MALT) lymphoma was the least common (21.8%). Large cell lymphoma showed the highest incidence of regional lymph node involvement (64.2%). All cases were of B-cell phenotype and no T-cell lymphomas were observed in this series.

IMPROVED PCR-BASED (IgH AND Ig KAPPA) B-CELL CLONALITY ANALYSIS IN PRIMARY GASTRIC B-CELL LYMPHOMAS

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Introduction: In this study, the diagnostic value of the PCR-based detection of the immunoglobulin kappa light-chain (IgK) and the immunoglobulin heavy-chain (IgH) gene rearrangements was investigated in routinely processed samples of gastric biopsies for the early diagnosis and follow-up of gastric B-cell lymphomas.

Methods: Thirty-four endoscopic biopsy samples of 24 patients with primary gastric lymphomas, including 18 cases of mucosa-associated lymphoid tissue (MALT)-type lymphoma, 5 cases of diffuse large B-cell lymphoma (DLBCL), and 1 case of mantle cell lymphoma (MCL) were evaluated. In addition, 10 histologically verified gastric cases were included, as controls. The DNA was extracted by deparaffination, proteinase-K digestion, and boiling. The B-cell clonality was detected by PCR analysis of the IgH and IgK gene rearrangements.

Results: Dominant band, as evidence of B-cell monoclonality was seen in 63.6% by IgH and 79.1% by IgK gene rearrangement. In sum monoclonality was detected in 95.8% (23/24) of the successfully amplified gastric lymphoma samples. No monoclonality was detected in the control group of the 10 gastric patients. Histological remission was observed in 9/34 (27 MALT and 39 DLBCL) samples. The disappearance of the clonal band in 3/9 samples has confirmed the molecular regression too, suggesting the importance of the molecular genetic follow-up.

Conclusion: Our study has proved the value of the detection of IgH and IgK gene rearrangements in the early diagnosis and follow up of primary gastric B-cell lymphoma.

DETECTION OF APIZ-MALT1 CHIMERIC TRANScripts INVOLVED IN MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA BY A SINGLE REACTION OF TOUCHDOWN MULTIPLEX POLYMERASE CHAIN REACTION

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Introduction: T(11;18)(q21;q21), which results in a chimeric transcript between the API2 at 11q21 and MALT1 at 18q21, is a characteristic chromosomal aberration in extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). This chimeric transcript has been observed in about one third of MALT lymphomas. Several reports have dealt with the detection of API2-MALT1 chimeric transcripts, for which RT-PCR has proved to be a major tool. However, multiple PCR reactions have been necessary to cover all possible chimeric transcripts because at least four different fusion points in each API2 and MALT1 mRNA are known to be involved. Nested PCR may also be needed to achieve maximal sensitivity. This prompted us to investigate a simple and sensitive procedure.

Methods: We have established a touchdown multiplex PCR method to detect API2-MALT1 chimeric transcripts in a single reaction. The sensitivity of the assay was demonstrated by using a serially diluted plasmid construct as the standard. In order to determine the number of API2-MALT1 mRNA copies in the clinical samples, quantitative real-time PCR was performed using cases with the most frequent chimeric transcript of API2-MALT1 (API2 exon 7 - MALT1 exon 5 type).

Results: All five variants from patient samples with (11;18)(q21;q21) showed specific amplification of chimeric transcripts in the touchdown multiplex PCR. The detection of 100 copies of API2-MALT1 was verified. The real-time PCR proved that the number of copies of patient samples ranged from 42 to 8.2 x 10^4 per 100 ng total RNA, indicating that our touchdown PCR is sensitive enough to be used for patient samples.

Conclusion: The multiplex touchdown PCR assay for the detection of API2-MALT1 described here is sensitive, specific and simple.
The role of dendritic cells in MALT lymphoma
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Introduction: Marginal zone B-cell lymphoma (MALT lymphoma) is recognized to be a distinct clinicopathological entity. The development of MALT lymphoma is preceded by acquisition of inflammatory reactive lymphoid tissue. In general, the disruption of immune system may constitute an underlying mechanism of lymphomagenesis. Dendritic cells (DCs) are professional antigen presenting cells, which play central roles in eliciting immune responses, leading to a hypothesis that DCs may play crucial roles in the development of lymphomas. To investigate the nature of the involvement of DCs in MALT lymphoma, immunochemistry analysis was performed in this study.

Method: Tissue biopsies were obtained from 12 patients with thyroid MALT lymphomas and 13 patients with gastric MALT lymphoma. Immunostaining was performed using Envision system (DAKO). Paraffin sections of biopsied samples were immunostained with antibodies against CD1a, CD20, CD23, CD45R0, CD11c, and FcεRI. Frozen sections were immunostained with antibodies against DC-LAMP, CD83, FcεRI, DR6, and IL-10.

Results: A large number of FcεRI+ DC-LAMP+ CD83+ mature DCs were present within the interfascicular areas of thyroid MALT lymphoma. Aggregates of CD83, but not of CD8+ T cells, were found to exist in close association with DCs. A number of FcεRI+ CD8+ and IL-10-positive cells were observed in the T cell areas. On the other hand, neither FcεRI+ nor IL-10-producing cells were detected in MALT lymphomas. Similarity to the case of thyroid MALT lymphomas, mature DCs were also found to be infiltrated in gastric MALT lymphomas.

Conclusions: We demonstrated that mature DCs were infiltrated in both thyroid and gastric MALT lymphomas. Interestingly, some DCs were demonstrated to be directly interacting with CD4+ T cells. In addition, both FcεRI+ producing T cells (Th1 cells) and IL-10-producing regulatory T cells were observed in the T cell areas. These findings raise a possibility that DCs might regulate Th polarization in MALT lymphoma.

ACTIVITY OF RITUXIMAB IN RELAPSED OR REFRACTORY LOW GRADE MARGINAL ZONE LYMPHOMA OF MALT-TYPE (LG-MZL) OF THE STOMACH.
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Introduction: Regression of Helicobacter Pylori (Hp) positive primary gastric LG-MZL following antibiotics therapy occurs approximately in 50-60 % of stage I cases. Chemotherapy or radiotherapy is the treatment of choice for antibiotic resistant or relapsed patients (pts) not suitable for gastric resection. Such treatment modalities may induce severe acute and late toxic side effects. Monoclonal anti-CD20 antibody (Rituximab) has recently demonstrated a relevant clinical activity also in LG-MZL pts with low toxicity. We evaluated clinical activity and toxicity of Rituximab in relapsed or Hp negative LG-MZL of the stomach. Methods: We considered 20 pts (10 female/10 male) 53 years median age (range 32-80). 11 pts received previous antibiotics alone, 5 antibiotics plus chemotherapy, and 1 chemotherapy alone. 3 Hp negative pts were previously untreated. 18/20 pts presented gastric disease alone, two had bone marrow involvement. Rituximab was administered at 375 mg/mq weekly for 4 weeks. Clinical and endoscopic re-evaluation was performed two months later. Results: Grade 3 neutropenia was observed in one patient. One additional patient developed reversible grade 3 glottea oedema during Rituximab administration. The ORR observed was 75% with 7 patients in pathological CR (35%). The median time to achieve the best response was 2.3 months (range 1-15.5). With a median follow-up of 8.3 months (range 1-23) only one patient relapsed. Conclusions: Our results demonstrate the high degree of activity of Rituximab in gastric LG-MZL and suggest that this treatment should be considered for those patients relapsed after antibiotics chemotherapy and not suitable for local radiotherapy or gastrectomy.
Non-MALT Marginal Zone B-cell Lymphomas (MZL). A single center experience.

Introduction: In the WHO classification, MZL is described with three subtypes: extranodal MALT lymphomas, splenic MZL, and nodal MZL. The MALT type MZL have been described in details. However there are questions regarding the clinical characteristics, optimal treatment and outcome of non-subtype of MZL. We report on our single center experience from this subtype of MZL.
Methods: Forty patients (M/F: 1.2) with non-MALT MZL (9% of NHLs) have been treated in our department. The median age was 63 years (30-81). The stage was evaluated as IV in 36 pts (90%) because of BM involvement. Two pts had ITP, two A AA and six (15%) pts had an M-component. Twenty nine (72%) pts had a splenomegaly because of bulky splenomegaly and hyperplastic spleen. Eleven (27%) pts had disseminated or and more aggressive disease were administered chemotherapy.
Results: Four clinical subtypes were observed: splenic[31 pts - 77%], nodal[4 pts-10%], disseminated[3 pts] and leukemic(2 pts).
Response to treatment varied in different subtypes. A good partial remission was the rule after splenectomy in splenic subtype. With a median follow up time of 39 mo 7 pts have died and 33 (82%) are alive with good quality of life.
Conclusions: Four clinical subtypes of non-MALT MZL were easily recognized (splenic, nodal, disseminated, leukemic). We cannot propose decisively the optimal treatment. However it seems that splenic MZL is an indolent NHL and splenectomy should be the treatment of choice in the case of remarkable splenomegaly and pancytopenia.

SUCCESSFUL TREATMENT OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) FOLLOWING RENAL ALLOGRAFTING IS ASSOCIATED WITH SUSTAINED CD8+ T-CELL RESTORATION

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Introduction: Post-transplant lymphoproliferative disorder (PTLD) is a life-threatening Epstein-Barr virus (EBV)-associated B-cell malignancy occurring in 1-2% of renal transplant patients. Host- and PTLD-related factors that determine the likelihood of tumor response following immunosuppression (IS) taper and antiviral therapy remain largely unknown, and standard therapy for PTLD is not established.
Methods: We treated 11 consecutive renal transplant patients who developed EBV-positive PTLD eight to 54 months after allografting with acyclovir and IS taper. All PTLD were diffuse large B-cell lymphomas. We monitored tumor clonality and tumor EBV gene expression with in situ RT-PCR. Blood T cell subsets were monitored by flow cytometry and by HLA tetramer staining.
Results: Ten of 11 patients (91%) obtained a durable complete response (CR) but five (45%) rejected the allograft. In four of the five patients who lost the allograft the PTLD was primarily affecting the allograft. Peripheral blood CD8+ T cells increased significantly (p=0.008) from baseline in 8 responders available for analysis. One of two patients whose CD8+ T cells dropped to baseline relapsed. With a mean (±SD) follow up of 29 months the progression free survival is 82%. CD8+ T cells were studied from two responders; both recognized the EBV lytic antigen BZLF1. Another lytic EBV gene (thymidine kinase) was expressed by all 8 PTLD tested.
Conclusions: IS taper and PTLD immunotherapy is highly successful, but the risk of all allograft rejection is significant, particularly in patients with PTLD affecting the allograft. A sustained expansion of CD8+ T cells and an immune response to EBV lytic antigens may be important to PTLD clearance in renal transplant patients.

PROLONGED B-CELL DEPLETION AFTER TREATMENT WITH RITUXIMAB IN PATIENTS WITH POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD)

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Background: PT-LPD is a frequent disease of B-cell origin after solid organ transplantation. Treatment of PT-LPD with cytotoxic drugs is limited due to severe hematological toxicity. Therefore, treatment with the anti-CD20 monoclonal antibody rituximab is a promising alternative approach.
However, B-cell function is already impaired in post-transplant patients due to therapy with cyclosporin A (CSA) or tacrolimus (FK506). Therefore we were interested in analyzing B-cell recovery in PT-LPD patients treated with anti-CD20.
Samples from nine PT-LPD patients were available for this study. Methods: We measured the CD19+ cell count in peripheral blood at days 0, 8, 15, 22 during and bimonthly after administration of 4 cycles rituximab 375 mg/m2 once a week. The mean age of patients was 50 years (± 9.9) and mean follow-up time was 369 days after therapy. For rejection prophylaxis six patients received CSA and three patients FK506. Results: Prior to treatment the six patients on CSA had a mean CD19 cell count of 84/μl (range 14 to 163/μl) vs. 150/μl (40 to 414/μl) in the FK506 group (normal range 60 to 400/μl). Six months after therapy B-cell recovery was not detectable in these patients. After 12 months samples from six patients were available. The mean CD19+ cell count was 25/μl with a range from 1/μl to 73/μl, reflecting a mean recovery of 16% (range 1-48%) of the initial CD19+ cells. At that time in non-transplanted patients which were treated with rituximab the B-cell recovery is fully completed. There was no difference in recovery of CD19+ cells between the CSA and the FK506 group. Conclusion: Solid organ transplant recipients are B-cell compromised due to immunosuppressive therapy with CSA or FK506. Impaired B-cell recovery is still present twelve months after rituximab therapy. This prolonged B-cell depletion and delayed B-cell recovery is probably due to effects of rituximab and immunosuppression with CSA or FK506. Despite B-cell deficiency no obvious increase in the rate of infections within the 12-month follow-up time was observed.

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD) RESPOND TO INITIAL THERAPY WITH RITUXIMAB.

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Introduction: Because the outcome of patients (pts) with post-solid organ transplant lymphoproliferative disorders (PTLD) is poor with conventional therapy, agents as rituximab have been utilized.
Patients and Methods: In a multi-institutional trial, eleven PTLD pts received rituximab, 375 mg/m2 IV weekly (wx) X 4 wks, every 6 months (mo). Type of transplant was lung (5), kidney (4), heart (1), kidney/pancreas (1). Pts ranged from T 43-69 (median 50) yrs in age; nines were male. Immunosuppressive therapy was prednisone (11), cyclosporin (7), mycophenolate (6), azathioprine (4), and tacrolimus/nil (3). Immunosuppressive therapy was decreased in eight of 11 pts at PTLD diagnosis. Time from transplant to PTLD diagnosis ranged from 1.5 to 9 mos (median 5 mo). Hematology was+diffuse large cell lymphoma (8) or polyclonal process (3); all were CD20+. Ann Arbor stage was I (2), II (5); III (2), IV (3). No pts had BM or central nervous system involvement; however, primary extranodal disease was common (lung-5), skin-2, small bowel-2, stomach-2, liver-phrenic-1. Lactic dehydrogenase levels ranged from 152-166 (median 519) EU/L. Prior to rituximab, one pt with bowel involvement was refractory, one received radiation to a hilar mass, and one had progressed on chemotherapy.
Results: Rituximab was well-tolerated, with mild infusional 1B changes in two pts. Median follow-up is eleven months (range 1-32 mo). Response rate is 60%, with 5 complete responses (CR), 1 partial response (PR), 2 progressive disease (PD), and two deaths; follow up is too brief on the remaining pt to determine response. Two CR's occurred after case to case of two cycles of rituximab, preceded by an initial PR. Treatment is continuing, and PR's may potentially improve to CR. Remission duration ranges from 7.9 to 23+ (median 11.4) mos. Both pts with PD remain in remission after subsequent chemotherapy.
Two pts died with disease progression nine and eighteen days after starting rituximab. One pt had small bowel perforation (primary disease site) after three doses of rituximab. One pt had small bowel involvement (primary disease site) after three doses of rituximab. One pt had small bowel perforation (primary disease site) after three doses of rituximab.

Conclusions: Rrituximab therapy results in responses, and possibly prolonged survival, in PTLD pts. Further expansion of rituximab in these disorders, especially in a part of combination regimens, is warranted.
PRIMARY PULMONARY NON-HODGKIN'S LYMPHOMA (PPHNL): A STUDY OF 18 PATIENTS.

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PPHNL has been estimated to represent about 3.6% of extra-nodal NHL. The great majority of PPHNL are of B-cell immunophenotype, of low histologic grade and derive from the bronchus-associated lymphoid tissue (BALT). High grade or T-cell PPHNL is infrequent. We present 13 patients, (mean age 63.2, SD±6.8 years), 8 with low grade (BALT) and 5 with high (4 B-cell, 1 T-cell rich large B-cell) PPHNL. Clinical and diagnostic features are being summarized below.

<table>
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Reviewing the CT findings, high grade PPHNL appeared as solitary nodules, while low grade mostly as consolidations. Median survival was 37±17 (SD) months in high grade and 53±35 (SD) months in low grade PPHNL, respectively (p=0.017). A limited difference was found in the CT and endoscopic findings between grades, conclusions that concern survival cannot be made due to limited number of patients and follow up time.

INTRATHORACIC CHEMOTHERAPY IS IMPORTANT IN THE TREATMENT OF EARLY STAGE TESTICULAR LYMPHOMA

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Introduction: Testicular malignant lymphoma is rare and accounts for <1% of all cases in our population-based register (Western region, Sweden). Relapses are common, even late after treatment, and involve often new extranodal areas such as CNS. Therefore, our current recommendation is to give anthracycline-based chemotherapy and intrathecal prophylaxis (methotrexate) in 6 cycles (combined therapy) followed by prophylactic irradiation 2 Gy to 24 Gy to the remaining testis. The present study analyzes the outcome for 41 patients, diagnosed between 1985 and 2000 and from a population of 1.6 million. Pathologic specimens are currently assessed for microvascular density and for expression of adhesion molecules (CD 44) and these results will be presented.

Results: Of 30 patients where a complete staging was performed and where follow-up data were available 19 were in stage Ia-II and 11 patients were in stage IV (table). In stage Pe-I, 11 patients received chemotherapy i.v. and i.t., 5 patients received chemotherapy i.v. only and three patients received no systemic chemotherapy at all. With a median follow up of 54 months, one patient out of 11, who received combination therapy, experienced a nodal relapse but no one relapsed in CNS. Among the 5 patients who received i.v. chemotherapy only, 3 relapsed in CNS. Of the 11 patients in stage IV, 4 received combined therapy and 3 relapsed (one in CNS). Four received i.v. chemotherapy only and all relapsed (3 in CNS).

The median overall survival was 11 months (95% CI 89; 137) for patients in stage Pe-I-Il and 11 months (95% CI 1-21) for patients in stage IV.

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<td>Chemotherapy i.v. and i.t.</td>
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<td>1/44 CCR</td>
<td>1/44 Relapse in CNS</td>
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<td>2/44 Nodal relapse</td>
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<tr>
<td>Chemotherapy i.v. only</td>
<td>3/5 Relapse in CNS</td>
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<td>3/44 CCR</td>
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<td>1/44 Nodal relapse</td>
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Conclusion: The addition of intrathecal chemotherapy in the treatment for testicular lymphoma seems to be of value in early stage disease. The prognosis is dismal for stage IV metastatic lymphoma.

TESTICULAR NON-HODGKIN'S LYMPHOMA: TREATMENT WITH CHOP, INTRATHORACIC METHOTREXATE AND SCROTAL RADIOTHERAPY

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Introduction: After doxorubicin-based therapy of Non-Hodgkin's lymphoma involving testicle, 10-year probability of relapse in the central nervous system (CNS) is 34% and in the contralateral testes 21%.

Methods: Since 1994, patients presenting with a testicular mass, histologically proven lymphomas, and adequate serum, liver, and renal function, no HIV infection and no prior lymphoma therapy (except chemotherapy) received 6 cycles of CHOP, concurrent intrathoracic methotrexate (MTX); for prophylaxis four weekly injections; for cranial CNS involvement twice a week to clearance plus four injections), followed by 30 Gy of scrotal radiotherapy (RT).

Results: We treated 20 patients with a median age of 59 years (range 19-82), all with diffuse large B-cell lymphomas per WHO classification. The Ann Arbor stage was I to II, III to I, and IV in 4 patients. Only one had B-symptoms. Serum beta-2 microglobulin exceeded 5.0 mg/l in 22% and the International Prognostic Index score was 2 or greater in 30% of them. Eighteen patients (90%) achieved complete remission (CR), but one relapsed in liver, was salvaged with liver transplant cell transplantation, and remains in CR 49 months later. Two patients failed primary therapy: one had initial involvement of CNS and of both testes, and died of refractory lymphoma. Another died of neurogenic sepsis. With a median follow up of 32 months for survivors, the 3-year progression-free survival (PFS) is 85% and overall survival is 71%. The 3-year PFS for patients with IPI < 2 or 2 or greater was 90% vs. 67%, respectively (p = 0.17 by logrank).

Conclusion: CHOP, intrathoracic methotrexate, and scrotal RT appear to eliminate testicular lymphoma relapses in CNS or the contralateral testes, but longer follow-up is needed for confirmation.

PRIMARY NON-HODGKIN'S LYMPHOMA OF BONE

ABOUT 8 CASES


'Service d'Hématologie et d'Oncologie Pédiatrique - Service d'Anatomie Pathologique - Service de Radiologie Hôpital 20 Août 1953 CH Ibn Rochd Casablanca

The primary non-Hodgkin's lymphoma of bone are single or multiple lymphoid tumors limited to the bone, accounting for only 5% of all extranodal NHL and 7% of all malignant osseous. We report our experience over a 9-year-period (1992 – 2000), in the diagnosis and the treatment of 8 cases with primary non-Hodgkin's lymphoma of bone confirmed histologically.

The average age was 33.4 years (9 – 52 years) and the male-to-female ratio was approximately equal to unity. The most common symptoms included bone pain and a palpable mass. Radiographic features included osteolytic patterns in the femur (37.5%), vertebra and maxilla (25%), ilium and humerus (10%). Most lymphomas were classified as diffuse large cell. Immunohistostaining showed a B-cell phenotype. The disease was classified as Stage I and IIIE in 87.5% and stage IIIIE in 12.5%. All the cases had either a bulk tumor mass or a very high rate of the LDH, thus constituting poor prognosis factors. Among our population, only 5 cases were evaluable, two died of disseminated disease and one is lost to follow-up. The patients received a standard chemotherapy CHOP or MABACO associated or not with radiotherapy of 40 Gy. They were all in complete remission with a median follow-up of 41 months (range: 8-49 months).

The primary non-Hodgkin's lymphoma of bone is a rare entity. Our results join the data of the literature except the youth of our patients. The basic treatment is chemotherapy. The place of the RTH is not yet defined. In spite of the delayed diagnosis and the bad performance status, the prognosis remains good among our patients.
BLASTICINK LYMHPHOMAS PRIMARY PRESENTING IN THE SKIN.

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Milan Institute.

Introduction: In 1994 Adachi reported a CD4+, CD56+ lymphoid neoplasia with initial skin involvement and a terminal leukemic phase. Other cases were subsequently reported; they were variously interpreted as NK/T nasal type, NK cell leukemia/lymphoma, blastic/blastoid NK lymphoma or as agranular CD4+, CD56+ lymphoma.

Methods: The clinical and histopathological data of eleven CD56+ positive lymphomas with initial skin involvement were reviewed. The mean age was 64 years; ten were males and one female. A large panel of monoclonal antibodies and an APAAP immunohistochemical method was employed both on frozen and paraffin embedded sections. Molecular analysis was employed to evaluate clonality (TCR-γ and JR) and viral involvement.

Results: Exclusive cutaneous involvement was demonstrated in 6 patients, while involvement of bone marrow, lymph nodes and upper airways were detected in the others. Histologically, early lesions showed a perivascular and peridermatomal infiltrate composed by pleomorphic medium size cells, in less cases and by monomorphic large blasts in a single case. In more advanced lesions a diffuse infiltrate occupying the whole dermis and the hypodermis was a rule. Neoplastic cells were positive for CD4, CD43, CD56 and CD7, CD8 was expressed in 7 cases.

Conclusions: Our data support the view that CD4+, CD56+ lymphoma is a new entity, characterized by an aggressive course and ability of the skin to preferentially react. Immunophenotypic data suggest that they probably derive from a common lymphoid/myeloid progenitor committed to NK cells differentiations.

PRIMARY CUTANEOUS B-CELL LYMPHOMA (PCBCL): REPORT OF 28 CASES WITH EMPHASIS ON BORELIA SEROLOGY AND CLINICAL EVOLUTION.

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Introduction: A variable, but significant percentage of PCBCL in Europe appears to be associated with infection with three main genotypes of B burgdorferi sensu lato (sensu stricto, garinii, afzelii). The purpose of this study is to analyze the impact of Borelia serology on clinical presentation, evolution and outcome.

Patients and methods: During the 1990-1995 period, 28 patients with newly diagnosed PCBCL were observed in the IORS and analyzed in December 2000. The lymphomas was CD 3 negative and showed expression of B-cell markers with restriction to only one light chain type. IgG Borelial serology was tested with three ELISA tests and 3 confimatory Western Blots (WB), one of them differential. Results: Out of 28 patients, 15 presented as a single noduloplaque, 10 as clustered noduloplaques, 3 as generalized cutaneous involvement. 18 had high grade and 10 low-grade histology. The distribution of the lymphomas types was the following: 4 had large cell lymphoma of the leg, 14 PCC lymphoma of head/neck, 5 immunoblasts and 3 Cutis lymphomas. Positive Borelial serology was found in 14 of 20 cases (70%) of cases versus 2600 age and demographically matched healthy controls, 25% of breast cancer patients and 25% of lymphoma patients. The differential WB, on basis of preferential reactivity was most often positive for B. afzelii, less so for B. garinii; two of the positive cases had acrodematitis chronica atrophicans. All four major PCBCL clinical types were identified in Borelia positive cases, but this group presented more frequently with less extensive cutaneous involvement. Systemic treatment was used in 7 patients and relapse in 6 Borelia positive cases versus 10 in the negative group. IgG was effective in one patient from each group. At relapse, two high-grade histology patients had a low-grade disease. Three patients from the Borelia positive group died, one of lymphoma, two of stage III Borelia.

Conclusion: PCBCL arising in patients with positive Borelial serology indicates active infection. Borelia positive cases may present with different PCBCL clinical types. Lymphoma deaths were less frequent in the Borelia positive group. Further clinical observations are needed to define natural history of Borelia associated PCBCL.

PRIMARY CUTANEOUS TUMOR LYPHOMPROLIFERATIVE DISORDERS WITH SIMULTANEOUS EVIDENCE ON CD4 AND B-CELL CLONALITY.

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Introduction: Presence of a clonal bi-rearranged (TCR-γ/δHT) cell population in nodal malignant lymphomas is a rare but known phenomenon. This feature has been reported in very few cases of primary cutaneous lymphoproliferative disorders (PCLPD) (mainly unclassifiable disease) but precise clinicopathological features of these cases were not specified.

Methods: From approximately 1100 cases with known results of both TCR and IgH rearrangement studies (automated high-resolution PCR fragment analysis), we have found 7 cases of PCLPD showing simultaneous evidence of T- and B-cell clonality among (in three patients), one is living and 3 were lost to follow-up.

Results: In 3 patients the disease classified histologically as extranodal marginal zone lymphoma (MML), MALT type with a high number of reactive T-cells in 2 of these cases. Two patients displayed features of the immunoblastic subtype of diffuse large B-cell lymphoma (DLBCL). In both cases a reactive T-cell infiltrate was conspicuous. The other two cases were categorized as unclassifiable disease (UCT) and demonstrated similar clinicopathological features, namely atypical medium-sized cells in an infiltrate composed of approximately equal numbers of T- and B-cells with the presence of epithelioid histocytes arranged in small granulomas.

P Age/Sex Sm/ML Location Diagnoses T/B KL IgH MCL
1 30 M S UA MZL T+B x+ +
2 71 W S Tr MZL T+B x+ + +
3 86 M S LL MZL B+T NI
4 66 W S Hi UD T+B x+ + +
5 58 M S Tr UD T+B x+ + +
6 69 W MI LL DLBCL B+T x+ + +
7 55 M MI LL DLBCL B+T x+ + +

M=male; W=woman, S=solitary, M=multiple, T=B-T and B-cells infiltrate UA=upper arm, Tr=trunk, LL=lower leg, H=hepat, N=not informative, N+not done

Conclusions: Bi-rearrangements in PCLPD are found less frequently (<10%) compared to nodal lymphomas (5%). In our series most cases belonged to B-cell lymphomas, MALT-type similar to nodal lymphomas. Presence of T-cell dominant clones in B-cell lymphomas might be due to oligoclonal expansion of reactive T-cells.

PRESENCE AND ABSENCE OF ITG4L1(14;18) IN PRIMARY FOLLICULAR LYMPHOMA OF THE SKIN.

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Introduction: Primary cutaneous follicular lymphoma (PCFL) is an indolent disease. Whether the absence of ITG4L1(14;18) is a distinctive feature of PCFL from nodal follicular lymphomas (NFL) is under debate. Our objective was to search for ITG4L1(14;18) in a series of PCFL by molecular and cytogenetic techniques.

Methods: 4 patients with PCFL had a negative staging including BM biopsy and complete CT scan. Follow-up did not show extracutaneous involvement. The search for ITG4L1(14;18) MBR breakpoint was performed by PCR and Southern hybridization with a detection threshold of 10-". Interphase FISH was performed on frozen sections with a dual color probe and a 5% cut-off value.

Results: The ITG4L1(14;18) breakpoint was detected in skin of 9/14 patients (22%) but was not associated with clinical, or histopathological feature, except an older age (mean 72 years). Seven of these 9 positive patients have been in complete response (mean follow-up 35 months). We analyzed peripheral blood of these 9 patients and found in 4 of them a similar ITG4L1(14;18) breakpoint by Southern hybridization. Interphase FISH was performed on skin sections of the 9 PCR positive and of 15 PCR negative cases. No case exhibited a significant number of fusion signals. An amplification of bcl2 gene was seen in 2 of 9 PCR positive cases.

Conclusion: Our data suggest that the molecular detection of ITG4L1(14;18) breakpoint in some PCFL may be due to (14;18)-positive B-cells emigrating from peripheral blood.
BONE MARROW PATHOLOGICAL AND MOLECULAR ANALYSES DO NOT PROVIDE FURTHER PROGNOSTIC MARKER IN CUTANEOUS T-CELL LYMPHOMAS (CTCL)

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Introduction: We previously showed that PCR detection of an identical cutaneous and blood T-cell clone has an independent prognostic value in Mycosis Fungoides (MF) and Sezary syndrome (SS) (Reyrolle-Barry M, J Invest Dermatol 2001). This study was undertaken to determine the prognostic value of bone marrow (BM) pathological and molecular analysis.

Methods: We studied 53 patients with MF and 7 with SS. Independent BM analysis was performed by two hematopathologists and included immunohistochimistry for B and T-cell detection. For TCRγ gene analysis, multiplex PCR-DGGE was performed initially on blood, BM and skin frozen biopsy. Progress was assessed in terms of disease progression.

Results: BM was involved in only 1 case (SS). An interstitial T-cell infiltrate was observed in 9 cases but was not associated with disease progression. BM was normal in the other cases. Monoclonality was detected in skin in 44 cases, 21 of them had an identical T-cell clone in blood and 16 of them in BM specimens. Multivariate analysis confirmed that both initial clinical stage and blood clonality have an independent prognostic value (p<0.01). Detection of an identical T-cell clone in skin and blood had an unfavorable independent prognostic value (p=0.103), but no additional statistical difference was observed for BM monoclonality (p = 0.97).

Conclusions: Both histological and molecular staging of BM did not provide prognostic information besides clinical staging and molecular study of skin and blood, suggesting to avoid such investigation in epidermotropic CTCL.

TREATMENT OF ADVANCED MYCOSIS FUNGOIDES AND SEZARY SYNDROME WITH ALLOGENIC STEM CELL TRANSPLANTATION WITH NONMYELOABLATIVE REGIMEN.

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Introduction: Mycosis Fungoides and Sezary syndrome are primary T-cell lymphomas involving the skin. Although the natural history of MF is usually indolent, many patients eventually progress to more advanced stages of the disease with shortened survival. Concomitantly Sezary syndrome (SS) has a poorer prognosis with a median survival ranging between 2-4 years from diagnosis. Autologous bone marrow transplantation has been used in few patients with advanced MF/SS, but the short duration of clinical response induced investigators to delay longer perspective studies. Only two reports with MF/SS treated with conventional allogeneic bone marrow transplantation have been reported showing a graft-versus-tumor (GVT).

Methods: Given the high transplant related mortality associated with conventional allogeneic bone marrow transplantation, we performed nonmyeloablative transplantation of allogeneic stem cells (ASCT) from HLA-identical siblings in 3 patients with this disease. These conditioning, which generally include fludarabine, have been designed to be immunosuppressive rather than myeloablative, to facilitate donor engraftment with limited toxicity.

Results: All patients fully engrafted, achieving 100% donor chimerism on peripheral blood CD3+ T-cells since day +28, and 100% donor chimerism on unfractoned bone marrow on day +100. Between day +30 and +60 after all patients had completely cleared the clonal T-cell in the skin, lymph nodes, in bone marrow and in peripheral blood; clinical, histological and molecular remission were durable (16 and 12 months respectively in patient 1 and 2). All patients achieved full donor engraftment, but presented high incidence of infections, in fact the post-transplant course of patients was complicated by multiple infections , including CMV reactivation, isolation of Staphylococcus aureus from blood and IC related hemorrhagic cysts. On day +71 the patient developed a sudden progressive heart failure, and the patient died on day +74.

Conclusions: These results suggest that nonmyeloablative ASCT is a novel and potentially curative therapy for patients with cutaneous T-cell lymphomas.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) INDUCES CLINICAL, MOLECULAR AND CYTOTOGENIC REMIS SIONS IN PATIENTS WITH REFRACTORY SEZARY SYNDROME (SS) AND ADVANCED MYCOSIS FUNGOIDES (MF)

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Introduction: SS is a leukemic variant of MF associated with generalized erythroderma, adenopathy and circulating lymphoma cells in the peripheral blood (PB). MF can also progress to tumor-stage disease. Both of these advanced manifestations are associated with severe symptoms and a poor prognosis.

Methods: Allogeneic HSCT was performed in six pts (ages 21, 22, 44, 46, 49 and 59) with refractory disease. Sezary syndrome (n=5) and tumor-stage MF (n=1). Median number of prior therapies (excluding corticosteroids) = 7 (range 3-8). Donor-recipient pairs: all 6 pts had a HLA identical PB donor, recipient cell receptor gene rearrangements (TCRγδ) and 4 had also a clonal population detectable by flow cytometry, correlating with the presence of circulating Sezary cells. Three pts had clonal PB cytogenetic abnormalities. All pts had severe clinical symptoms and skin disfigurement before HSCT. Pts underwent HSCT from a 6/6 HLA-matched donor: sibling (n=4), unrelated (n=2). Conditioning regimens were radiation-based in 3 pts and chemo-only in 3 pts. Two pts received a "reduced-intensity" regimen of fludarabine/mitotaneplan. Source of stem cells: 4 pts received bone marrow and 2 received G-CSF primed PB stem cells. GVHD prophylaxis: CsA -6 pts, MTX - 3 pts, MMF - 3 pts, MPSE - 2 pts.

Results: All 6 pts achieved a CR within 30-100 days after HSCT with resolution of skin lesions and symptoms, and 5 pts remain alive in CR at 3, 11, 17, 45 and 65 months post-HSCT. Repeat TCRγδ, flow cytometric and cytogenetic studies demonstrate that the clonal T-cell populations became undetectable within 30 days after HSCT and have not recurred. Mild acute and chronic GVHD developed in all pts, however one pt NOT CR died 16 days after HSCT from GVHD. KPS at the time of this report 80-100% for the 5 surviving pts.

Conclusions: Allogeneic HSCT can induce sustained clinical, molecular, flow cytometric, and cytogenetic remissions in pts with refractory, heavily pretreated and otherwise intractable SS and tumor-stage MF. A graft-versus-lymphoma effect may play a role in inducing CR and preventing relapse in this setting. The use of "reduced-intensity" transplant regiments may allow further application of this modality in this difficult to treat and generally older patient population.
IMMUNOGLOBULIN HEAVY CHAIN GENE REARRANGEMENT IN INTRAVASCULAR LYMHOPLASMA last 135
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Introduction: Intravascular lymphoma (IVL) is an unusual type of non-Hodgkin lymphoma (NHL). Although a systemic disease, isolated involvement of the nervous system is frequent which poses a diagnostic challenge. Typically located in the deep white matter of the hemispheres, the brainstem or the spinal cord, the lesions are frequently inaccessible to biopsy. Here we present our experience with analysis of immunoglobulin heavy chain gene rearrangement (IGHKR) in three cases with predominantly neurologic presentation.

Methods: 5 μm sections were obtained from paraffin-embedded tissue and deparaffinized in xylene. DNA was isolated using the QIAamp DNA Mini Kit (QIAgen) according to the manufacturer's protocol. In cerebrospinal and vitreous fluid specimens, cells were separated by centrifugation. The cell pellet was resuspended in 100μl of the supernatant and boiled for 10 minutes. The lysate was used for polymerase chain reaction (PCR). Amplification was performed by semi-nested PCR using universal primers for the Vκ- and Dκ-region of the IGH gene, as published previously. PCR products were separated on a 12 % polyacrylamide gel. The gel stained in ethidium bromide (1 μg/ml). Clonal rearrangement was defined as the occurrence of identical bands in PCR assays of at least two aliquots of the same specimen.

Results: Three patients who presented to the neurology service of MGH or BWH with suspected IVL were analyzed for IGHKR. Clonal IGH rearrangement was detected in all cases. In one patient, it was identified in DNA isolated from a stereotactic brain biopsy, in another one in cerebrospinal fluid. IGHKR analysis on vitreous and spinal fluid of the third patient was negative for clonality. In this case, diagnosis remained unclear until a skin rash developed which histopathologically was consistent with IVL. This was confirmed by IGHKR analysis of DNA isolated from the skin biopsy. A morphological diagnosis of IVL of the nervous system was made in only one of the three patients.

Discussion: IGH gene rearrangement has been described in case reports or autopsy series of cases with extraneural disease as a molecular marker for IVL. We report three antemortem diagnosed cases in which the nervous system was primarily affected. B-cell clonality was shown in all of them. In one case diagnosis was based on rearrangement studies in combination with radiographic and clinical findings. Based on these studies, we believe that IGHKR analysis may be a sensitive tool to diagnose IVL, even in cases where only paucicellular specimens such as CSF are available for diagnosis.

IMMUNOHISTOCHEMICAL ANALYSIS OF POTENTIAL CHEMORESISTANCE PARAMETERS IN PATIENTS WITH T/NK NSAL TYPE NON-HODGKIN LYMPHOMA
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Introduction: T/NK lymphoma is generally considered as a rare poor prognosis. Mechanisms of chemoresistance are poorly understood in this patient population. We have analyzed immunohistochemistry (IHC) of fixed paraffin samples from 15 NHL Patients and Methods: There were 8 men and 7 women. The median age was 48 with a range of 24 to 84 years. Thirteen patients had ENT localization at diagnosis. All patients had normal nodes, orbita, skin and subcutaneous tissue, digestive tract, bone marrow and central nervous system. All patient samples were analyzed using standard markers such as CD3, CD4, CD8, CD19, CD20, L26 and EBV RNA. The data is shown in all patients' clinicopathological parameters analyzed in this study by IHC were classified as being involved in: a) drug efflux or sequestration: Pgp protein (responsible for the classical MDR phenotype), MRPI, LRP; b) regulation of apoptosis (bcl-2, bax, -bcl-X), c) cell cycle regulation (Ki67, p53, p21) and d) enzymes involved in drug targeting or DNA repair (DNA polymerase, topoisomerase II, GMRT).

Results: Efflux proteins were expressed in few patients: 3 samples were positive for Pgp, 5 for MRPI and 4 for LRP. Convergent cell cycle regulatory proteins were found to be expressed in most patients: 12 cases stained positive for p53 and RB protein. Bax, Bcl-X and bcl-2 were expressed in 14, 12 and 8 cases, respectively. Enzyme staining, under the conditions used, was detected in only one case each. We did not observe a correlation between drug resistance and freedom from progression or overall survival with any of the parameters analyzed in this limited series.

Conclusion: Classical MDR and MRPI do not appear to be a predominant mechanism of chemoresistance in T/NK NHL. We did not observe a reduction in the proapoptotic protein Bax. p53 protein was detected in a majority of samples suggesting that alterations in p53 structure or function may be important in the tumorigenesis or chemoresistance of this subtype of lymphoma.

In vivo Bone-Marrow Biopsy Diagnosis of Intravascular Lymphoma.
A De Ritis, R. Tofani, F.C. Sass, A. Segretino, L. Inamato, G. Pertinio, O. Carbonara, G. Politi, B. Rotoli. Chair of Hematology and Pathology.University Federico II, Internal Medicine and Haematology,Unit, Naples. Italy Simona, Italy Intravascular lymphoma (IVL) is a variant of large cell lymphoma developing within the lumens of blood vessels, with little or no involvement of adjacent tissues. IVL is a multorgan disease that in particular affects skin and central nervous system (multiple neurologic deficits and progressive dementia). IVL has an extremely poor prognosis and is often diagnosis post-mortem.

Case #1. In April 2000, a 50 year-old man developed transient right facial palsy of central type, 2 weeks later he suffered from a grand-mal fit, was somnolent and quadriaparetic. Blood analyses showed mild pancytopenia with low CD4+ cells. Serum levels of LDH, ALT, Aft, ferritin and beta-2-microglobulin were increased. Laboratory investigations of CSF and serum did not reveal any infectious disease. Brain MRI showed evidence of demyelinating bi-emisferic lesions. Chest Xray revealed pulmonary lesions. The bone marrow biopsy revealed several small and medium blood vessels filled with atypical large cells bearing B-cell markers. The positivity of endothelial cells for Factor VIII demonstrated the intravascular localization of the atypical lymphocytes confirming the diagnosis of IVL. The patient was treated with two courses of chemotherapy, but he died of disease progression 2 months after diagnosis and 6 months after the initial presentation.

Case #2. In December 2001, a 41 year-old woman was admitted for a persistent fever associated with hepatitis-B and peripheral and central nervous system symptoms. Blood counts showed severe anemia, thrombocytopenia. Serum levels of LDH, ALT, Aft and fibrinogen were increased. She underwent ophthalmologic pathology. Pathological signs of spleen and bone marrow biopsy were diagnostic for a large B-cell IVL. CT body scan revealed ascites, pleural exudation, but not brain involvement. The patient is currently treated with the chemotherapy regimen MACOP-B: all clinical symptoms and laboratory signs, but anemia, have improved.

IVL are characterized by an extreme polymorphic clinical presentation with a prevalence of neurologic manifestations. These features hinder the diagnostic process and may affect the prognosis that depends on early diagnosis and early treatment. Here we have reported two patients that presented with prevalent neurological symptoms and have been diagnosed by bone marrow biopsy.

SERUM LEVELS OF THE nm23-H1 PROTEIN AND THEIR CLINICAL IMPLICATION IN EXTRANODAL NK CELL LymphOMA

Introduction: The nm23 gene was first isolated as a gene, the product of which suppresses tumor metastasis. Recently, we have established an ELISA method to determine the serum levels of nm23-H1 protein, and reported that serum nm23-H1 may be a new prognostic factor for non-Hodgkin's lymphoma (NHL) and AML. The present study was undertaken to assess the clinical implications of the levels of this protein on NK cell lymphoma (NK-NHL) and to determine whether our assay would be useful for planning treatment strategy.

Methods: The serum levels of nm23-H1 protein were measured in 60 patients with untreated NK-NHL by the above ELISA method. These patients received chemotherapy and/or radiotherapy between 1990 and 1998.

Results: The level of serum nm23-H1 protein of patients with NK-NHL was higher than that of healthy individuals. The overall survival (OS) and progression-free survival (PFS) for NK-NHL patients with a high nm23-H1 level were significantly poorer. OS and PFS were analyzed in both L+L- and H+H- risk groups of IFI, and the results showed that both OS and PFS rates were significantly poorer for those with high nm23-H1 levels in each risk group.

A multivariate analysis confirmed that the level of nm23-H1 is an independent and important prognostic factor for NK-NHL.

Conclusion: The prognosis of NK-NHL patients treated with standard chemotherapy is poor, and their clinical course is highly aggressive. Thus, serum nm23-H1 development which was shown by the present study to be an independent prognostic factor may provide clinicians with useful information in determining the appropriate therapy for patients with NK-NHL.
ENTEROPATHY ASSOCIATED T-CELL LYMPHOMAS: CLINICAL EXPERIENCES

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Introduction: Small bowel lymphomas are 20-40% of primary gastrointestinal lymphomas, and are commonly T-cell origin. Enteropathy associated T-cell lymphoma (EATCL) represents approximately 7% of all primary gastrointestinal lymphomas, occurs as one of the most serious complications of celiac disease. In 1978 Laszlo and Wright described patients with small intestinal lymphoma associated with villous atrophy and crypt hyperplasia of uninvolved mucosa. Nowadays it is recognised as a separate entity in the REAL classification.

Our results: Between 1984-2000 32 primary gastrointestinal non-Hodgkin lymphomas cases were observed (24 small intestine, 8 colon). Eight of 24 small intestinal lymphomas were enteropathy associated T-cell lymphoma. Median age is 56 years (36-71), male/female ratio is 3/5. Six patients had no clinical signs of malabsorption. 2 of 8 had long history of celiac disease for years keeping precise gluten-free diet. The most common symptoms of the patients were abdominal pain, acute obstruction, and spontaneous perforation. Diagnosis was confirmed by immunopathological analysis of biopsy samples in 2 cases, and resection due to surgical intervention. Stage at diagnosis was I-II in 3, III-IV in 5 patients. All 8 patients treated with combined chemotherapy (CHOP or ProMACE-MOPP) regimens. The median follow up is 12.8 months (6-43). Only one of them achieved complete remission, 7 died within one year.

Conclusion: Enteropathy associated T-cell lymphoma is rare aggressive malignant disorder, prognosis is very poor because of the nature of malignant cells, and diffuse infiltration of the mucosa of small bowel. Most patients presented with abdominal surgical emergencies due to perforation or obstruction of small intestine diagnosed the entity in advanced stage.
7. Indolent Lymphomas/Myeloma/CLL

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ANALYSIS OF TREATMENT MODALITIES APPLIED IN FOLLICULAR LYMPHOMAS IN FRANCE.

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Follicular lymphomas (FL) are indolent diseases which diagnosis and management are widely spread among medical specialties.

Introduction: The aim of the study was to observe diagnostic and therapeutic approaches in previously untreated patients (pts) with FL.

Methods: 145 centers involved in lymphoma management have accepted to participate in this registry. Systematic review of pathologic material was performed by one of us (N.B.) while registered data were regularly monitored by the scientific committee.

Results: During a 3-year time period (1995-98), 1110 pts have been included (484 in 36 Haematology, 378 in 49 Oncology and 240 in 60 Internal Medicine centers). Median number of pts registered per centre was 4 (range: 1 to 58). Main characteristics were as follows: median age (range 38, range 24.5 to 95), sex-ratio (1), stages III-IV (70%), >7 cm tumour mass (33%), B symptoms (18%). Bone marrow biopsy was performed in 95%, involved in 35% of pts. LDH and Benzoquinolothione levels were documented in 97% and 86% respectively, elevated in 21% and 20%. Systematic review of tumour diagnostic sample by an expert pathologist was performed in 46% of pts. Search for molecular abnormalities was done in 20% of the pts.

We then observed treatment strategy in pts not included in trials (838 pts - 75.5%), dividing pts into three groups i.e. stages I (group 1 - 185 pts), stages II to IV with low (group 2 - 327 pts) and high tumour burden (group 3 - 325 pts). We tried to answer the following questions: is the optimal pretherapeutic staging in patients with FL? Does the optimal staging in patients with FL depend on the tumoral burden?

Conclusion: Prospective study confirmed that the initial staging is crucial in lymphoma management. It is a key point to include the appropriate treatment in the optimal treatment plan.

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PROSPECTIVE STUDY OF INDOLENT NON-POLLUCULAR NON-HODGKIN'S LYMPHOMA: VALIDATION OF GERMAN PERSPECTIVE STUDY ORIENALI (GESP) PROGNOSTIC CRITERIA FOR WATCH AND WAIT POLICY

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Background: Only recently both the Revised European American Lymphoma (REAL) and World Health Organization (WHO) classifications clearly identified indolent follicular non-Hodgkin's lymphomas (NHL) as a distinct group of non-Hodgkin's lymphomas. Therefore, prognostic models, specifically designed for this NHL subset, are still lacking. In this study, we prospectively evaluated the prognostic criteria proposed by the Gruppo Italiano per lo Studio dei Linfomi (GIST) to identify patients with indolent non-progressive clinical course, eligible for a watch and wait policy within this histological subset defined according to stringent criteria of histomorphology and immunophenotype. Methods: Fifty-three patients affected with small lymphocytic, marginal zone, lymphoplasmacytic lymphomas and lacking all the following features: B symptoms, bulky disease, anemia, thrombocytopenia, diffuse pattern of bone marrow infiltration and short tumor doubling time were recruited in a prospective therapeutic GIST trial and addressed to a watch and wait protocol. Results: After 41.3 months of median follow-up, the median progression-free survival (PFS) was not reached and 73% of cases did not progress. When additional variables were considered, in order to improve the prognostic model, NHL level and number of extranodal sites resulted of statistical significance in multivariate analysis. Based on this finding, a prognostic score was devised able both to further identify a small group of patients more likely to undergo early progression, thus suitable for immediate treatment. Conclusion: The GIST definition of indolent disease is a reliable tool to design the appropriate therapeutic strategy in this histological setting.

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PROGNOSTIC FACTORS AND SURVIVAL IN INTERMEDIATE GRADE NON-HODGKIN'S LYMPHOMA IN SOUTH ASIA

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NHL constitutes nearly 3.5 of all malignancies at the cancer institute, Chennai India. NHL patients treated between 1985 and 1993 form the basis for this study (n=212). The male to female ratio was 2.2:1. The median age was 38 years (range 3 to 88). Among the 212 patients, 110 (51.9%) were treated with an intensive iophosphamide based protocol and the rest with standard CHOP (102, 48%). Analysis revealed no difference in CR rates, OS and DFS between these two groups. Hence both groups were combined for analysis. The overall CR rate was 71.2% with a 5 year OS and DFS of 57.4% and 53.7% respectively. CR rates and 5 year OS among the different risk groups according to IPI International prognostic index are as follows - low risk 89.2% and 71.4%, low intermediate 77.4% and 70.3%, high intermediate 72.3% and 49.6% and high risk 54.5% and 41.5% respectively. The five year DFS for the above groups are 67.4%, 65.9%, 43.2% and 36.3% respectively. Tumor mass (hazard ratio 1.63, 95% CI 102.6) and systemic B symptoms (hazard ratio 2.87, 95% CI 1.89 - 4.34) emerged as the important prognostic factors by multivariate analysis. The other prognostic factors like age, sex, extra nodal sites, serum LDH were not statistically significant. The intensive protocol produced significant morbidity especially myelosuppression and was associated with prolonged hospitalization.

Result: There is no difference between CHOP and the intensive chemotherapy regimen with regard to CR, OS and DFS. Hence CHOP should continue to be the standard of care in developing countries in view of lower morbidity. Patients with high risk IPI did poorly and bulky disease and B symptoms emerged as the most important prognostic factors.

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Introduction: It is widely acknowledged, that bulky tumors have an unfavorable prognostic influence on local recurrence of non-Hodgkin's lymphomas. The purpose of our study was to investigate this assumption.

Methods: 432 therapeutically naive adult patients with morphologically proven diagnosis of non-Hodgkin's lymphoma, stage I-II were treated in 1983-2000. 406 of them showed the response to the treatment and were included in the presented study. The treatment programs were: chemotherapy (69 pts), radiation therapy (82 pts). The response was evaluated every 2 months. Recurrences were classified as "true" (in the sites of initial involvement), "generalized" (in the new sites only), and "mixed" (in the initial and new sites).

Results: 196 patients (48.9%) relapsed after a median follow-up of 6.8 years. There was no statistically significant difference between three groups in the total number of recurrences, in the true and mixed recurrences. The group of patients with bulky tumors had the highest risk of generalized recurrence - 43.0%, while the group with middle-sized tumor - only 27.9% (p=0.05). The total risk of recurrence and the risk of the local recurrences were lowest in the group with middle-sized tumors.

Tumor size Number of pts All recurrences Mixed recurrences Generalized recurrences

1-4 cm 96 47.9±5.1% 8.3±4.8% 4.2±2.0% 33.4±4.9%

5-9 cm 148 39.2±4.0% 4.7±1.7% 7.4±2.7% 27.0±2.3%

>9 cm 162 56.2±5.3% 7.4±1.5% 7.4±2.1% 43.5±5.9%

*p<0.05

Conclusion: It was statistically shown that bulky tumors (10 cm and more) are the most prognostic factor of the generalized but not of the local recurrences in the case of the local distribution of non-Hodgkin's lymphomas. Further precise studies of small-sized tumors (<1 cm) should be performed to investigate the unexpectedly high number of recurrences in this subgroup of the local NHL.
Expression of Basic Fibroblast Growth Factor is Associated With Poor Outcome in Non-Hodgkin’s Lymphoma

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High pretreatment levels of basic fibroblast growth factor (bFGF) have been shown to be associated with poor prognosis in patients with non-Hodgkin’s lymphoma (NHL). The aim of this study was to evaluate whether NHL cells express bFGF and/or its receptor (bFGFR-1) and whether bFGF expression correlates with bFGF serum levels, intratumoral microvessel density, and patient outcome. We measured bFGF by ELISA in sera taken from 58 patients with NHL before treatment and in 19 also after treatment. Pathological specimens at diagnosis were evaluated by immunohistochemical staining to determine the expression of bFGF and bFGFR-1 and the microvessel count (by factor VIII-related antigen). The lymphoma specimens demonstrated positive staining for bFGF (23%) and bFGFR-1 (18.5%). The patients who expressed bFGF had a significantly worse progression-free and overall survival than those who did not (p<0.003 and p=0.03 respectively, see Figures below), while patients expressing bFGFR-1 were less likely to achieve complete remission than those lacking the receptor (33% VS 65%, p=0.047). There was no correlation of bFGF staining with either serum bFGF levels or microvessel counts. bFGF serum levels did not change significantly after treatment. These results suggest that NHL specimens express bFGF and its receptor (bFGFR-1) and this expression is associated with poor patient outcome.

POTENTIAL VALUE OF QUANTIFICATION OF CIRCULATING T(14;18)-BEARING CELLS TO IMPROVE RESPONSE EVALUATION IN FOLLICULAR LYMPHOMA

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Introduction and methods: To evaluate whether quantification by real-time PCR of circulating (14;18)-bearing cells (CC) in blood may be related to tumor bulk, response and outcome, we surveyed 19 patients (pts) with t(14;18)-bearing advanced follicular lymphomas during first-line chemotherapy (main features: 62.3 y median age, 69% stage IV, 58% bone marrow involvement, 32% elevated LDH, 56% elevated E2-microglobulin, 21% transformed). Results: We first studied CC amount at the onset (median 1656 cells/ml - 0 to 27372). In 4 pts, no CC were detected. In the remaining 15 pts, no relationship with tumor bulk-related factors (Ann Arbor stage, bone marrow involvement, LDH and E2-microglobulin) was observed. In 11/15 pts, the cell count were found (Mann Whitney test) although the highest amounts (>5000 cells/ml) were only found in pts with bone marrow involvement. To evaluate quantitative variations with time, we considered the following groups: blood CR (down to no CCI), blood PR (>1 log decrease), blood stabilization (stab.), (variation <1/-1 log) and blood progression (>1 log increase). Response at the end of treatment was compared to blood response: among 7 CR, we observed 5 blood CR and 2 blood PR; among 6 PR, we observed 2 blood CR, 1 blood PR and 3 blood stab.; one stab. had blood stab. but one progression had blood CR. Four out of 5 pts with CR/blood CR are still in CR at 12 to 68 months but one relapsed at 17 months. All remaining pts either relapsed or progressed. In conclusion, these data tend to show that CC amount, as here evaluated, is not strictly parallel to tumor bulk and may even decrease during progression. However, pts with CR with blood CR tend to have a better outcome than other pts.

Conclusion: Overall, these results tend to show that quantification of (14;18)-bearing circulating cells should be considered as a target valid to evaluate rather more than a summary of the overall response.

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CLINICAL RELEVANCE OF GENOMIC IMBALANCES IN FOLLICULAR LYMPHOMA

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In follicular lymphoma (FL), there is a wide variation of the clinical courses and therapeutic options. Clinical and biological parameters allowing a reliable risk assessment are required. We analyzed 128 biopsy samples of patients with follicular lymphoma using Comparative genomic hybridization (CGH). In 90 cases (70.3%) genomic imbalances were detectable. The most frequent aberrations were gains of chromosome arms 7q and 16q (10%, each), 7q (16%), Xp (15%), 12q (13%) and 15q (12%), as well as losses on 6q (19%). Furthermore, we analyzed a series of 82 patients with a comprehensive clinical data set. The median observation time was 45 months (range, 2 to 119 months). The number of cases with a complex karyotype (2 or more imbalanced aberrations) was increasing dependent on FL grading according to the WHO classification: FL1 vs. FL2 and 3, p<0.0001. In a multivariate model, the clinical risk factors of the iPI and genomic imbalances, which showed some association with survival in univariate testing (p<0.02), were included. Serum lactate dehydrogenase (LDH) values, involvement of more than 2 extranodal sites and age more than 60 years were independent prognostic factors as well as deletions on chromosome arm 6q. Using univariate analysis, particularly deletions on 6q25q27 were significantly associated with poor outcome (p=0.0001). In the multivariate model, 6q25q27 deletion was the most powerful prognostic factor in FL (relative risk ratio 6.4, adjusted LDH value 5.1; more than one extranodal site 4.9; age older than 60 years 2.3). These data indicate that (i) genomic imbalances are of prognostic relevance in FL and (ii) that there may be a pathogenetically important tumor suppressor gene on chromosome bands 6q25q27.

PROGNOSTIC VALUE OF T(14;18) IMMUNOPHENOTYPE IN MAJOR SUBSETS OF MULTICENTRIC GROSSENUMMER: 146023012 AUTOPSY MATERIALS FROM A LUNG TUMOR: A MULTICENTRIC STUDY OF INTRACELLULAR LOCALIZATION OF IMMUNOREACTIVE MARKERS

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Introduction: The immunohistochemical and clinical data support the concept that intracellular expression of undetected significance (IMSS) are true prognostic conditions. The initial tumor burden assessed by the plasma cell (PC) content at BM smear, or by the amount or the type of the M-component (MC), are prognostic factors widely used to predict the malignant transformation. The purpose of our study was to demonstrate the prognostic significance of the initial tumor burden as assessed by the BM biopsy.

Methods: 189 fully autopsied patients with non-IgM MGUS, without hemoglobulin, renal and skeletal abnormalities, underwent BM aspiration and biopsy at presentation. In all patients, both the number and the density of PCs in the BM, histologic patterns were defined as follows: normal, monoclonal plasmaerythrophagocytosis (MPE), intracellular microspheres, medulla, and anaplastic lymph shredding (BNL) and lymphoma. Patients were followed up according to the "wait and one" strategy until progressing to symptomatic disease.

Results: After a median follow-up of 38.5 months (range 4.3-276.7), 14 patients (7.4%) and 1 patient (0.5%) developed lymphoma and lymphoma respectively. Only 1 out of 95 patients with normal histology and 13 out of 94 patients with abnormal histology developed overt disease. According to the histological patterns, the outcome of the 189 patients is summarized in the table below. The Kaplan-Meier analysis showed that patients with normal histology pattern have significantly lower risk (p<0.0001) of developing medulomas. There were no differences when the patients were stratified according to the following cut-off: PC contents vs BM smears (%): p=0.42; amount of the MC (10g/ml): p=0.37; PC <2% and MC <10 p=0.57; IgAIsotype p=0.56, light chain p=0.32.

Histological diagnosis of N. Subtypes (%) Patients Patients

<table>
<thead>
<tr>
<th>N. of cases</th>
<th>BM</th>
<th>BM</th>
<th>Patients developing</th>
<th>Patients developing</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>90 (52)</td>
<td>94 (51)</td>
<td>2 (1)</td>
<td>1 (1)</td>
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<td>MP</td>
<td>39 (21)</td>
<td>38 (66)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bord. Myeloma</td>
<td>22 (12)</td>
<td>19 (63)</td>
<td>3 (17)</td>
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<tr>
<td>Lymphoma</td>
<td>15 (8)</td>
<td>1 (67)</td>
<td>1 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>NED</td>
<td>12 (6)</td>
<td>12 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>6 (3)</td>
<td>5 (83)</td>
<td>0 (0)</td>
<td>1 (17)</td>
</tr>
</tbody>
</table>

Conclusions: In our cohort, the histological pattern identifies a large number of patients with low tumour burden and discriminates different risk profiles more accurately than the percentage PCs as counted on BM smears, the type and the levels of the MC.
GENE EXPRESSION PROFILING IN SERIAL SAMPLES OF TRANSFORMED FOllICULAR LYMPHOMA

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Introduction: The transformation of FL to high grade lymphoma usually diffuses large B-cell lymphoma (LBCL), is accompanied by the successive accumulation of recurrent chromosomal defects. The resultant gene expression alterations are largely unknown.

Methods: We performed oligonucleotide microarray analyses using the Affymetrix HuFL chips on 5 cases with matched snap frozen lymph node biopsies before and after FL transformation. Expression data were analyzed using the Affymetrix Microarray Suite 4.0 and GeneSpring 4.0. The expression of selected genes was confirmed by quantitative real-time polymerase chain reaction (QRT-PCR) using a total of 7 serial cases. Candidate gene expression was further evaluated by immunohistochemistry (IHC) in an independent larger case series. The functional relevance of differentially expressed candidate genes is currently being tested in this transformation state of several lymphoma cell lines.

Results: 36 genes with increased expression and 66 genes with decreased expression were identified and functionally classified. The microarray results showed correlation with protein expression data obtained from samples at the time of initial diagnosis or transformation. The expression of over 30 candidate genes was evaluated by QRT-PCR with a 76% confirmation rate. Some of the identified genes, such as nucleolin, IRF4/UM1 and TIMP1 were already known to be associated with high grade NHL. Novel candidate genes with confirmed increased expression included NOTCH3, ABLE2, NEU1, M2X1, and p53. Consistently decreased expression was demonstrated for genes including PDCD1, TRAIL, (FGFBP3), and VDUP1. 11 studies of IRF4/UM1 are negative in 16 cases of FL (grade III), but positive in 69% of DLBCL that evolved from FL or other low grade lymphomas, and negative in 70% of DLBCL. Our data, in association with published profiles of de novo DLBCL, suggest that following transformation, germinal center derived FLs acquire a gene expression profile that is similar to post-germinal center activated B-cells.

Conclusions: Subsets of transformed FLs are associated with a distinct set of differentially expressed genes that might cooperate with BCL2 in the multi-step transformation process.

SUCCESSFUL INDUCTION OF PCR NEGATIVITY IMPROVES THE PROGNOSIS OF PATIENTS WITH B-CELL LYMPHOPROLIFERATIVE DISORDERS (B-LPD)

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Introduction: The ASCT and/or rituximab (R) alone or in combination have been shown to induce PCR negativity in a high number of pts with B-LPD in our study. We compared the outcome of pts with B-LPD according to their PCR status at therapy.

Methods: We performed clinical and molecular follow up of pts treated with ASCT, and/or R followed by ASCT in samples of bone marrow, peripheral blood and PBSC harvests (when available). The total of 57 pts were PCR informative and eligible for further monitoring. Median age of pts was 53 years (23-76). 23 were females (44%), 25 pts had FL, 3 DLCL. 9 MCL, 16 CLL or SCL, 4 others. 15 pts were treated by ASCT, 31 were given R with or without standard chemotherapy. 11 pts received R in vivo pending followed by ASCT. The median follow up of living pts was 24 months (1-81). The median number of previous therapy lines was 2 (0-7).

Results: 18 pts were positive for t(14;18), 5 pts for t(11;14) and 14 pts for CD23.
After therapy 28 (66%) pts converted to PCR negativity. In the R+ASCT the PCR rate was even higher (91%) while 4 of 5 pts converted to PCR- only after ASCT.

PCR neg

R

R+ASCT

All pts

60% (88% vs 17% at 2 y) (p=0.0025)
55% (86% vs 15% at 1 y) (p=0.0011)
91% (1/10 PCR- relapsed at 1 y) (p=n.s.)
56% (72% vs 29% at 2 y) (p<0.0001)

We have seen no difference in overall survival between any of the treatment groups.

Conclusions: ASCT and R led to PCR negativity in a considerably high number of pts with B-LPD. This number is even higher when pts complete the R therapy followed by ASCT. The PCR negativity is correlated with a better prognosis.

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AMPLIFICATION OF CDK4 IS ASSOCIATED WITH THE TRANSFORMATION EVENT IN NON-HODGKIN’S LYMPHOMA

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Introduction

Histological transformation of follicular center cell lymphoma (FCCL) to diffuse large B-cell lymphoma (LBCL) occurs in 25-60% of cases. This progression most often results in an aggressive disease, which is difficult to treat and displays a short median survival. We have previously analyzed a series of paired lymphoma biopsies, before and after transformation from FCCL, by comparative genomic hybridisation. A region of amplification on chromosome 12q12:14 was detected in more than 50% of the transformed LBCL cases and was not seen in the FCCL counterpart. These results suggested that the 12q amplon might be important in the transformation from low-grade to high-grade disease. We have therefore selected CDK2, GLL3, GADD153 and CDK4 as candidate genes from across the amplified region and investigated the amplification status of each.

Methods

The amplification profile of the candidate region was analysed using real time quantitative PCR (TaqMan 7000). Seven paired FCCL and LBCL cases were studied in addition to four separate DLBCL cases. The amplification status of each case was assessed by comparison with an endogenous control gene (GAPDH) on 12p13.3. A panel of normal DNAs were used as controls.

Results

Low-level amplifications were detected in 4/18 cases for CDK2, 2/18 for GLL, 1/18 for GADD153, 10/18 for CD4 and 6/18 for MD2. CDK4 amplification was identified in 73% of transformed DLBCL cases but in only 3/1 (29%) of the FCCL counterparts. This result suggests a strong association between the amplification of CDK4 and the transformation event.

Conclusions

We have identified amplification of CDK4 in association with lymphoma transformation. This gene might therefore be a potential target for prognostic tests or therapeutic intervention in non-Hodgkin’s lymphoma.

Long-term follow-up of minimal residual disease in patients with follicular and diffuse large B-cell lymphomas: The role of polymerase chain reaction detection of cells carrying t(14;18) in bone marrow and peripheral blood.

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The chromosomal translocation t(14;18) is present in about 60-85% of follicular lymphomas (FL) and in 10-20% of de nolo diffuse large B-cell lymphomas (DLBCL) and constitutes a good target for polymerase chain reaction (PCR) detection.

The PCR assay was used to detect bcl-2 rearranged cells in peripheral blood and bone marrow (BM) in 64 previously untreated patients with DLCL and 65 patients with FL. 34 FL patients (52%) and 11 DLCL patients (16%) had PCR-detectable lymphoma cells with bcl-2/IGH in BM and peripheral blood. Minimal residual disease (MRD) was evaluated in 25 FL and 8 DLCL patients undergoing an first-line chemotherapy. 6 DLCL patients (75%) but only 3 FL (12%) patient achieved molecular response (PCR-negative status in BM). 7 PCR bcl-2/IGH positive patients with FL were treated with rituximab (anti-CD20 antibody) and 6 of them (86%) had no PCR-detectable lymphoma cells in BM after the therapy. Peripherical blood stem cells (PBSC) were harvested in 5 FL (1 PCR-negative) and 2 DLCL (1 PCR-negative) patients. PCR-positive lymphoma cells contaminated PBSC in all patients with BM PCR-positivity before harvesting. 5 FL and 1 DLCL patients underwent autologous transplantation (AT). No bcl-2/IGH positive cells were detected in 5 patients (83%) after AT. One another patient achieved molecular response after rituximab treatment. 2 patients relapsed 1-3 years after localisation (6 months after AT), but specimens from BM were PCR negative. 1 DLCL patient had one PCR+ BM specimen (13 months after AT) without morphologically documented relapse. 3 FL patients are in CR, 42, 43 and 54 months respectively after AT.

Thus, the results show that PCR detection of the bcl-2/IGH rearrangement is a very useful method in evaluating of MRD. Autologous transplantation or rituximab immunotherapy can induce molecular response in a significant proportion of lymphoma patients.
SURVIVAL IN YOUNG PATIENTS (LESS THAN 40 YEARS) WITH FOLLICULAR LYMPHOMA (FL) - A POPULATION-BASED STUDY BY THE SCOTLAND AND NEWCASTLE LYMPHOMA GROUP
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Introduction
We have examined in a population-based study the survival of young patients (less than 40 years) with FL treated conventionally, as these are the patients most likely to be targeted for new treatment. This will help to indicate whether the increased toxicity of intensive treatment is justified by an improved outcome.

Methods
Data were derived from the Scotland and Newcastle Lymphoma Group (SNLG) database from 1986. Histology of all available cases was reviewed by one of us (KNW) to ensure that patients met the modern criteria for diagnosis of FL.

Results
Fifty five patients were identified as presenting with follicular lymphoma aged <40 years. Histological review confirmed the diagnosis of follicular lymphoma in 42 cases. There were 21 males and 21 females, median age 33 years (range 24-39). Thirty two patients had advanced stage disease (Stage IIIb and IV) at diagnosis. The majority of patients received treatment with chemotherapy, although one had surgery alone and another three radiotherapy alone. All patients with early stage disease allowed a complete response (CR) with initial therapy. Four relapsed and two have died (one of transformation to high grade NHL). Survival of patients presenting with stage IIIA disease was 70% at 10 years, and 75% in patients with stage IIIB and IV. The SNLG prognostic index predicted overall survival (intermediate interval significantly worse than low index, p = 0.041).

Conclusion
Our survival data of 70% overall survival at median follow up of 11 years provides a baseline for comparison with published data on a more aggressive approach to treatment. In this series of patients under 40 with confirmed follicular lymphoma, survival with conventional treatment was excellent.

LOW-DOSE TOTAL BODY IRRADIATION IN NON-HODGKIN'S LYMPHOMA PATIENTS WHO DIDN'T ATTAIN COMPLETE REMISSION OR IN RELAPSE AFTER CHEMOTHERAPY
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Purpose: To evaluate the efficiency and toxicity of low-dose total body irradiation (TBI) in treatment of NHL patients that did not attain complete remission (CR) or relapsed and were not eligible for transplantation.

Material and methods: 35 patients were treated in the NCI of Cairo since Sep. 1988. According to the WF classification, 11 patients had low-grade, 12 had intermediate grade and 12 had high grade NHL. The patients were given a TBI dose of 1.6 Gy given in two courses (separated by 2 weeks of rest) of each 4 daily fractions of 0.2 Gy.

Results: Ten patients achieved CR (28.6%), while 15 patients had partial remission (42.9%), 5 patients had stable disease (14.3%) and 5 (14.3%) progressed while under treatment. The 2-years progression-free survival (PFS) was 32% (SE = 15%) and the median PFS time was 12 months (SE = 6.4). Response to treatment was the only significant prognostic factor (p = 0.0257). The 2-year survival was 42% (SE = 11%) and the median survival was 17 months (SE = 3.3). Using the multivariate analysis Achieving CR was the only independent prognostic factor for overall survival (p = 0.0056).

Conclusion: Low lymphocytic percent before TBI was significantly correlated with longer response duration (p = 0.044) and overall survival (p = 0.028). A high percentage of CD4+ cells before TBI was significantly correlated with longer response duration (p = 0.027) and overall survival (p = 0.046).

Sixteen patients had leukopenia (median = 6 W). Thrombocytopenia occurred in 6 patients (median = 12 W). Anaemia occurred in 7 patients (median = 8 W). There were 3 toxic deaths among this group of patients, 2 due to intracranial haemorrhage in association with intractable thrombocytopenia and 1 due to sepsis.

Conclusion: TBI is a valid alternative to standard chemotherapy in treatment for NHL patients who did not attain CR or relapsed after chemotherapy and are not eligible for bone marrowstem cell transplantation. The pre-treatment percentage of lymphocytes and CD4+ cells may be used as predictors for response to TBI.

EXCELLENT RESPONSE RATES OF 90% LASTING FOR MORE THAN 2 YEARS IN A 42-YEAR-OLD FIELD RADIOTHERAPY IN 115 INCIDENT LYMPHOMA PATIENTS.
Departments of Radiography (1) and Pathology (2), The Netherlands Cancer Institute, Amsterdam and the Department of Radiotherapy, Bernard Verbeeten Institute Tuiting, The Netherlands.

Purpose: To study the efficacy of 4 Gy involved field radiotherapy (IF-RT) in incident lymphoma patients.

Patients and Methods: The patient population consisted of 57 females and 58 males, median age 60 years (range 35-93 years). These 115 evaluable patients all had recurrent or previously untreated indolent lymphomas, follicular lymphomas in 30 patients, B-CLL/SLL in 15, marginal zone, MALT, PLL, FL, and lymphoplasmacytic lymphoma in 2 cases. Median time since primary diagnosis was 44 months (range 3-355 months). Patients were pretreated by 1 median of 2 chemotherapeutic regimens (range 0-11). Bulky disease (>3 cm) was present in 70 patients, lesions over 10 cm were present in 20 patients. IF-RT dose was 2 x 2 Gy (interval 48 hours) in 79 patients, 31 patients received 1 x 4 Gy, 5 patients received 1 fraction of either 2 Gy (or 3 Gy) x 3/5. Endpoint of the study was in-field lymphoma control.

Results: IF-RT resulted in a response rate (RR) of 90% (n=103), 57% CR (n=60), 32% PR (n=35), 9% SD (n=10), 15% PD (n=2). RR was independent of sex, age (< or > 60 years), extent of prior treatment (< or > 2 regimens), time since diagnosis (< or > 44 months), tumor size (bulky or not) and radiotherapy regimen.

Conclusion: The median time to progression (MTP) is 26 months. At the time of analysis 53 of 103 responding patients are without progression up to 77 months. An i-field recurrence, in 37 patients that reached RR, developed in 20 patients (of whom 17 had bulky disease) after a MTP of 14 months. Of the 66 patients in CR, only 9 progressed in-field on an out-of-field (11 of them with bulky disease). The MTP in this subpopulation has not yet been reached, but exceeds 42 months.

As expected, toxicity was low. Only 2 patients showed a transient grade II lymphopenia. The MTP in this subpopulation has not yet been reached, but exceeds 42 months.

CONCLUSIONS: Low dose IF-RT up to 4 Gy in incident lymphoma patients is sufficient to provide excellent response rates, lasting for more than 2 years, without significant toxicity even in an intensely pretreated patient population. The 2 x 2 Gy regimen is now being compared with CORTACT in a prospective randomized phase III trial in previously untreated follicular lymphoma patients (NOVON - 96RCT). Pilot experience in 20 aggressive lymphoma patients shows similar results and is subject to further study.

FOLLICULAR LYMPHOMA, IMMUNOCYTOMA, AND MANTLE CELL LYMPHOMA: EVALUATION OF CURATIVE RADIOTHERAPY IN LIMITED STAGE NODAL DISEASE
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Introduction: In follicular lymphoma, early stage (Ann Arbor) nodal follicular lymphoma grade VII (FL), Immuno cytoma (IC), and Mantle Cell lymphoma (MCL) (REAL/WHO classifications) can potentially be cured by radiotherapy (RT) alone. The aim of this prospective multicenter study is to determine adequate irradiation for the more common FL (randomised trial) and to evaluate standardisation RT in the rare cases of early stage IC and MCL (prospective observation trial).

Methods: In nodal FL stage I (10-14 cm) and limited (<4 involved regions) stage III disease, patients (n=68) aged 18-65 years are randomly assigned to Extended field (EF) or Total lymphatic irradiation (TLI) with doses of 30 Gy (5x2.5Gy/week) supplemented by a boost of 10 Gy to areas of macroscopic lymphoma < 3 cm G, and 14 Gy to those of 3-10 cm G. In the abdominal bath, total dose is restricted to 25.5 Gy (Sx1.5Gy/week) with boost doses of 16 and 20 Gy, respectively. Pts aged 66-75 years are treated exclusively in the EF study arm.

In nodal IC or MCL stage I VII pts aged 18-75 yrs consists of a modified EF limited to the involved side of the diaphragm with the same doses as in the randomised trial. In pts >75 yrs with FL, IC, and MCL involved field (IF) RT is applied with 40-44 Gy.

Results: From Feb. 2000 to Jan. 2002, a total of 109 pts was recruited, 87 to the randomised study. In the latter pts, median age was 53.3 (65) yrs, the m/f ratio 1.2, FL were grade I in 64% of the pts, stage I, II and III were present in 55%, 24, and 21% of the pts, respectively, with cervical and/or inguinal involvement in the majority of the pts. Twenty-two pts, median age 67 (66-71) yrs, were included in the observation trial, 19 with FL and 3 with MCL.

Conclusions: In comparison to the earlier observation study in FL by the same group and historic series collected worldwide, this ongoing randomised trial (aimed to recruit 24100 pts) will contribute to the current questions, and curability of early stage FL disease and the adequate irradiation volume necessary.
FLUDARABINE & CYCLOPHOSPHAMIDE & IDAURIBIN (FLICU) AS FIRST-LINE TREATMENT IN PATIENT WITH NON-FOLLUCULAR INDIFFERENT LYMPHOMA

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Introduction: In our previous report (UCO 2000, 18, 773-9) the combination Fludarabine and Idarubicin resulted to be more effective than Fludarabine alone in treating non-follicular indolent lymphoma (NL, lymphoma (GL).) On the basis of these data, we have modified this regimen including Cyclophosphamide for improving these results. The aim of this study was to evaluate the efficacy and toxicity of Fludarabine combined with Cyclophosphamide and Idarubicin (FLICU).

Methods: From October 1999, from 8 Italian centers 58 patients with untreated FL, and MZ indolent lymphoma were enrolled for receiving a three-drug combination of Fludarabine 25 mg/m²/day IV on days 1 to 3, Idarubicin 12 mg/m² IV on day 1, and Cyclophosphamide 200 mg/m² IV on days 1 to 3 for 3 cycles. Patients were required to have a histologically proven diagnosis of CD20 positive, according to the REAL classification, age 15 to 70, stage II-II and an ECOS performance status of 0-2.

Results: Of the 58 evaluable patients, 15 (26%) achieved complete responses (CR), 10 (38%) partial responses (PR) with an overall response rate of 96%. Regarding the histologic subtypes, CR rates were 49% (6/13) among those with MZL, 60% (6/10) among those with RL, and 100% (3/3) among those with MZL lymphoma. Hematologic grade 4 toxicity was seen in only 2 (7%) patients; no opportunistic infections or deaths were associated with the administration of the FLICU regimen.

Conclusions: These preliminary data show that the FLICU regimen is a very active, well-tolerated combination chemotherapy for untreated patients with advanced non-follicular indolent lymphoma.
BRIEF CHEMO-IMMUNOTHERAPY WITH FLUDARABINE/MITOXANTHONE (FLU/MITO) FOLLOWED BY RITUXIMAB (RTX) IN ELDERLY PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA (FL) AT DIAGNOSIS: SAFETY, CLINICAL AND MOLECULAR RESPONSE.


On the behalf of "Gruppo Multigireale e Toscana per i Linfomi non Hodgkin" / Hematology. Az Osp S. Giovanni Battista and University. Turin, Italy.

Introduction: the combination of Fludarabine containing regimens with Rituxin is attractive because of its potential additive effect. but its feasibility, safety and response need to be explored. Patients and methods: from March 1999 to December 2001, 30 elderly pts (age >60) with advanced stage FL at diagnosis were enrolled in this study. Treatment consisted in: 4 courses of FND (Fludarabine 25mg/m² days 1-3, Mitox 10mg/m2/day 1 and Dec 20 mg days 1-3) followed by 4 Rituxus infusions at 375mg/m²/week. Pts in partial remission after this treatment received 2 additional FND courses and 2 Rituxus infusions. PCR molecular monitoring for the presence of IgH/BCL2 rearrangement and/or Ig heavy chain gene rearrangement was performed at the beginning of the treatment and on bone marrow after FND and after Rituxus. Results: median age was 66 (range 60-78); 23 males and 7 females; 6 had stage II, 7 stage III and 7 stage IV disease; 31 BM involvement, 24 bulky disease and 8 had >1 extranodal site. Up to date, 29 pts are available. Twenty-one of 24 patients achieved a complete remission (CR) and 4 patients relapsed after 18 months without further treatment. Two men developed progressive disease after 2 and 14 months of treatment, respectively. The other 2 patients achieved a partial remission with a disease-free survival of 14 and 7 months, respectively.

Conclusion: the combination of Fludarabine and Rituxus is feasible and potentially useful for elderly patients with advanced stage FL.

RITUXIMAB COMBINED WITH CHEMOTHERAPY (CVP/CHOP) TO INDUCE CLINICAL AND MOLECULAR RESPONSE IN PREVIOUSLY UNTREATED INDOLENT LYMPHOMA.


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Introduction: Indolent lymphoma is currently considered to be an incurable disease, with median survival of 6-8 years. In the absence of a cure, the variety of therapeutic options available for the patients (pts) with indolent lymphoma range from watch- and-wait to high dose therapy with autotransplantation. New treatment modalities providing tumor reduction with less toxicity are necessary. Rituximab has been shown to mediate cytotoxicity in the lymphoma cells via direct proapoptotic and apoptotic effect and by indirect- CDC and ADCC. Rituximab might be an ideal agent for the combinations with chemotherapy because of non- cross resistant efficacy and differential toxicity.

Aim: The aim of this study was to determine safety and efficacy of Rituximab combined with chemotherapy (CVP/CHOP) to induce clinical and molecular response in the previously untreated indolent lymphoma.

Methods: Characteristics of the 47 pts enrolled in this study was: male/female 33/14, median age 47 (26-76), all pts had stage of disease III or IV; histology - follicular gr III 14, SLUM-CALL 14/8 (22), marginal 11. Pts received Rituximab 375mg/m² on day 1 and standard CHOP (5), or CVP protocol (42). Cyclophosphamide 750mg/m², Vincristine 1,4mg/m² on day 1, L-Methylprednisolone 45mg/m² on day 1-5, repeated in three weeks for 8 cycles.

Results: Rituximab was extremely well tolerated. Only 11 pts out 47 assessable experienced a grade II infusion- related toxicity (mild chills, rash). Infections were rare, of moderate intensity (febrile neutropenia in 3 pts), 6 pts were take out of study. stable (7) or the disease (3). The overall response rate in 47 evaluable pts was 87,3% (41/47), with 38,5% (18/47) CR and 49% (23/47) PR. Median duration of response is 17 (3-33 months). Fourteen CR pts received maintenance therapy (7). Rituximab every 6 months, 7 IFN-alpha 9 MV/m².

Conclusion: Results of Rituximab chemotherapy combination demonstrate excellent antitumor activity with acceptable toxicity and represent a new approach for the treatment of indolent lymphoma. We need complete analysis (molecular) and longer follow-up for the final conclusions.

TREATMENT OF ADVANCED STAGE INDOLENT LYMPHOMA AT DIAGNOSIS WITH CCA/DM (CYCLOPHOSPHAMIDE AND CHLADRINE).


Introduction: Between 02/95 and 12/98, 49 consecutive patients (pts) (M/F: 25/24) up to the age of 64 yrs (median age, range 48, range 32 to 64) with advanced stage indolent lymphoma were enrolled at diagnosis on treatment with CCA/DM: cyclophosphamide 1.2g/m² iv in week followed in 3 weeks by cladribine (2-Cda) 0.14mg/kg/day by 2hr iv infusion x 5 days. C was reintroduced 4 weeks later and therapy was alternated to a total of 8 cycles (4 of each). 30% of the whole treatment program was cladribine, 4 small lymphocytic, 4 lymphoplasmacytoid and 4 lymphoma of MALT. 39 pts had stage IV disease, 6 stage III and 4 extensive stage II. 4 pts had B symptoms, 31 bone marrow involvement, 5 serum LDH > normal and 10 bulky disease (> 10 cm). 45 pts had an ECOG performance status of 0 or 1, 1 pt. had 2, 1 pt. had IPI score of 0 or 1 in 32 pts and 2 in 15 pts.

Results: 48 pts completed planned treatment. There was minimal alopecia, 1 serious infection with candida pneumonitis, and no deaths due to toxicity. 43pts achieved CR or good PR (residual imaging adenopathy < 20% of pretreatment, but > 1.5 cm). With a median follow-up of 23 months (range 2 to 76), 21 pts remain in first remission, 21 are alive after 1 or more recurrences, 6 have died of disease and 1 is lost to follow-up. The failure-free survival for all pts is 75% at 3 yrs and overall survival (OS) at 5 yrs is 84%. This compares favorably to a 69% OS for a matched historical control group (130 pts) treated between 1980-85 with an initial plan of observation followed by oral Chlorambucil for symptomatic disease progression (p<0.05).

The results are similar to those produced at our institution with aggressive chemotherapy (BP-VACOP) and intensive radiotherapy, and by others with high dose chemotherapy and autologous stem cell rescue.

Conclusions: CCA/DM is well tolerated and produces survival outcomes similar to more aggressive combined chemotherapy/radiotherapy strategies. Exploration in combination with targeted molecular therapies or immunotherapy would be feasible.

FLUDARABINE/CYTOKINE/MITOXANTHONE (FLU/MITO) AS FRONT-LINE THERAPY IN LOW-GRADE NHL (LGL).


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Following our previous experience with FLUCY/MITO combination in recurrent LGL, we started a new study in which the same treatment was used as front-line therapy in this category of patients. Sixty-seven successive patients with LGL (R.E.A.L. classification) entered study and 54 had follicular lymphoma. Patients: median age 58 years (range 36 to 76); stage II, 3 patients, stage III, 14 patients, stage IV, 43 patients, increased LDH level, 22 patients. Patients received FLU 25 mg/m² days 1 to 3, CY 300 mg/m² days 1 to 3, and MITO 10 mg/m² day 1. Courses were given every 28 days for a maximum of 6 courses. Patients received antibiotic prophylaxis throughout treatment and growth factor (G-CSF) when grade 3 granulocytopenia (WHO) occurred. At present fifty-four patients are evaluable for response: 33 achieved CR (61%), and 16 PR (30%), with an overall response rate of 91%. Follicle grade 1-2 episodes occurred in 16% of patients, 9% of whom suffered grade 1-3 infection. Myelosuppression was the most evident toxic effect and grade 3-4 leukopenia occurred in 42% of treated patients. Anemia and thrombocytopenia (grade 1-3) occurred in 25% and in 27% of patients, respectively. The overall 3-yr probability of survival and FFS are 91% and 74%, respectively. Light gene rearrangement and bcl-2 translocation were detected with PCR technique in BM and PB of 23 out of 32 patients, available for molecular study at diagnosis. At the end of treatment, the PCR-based analysis was negative in 14 out of 19 CR patients (74%). The clinical follow-up of patients with PCR undetectable disease will help to clarify the meaning of "molecular remission". This study confirms that FLU in combination provides an effective and safe treatment for LGL. The antibiotic prophylaxis with G-CSF support seems to reduce treatment-related infection.
MITOXANTRONE/BENDAMUSTINE/PREDNISOLONE (MBP) IN INDOLENT LYMPHOMAS – CLINICAL RESULTS OF A PHASE II STUDY

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Introduction: The clinical course of indolent lymphomas is characterized by frequent relapses and a continuously declining survival curve. However, even in relapse, the disease is sensitive to conventional chemotherapy. Bendamustine is a well-characterized alkylating drug which has been proven to be highly effective in indolent lymphomas.

Methods: A phase II study was initiated to define the maximally tolerated dose of Bendamustine in a combined chemotherapy regimen (MBP) based on the previous MGP scheme of the German Low Grade Lymphoma Study Group (Mitoxantrone 80 mg/m² d1+2, Bendamustine 80-120 mg/m² d1+2, Prednisone 25 mg/m² d1-5). Bendamustine dose was started at 80 mg/m² and increased in 20 mg/m² steps if no dose limiting toxicity was observed in a 3 patient cohort.

Results: Currently, 12 patients with relapsed malignant lymphomas have been treated (4 follicular lymphomas, 3 lymphoplasmacytoid immunocytomas and 4 mantle cell lymphomas, 1 transformed lymphoma). Whereas generally, MBP was well tolerated, a subset of patients showed a significant hematotoxicity grade 4 after subsequent cycles of chemotherapy (4/6 pts at 80 mg/m², 1/3 pts at 100 mg/m² and 1/3 pts at 120 mg/m² Bendamustine). Thus, the mitoxantrone dose was fixed at 100 mg/m² d1 and the Bendamustine dose was reduced to 80 mg/m². Interestingly, 7 of 9 patients (78%) evaluable of this heavily pretreated patient population achieved a remission (2 CR, 4 PR, 1 MR). Especially, one ongoing complete remission was observed in a patient with relapsed mantle cell lymphoma.

Discussion: Our study results confirm a Bendamustine-containing chemotherapy as a highly effective and generally well tolerated therapeutic regime. However, because of cumulative hematotoxicity, the optimal Bendamustine dose should be defined.

Based on the results of the current phase I/II study, a multicenter phase II study is planned to evaluate a combination immuno-chemotherapy (Rituximab-MBP) in relapsed indolent lymphomas.

FLUDARABIN AND BENDAMUSTINE IN REFRACTORY / RELAPSED LOW-GRADE LYMPHOMA – A MULTICENTER PHASE II TRIAL BY THE EASTERN GERMAN STUDY GROUP FOR HEMATOLOGY AND ONCOLOGY (OSHO)

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Background: Bendamustine is a bifunctional alkylating substance with additional activity as purinomimetic. Its effect is both cytotoxic and immunomodulatory. Clinical activity in low-grade lymphoma has been previously shown in single agent as well as in combination trials with steroids, antiretroviral and vinca alkaloids. In a multicenter clinical phase II trial we combined Bendamustine with Fludarabin for patients (pts) with relapsed or refractory low-grade lymphoma.

Treatment plan: Bendamustine was given within phase I of the study at 30 or 40 mg/m²/d (dose levels I and II), Fludarabin constantly at 30 mg/m²/d, both drugs iv infusion over 30 min days 1-3. Six cycles were to be given every 4 weeks.

Results: Within phase I of the study 7 pts were treated at dose level I and 7 pts at dose level II. Another 6 pts have been included within phase II so far, yielding altogether 20 pts with low grade mantle cell lymphoma, 12 pts follicular lymphoma.

Median age was 64 years (range 56-73). Stage of disease at relapse was III in 3 pts and IV in 17 pts. Before study entry, all 20 pts had prior chemotherapy with or without additional radio- or immunotherapy. Dose level I was established as maximum tolerated dose by toxicity testing during phase I. Dose limiting toxicities were mainly hematologic, with CTCl 4/5 in 2/7 pts at dose level I and prolonged cytopenia in 3/7 pts at dose level II. Bacterial and viral infections, CTC grade II (nausea, anorexia) and IV (infection, n=1) occurred only at dose level II. Six of 7 pts in phase I at dose level I completed chemotherapy with 1 CR and 5 PR, while in 1 pt therapy was halted due to progressive disease (PD). At dose level II, 7/20 pts were treated. Four of these pts completed all 6 cycles, 1 pt dropped out due to PD, and 3 pts therapy was discontinued due to DLT. Three pts entered CR, 3 pts PR. At a mean follow-up time of 368 days (54-743) for the 14 pts in phase I, 6/7 pts were free from progression at disease level I, and also 6/7 pts at dose level II.

Conclusions: The leading toxicity of Fludarabin in combination with Bendamustine is hematotoxicity. Dose level I was defined as maximum tolerable dose. Despite unfavorable prognostic features (histologic subtype, stage of disease, performance status) response rates appear to be good with this regimen. The protocol is especially appropriate for elderly pts. Efficacy is being further studied at dose level I.

MITOXANTRONE, PREDNISOLONE, PENTOSTATIN AND BLEOMICIN (MIPPEH) REGIMEN FOR PATIENTS WITH INDOMENT NON- HODGKIN'S LYMPHOMA RELAPSED OR UNRESPONSIVE TO PREVIOUS TREATMENTS. FINAL RESULTS OF A PHASE II STUDY CONDUCTED BY THE GRUPPO ITALIANO PER LO STUDIO DEI LINFOMI (GISL)

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Introduction. Although patients with indolent NHL usually respond to initial therapy, they exhibit tendency to relapse, with subsequent responses of progressively shorter duration. Taking into account the promising results achieved with purine analogs in combination regimens in patients with indolent NHL, we designed a phase II study in patients with previously relapsed indolent NHL, comparing the effectiveness of purine analogs, MIPPEH, and mitoxantrone. Patients and Methods. 30 patients with indolent NHL registered to the study and treated with MIPPEH. Nine patients had already received one, 10 patients 2 and 11 patients 3 or more lines of therapy respectively. The treatment consisted of Pentostatin 5 mg/m² on days 1 and 9, Mitoxantrone 10 mg/m² on day 1, Bleomycin 8 mg/m², on day 8, Prednisone 100 mg, on days 1 to 8; cycles were administered at 3 weeks interval for a maximum of 6 cycles. Results. Between November 1997 and July 2000, 30 patients (18 males and 12 females) were evaluated. A median of 3 cycles (range 2-6) was administered. Out of 29 patients evaluable for response, 9 CRs and 8 PRs were observed, with an overall (CR+PR) response rate of 59%. After a median follow-up of 16 months the 3 year overall and failure-free survival were 50% and 37% respectively. The actuarial 3-year relapse-free survival for 9 patients in CR was 51%. Treatment was well tolerated in the majority of cases. Toxicity was mainly hematological with grade 3-4 neutropenia in 37% and grade 3 thrombocytopenia in 7% of cases. Conclusion. MIPPEH is an active regime in patients with relapsed indolent NHL, and resulted in limited remissions. Moreover, the regimen was well tolerated, with minimal toxicity.

Updated results of Rituximab as single first-line therapy for patients with follicular lymphoma (FL) and a low tumor burden: Clinical and molecular evaluation


Introduction. No treatment has demonstrated that it modifies the natural history of patients with FL and a low-tumor burden. Because of the clinical efficacy of rituximab (R) in previously treated patients (pts), the short treatment duration and its low toxicity profile, we have conducted a phase II trial of R in previously untreated patients with FL and a low tumor burden (GELF criteria).

Methods. Forty-nine were treated with 4 weekly infusions of R 175 mg/m². All pts had a PCR evaluation for bcl-2/3 rearrangement in the blood and in the marrow, before treatment, one month after treatment and then every 6 months. Clinical characteristics, response rates (RR) and short-term follow-up were recently reported (Blood 2001; 97:101).

Results. Overall RR, complete/unconfirmed RR, partial RR according to NCI criteria one month after the end of R treatment were 73%, 26% and 47% respectively. Some responses were delayed and the RR at any staging during the year following treatment was 80% with 41% CR/CRR. Out of the 32 pts PCR positive before treatment, 17 became negative 1 month after R in the blood. All pts were followed at least 3 years. At 3-year staging, 31/35 (88 %) have progressed and/or relapsed and the median time to progression (TPP) was 18.4 months. There was a strong relationship between PCR results after R and risk of progression : median TPP of 37 months for pts who became negative as opposed to 12 months for pts who remained PCR positive (p = 0.006). Out of 17 patients who became PCR negative, 8 are still in complete molecular and clinical remission 3 years after R treatment.

Conclusion. Some pts enjoy a long-term clinical and molecular remission after a single rituximab course in this subgroup of FL. A persistent PCR positivity after treatment is predictive of early relapse. TTP appears to be similar to that of pts treated with standard chemotherapy. However, the duration of treatment is much shorter and there is no longer toxicity after R treatment. Other modalities are under investigation: combination chemotherapy, IFN and repeated treatments.
Lymphocytes Population Changes During Therapy With Rituximab® in Patients with B-Cell Non-Hodgkin's Lymphoma

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Introduction: The purpose of the study was to monitor the changes of peripheral blood lymphocyte subpopulations during the RITUXIMAB® therapy of non-Hodgkin's lymphoma patients.

Methods: 40 patients with immunophenotypically proven B-cell non-Hodgkin's lymphoma were treated between 1/2001-12/2001 by RITUXIMAB® in doses 375 mg/m² once a week (4-6 infusions for course of treatment). Age of the patients - 32-78 years (mean 56 yrs), 29 (72.5%) patients were with follicular lymphoma, 6 (15.5%) - with MALT-lymphoma, 5 (12.5%) - with small lymphocytic lymphoma. Before every RITUXIMAB® infusion we study the lymphocytes subpopulation (CD3, CD4, CD8, CD16, CD19, CD20) in peripheral blood by flow cytometry.

Results: In 39 (98%) pts. the CD19/CD20 lymphocytes was not identified by flow cytometry in peripheral blood after 4 injections of RITUXIMAB®. Also we find the trend of increase in the percentage of CD19 lymphocytes at the beginning of the therapy. All this changes didn't correlate with effect of treatment. Median time to the start of tumor regression was 48 days (range 41-76). Observed overall response: complete remission - 40%, partial remission - 22.5%, didn't correlate with CD19/20 lymphocytes disappearance from peripheral blood.

In one case transformation of a initial tumor clone (CD19+/CD20+CD5+/CD23+) with disappearance of CD19/CD20 and the generalization of the small lymphocytic lymphoma clone (CD19+CD20+CD5+CD23+) was registered.

Conclusion: Flow cytometry is very useful in monitoring the cells response to RITUXIMAB® therapy and detecting the appearance of new clones of circulating lymphoma cells in peripheral blood.

MABThera® Followed by Roferon® Induces Prolonged Responses in Patients with Refractory/Relapsing Low Grade Lymphomas. A Phase II Study from the Belgian Hematological Society

For the Belgian Hematological Society (BHS) and Roche.

In patients with relapsing/refractory indolent lymphoma, Mabthera® (Rituximab) chimeric anti-CD20 monoclonal antibody induces a 49% objective response rate, with a median time to progression for responders of 13 months. (McLaughlin et al, JCO 1998; 16: 2825). In order to prolong the duration of response and because of the proven anti-tumoral efficacy of interferon – the BHS conducted a phase II combination study of 4 weekly i.v. doses of RITUXIMAB® (375 mg/m²) followed by interferon given at 3 MU/m² subcutaneously TIW for 6 months, starting 2 weeks after the last infusion of Rituximab. A total of 70 patients were treated from 3/98 to 28/11/00.

Response rate and survival were analyzed on an intent-to-treat basis. After Mabthera®, 34 patients (49%) obtained a response: 7 complete remission (CR), 27 partial remission (PR). After Roferon®, 28 (41%) patients remained in objective response with 13 CR and 15 PR. The best response before progression was 14 CR (20%) and 21 PR (30%). The median time to progression has not been reached yet in complete responders (18+ months). The median duration of overall response (CR + PR) was 20 months. The median duration of overall survival was 35 months (95% CI 25-49) with 56% (± 12) of patients alive at 2 years. Serious adverse events during Mabthera® and Roferon® treatments occurred in 13% and 23% of patients, respectively, but no toxic death was observed. In conclusion, the sequential administration of Mabthera® and Roferon® may increase the CR rate and prolong response in patients with advanced indolent lymphomas, in comparison with Mabthera® alone. This combination should be further investigated in randomized trials.

LYMPHOCYTES POPULATION CHANGES DURING THERAPY WITH RITUXIMAB® IN PATIENTS WITH B-CELL NON-HODGKIN'S LYMPHOMA

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Russian Scientific Center of Rerentgenodiagnosis, Moscow, Russia.

Introduction: The purpose of the study was to monitor the changes of peripheral blood lymphocyte subpopulations during the RITUXIMAB® therapy of non-Hodgkin's lymphoma patients.

Methods: 40 patients with immunophenotypically proven B-cell non-Hodgkin's lymphoma were treated between 1/2001-12/2001 by RITUXIMAB® in doses 375 mg/m² once a week (4-6 infusions for course of treatment). Age of the patients - 32-78 years (mean 56 yrs), 29 (72.5%) patients were with follicular lymphoma, 6 (15.5%) - with MALT-lymphoma, 5 (12.5%) - with small lymphocytic lymphoma. Before every RITUXIMAB® infusion we study the lymphocytes subpopulation (CD3, CD4, CD8, CD16, CD19, CD20) in peripheral blood by flow cytometry.

Results: In 39 (98%) pts. the CD19/CD20 lymphocytes was not identified by flow cytometry in peripheral blood after 4 injections of RITUXIMAB®. Also we find the trend of increase in the percentage of CD19 lymphocytes at the beginning of the therapy. All this changes didn't correlate with effect of treatment. Median time to the start of tumor regression was 48 days (range 41-76). Observed overall response: complete remission - 40%, partial remission - 22.5%, didn't correlate with CD19/20 lymphocytes disappearance from peripheral blood.

In one case transformation of a initial tumor clone (CD19+/CD20+CD5+CD23+) with disappearance of CD19/CD20 and the generalization of the small lymphocytic lymphoma clone (CD19+CD20+CD5+CD23+) was registered.

Conclusion: Flow cytometry is very useful in monitoring the cells response to RITUXIMAB® therapy and detecting the appearance of new clones of circulating lymphoma cells in peripheral blood.

MABThera® (Rituximab) in Treatment Relapses and Resistant Low-grade Non-hodgkin Lymphomas (NHL)

A Hellenic Co-operative Oncology Group Study.

Introduction: It is well known, that the majority of NHL patients have systemic lymphoma at presentation, which is not cured by primary therapy. But, in many cases, in the first and sometimes in the second relapse, they can be cured by conventional chemotherapy and allogeneic hematopoietic stem cell transplantation. In contrast, patients with relapsed or refractory NHL usually have an aggressive disease and have a poor prognosis. As a result, there is an urgent need for new, effective and well tolerated treatments for such patients. Rituximab, a chimeric anti-CD20 monoclonal antibody, is currently considered the standard treatment of choice for patients with relapsed or refractory NHL. The combination of rituximab with standard chemotherapy or other immunotherapies has shown improved outcomes compared to chemotherapy alone, and has become the standard of care for many NHL patients. The goal of this study is to evaluate the efficacy and safety of a combination of rituximab with chemotherapy in the treatment of patients with relapsed or refractory NHL.

Methods: 51 patients with relapsed/refractory low-grade NHL were treated with a combination of rituximab and chemotherapy. The patients were randomized to receive either a rituximab-containing regimen or a chemotherapy-containing regimen. The primary endpoint of the study was overall response rate (ORR), which was defined as the proportion of patients achieving a complete or partial response. Secondary endpoints included progression-free survival (PFS), time to progression (TTP), and overall survival (OS).

Results: The ORR was 62% for the rituximab-containing regimen and 38% for the chemotherapy-containing regimen. The median PFS was 12 months for the rituximab-containing regimen and 9 months for the chemotherapy-containing regimen. The median OS was not reached for either group. The combination of rituximab with chemotherapy was well tolerated, with the most common side effects being fatigue, infections, and lymphocytopenia. The results of this study suggest that the combination of rituximab with chemotherapy is an effective and well tolerated treatment for patients with relapsed or refractory NHL.

Conclusion: The combination of rituximab with chemotherapy is an effective and well tolerated treatment for patients with relapsed or refractory NHL. Further studies are needed to determine the optimal duration and schedule of rituximab therapy, as well as to identify subgroups of patients who may benefit the most from this treatment approach.

Introduction: In a prospective randomized trial the efficacy and toxicity of rituximab (375 mg /m² i.v.) plus MCV4 (methylasparagine 8 mg/m² i.d. 3x, chlorambucil 3x mg/m² 6-7, prednisolone 25 mg/m² 3x 7-37) was studied in comparison with MCV4 alone (i.d. 3x 4x 4 methods). Methods: The study includes patients with advanced follicular lymphoma (G1-4). The trial was conducted in the experimental arm of the study. Results: In the second interim analysis of 124 randomized patients 106 patients were evaluable on an intent-to-treat basis. 53 in the rituximab plus MCV4 group and 51 in the MCV4 group. The response rate (CR plus PR) for all patients was 81% (95% CI 72 - 88%), at present there is no statistically significant difference concerning the response rate between the treatment arms, and so the respective numbers are still blinded. CR for all patients was 40%, PR rate 41%. Toxicity analysis was made on the basis of 414 treatment cycles in the Rituximab group and 347 cycles in the MCV4 group (78 vs. 58 patients). Counting all adverse events (CTC grading 1 - 4) there is a tendency to more events in the MCV4 group (375 vs. 260; 0.9 vs. 0.76 per cycle), however this is not statistically significant (p=0.612). Myelosuppression is the main cause for CTC-grade IV toxicity, there is no statistically significant difference for hemorrhage and platelets, but a significantly higher proportion of CTC-grade IV leukopenia occurred in the MCV4 group (13% vs. 6% of cycles, p=0.000), however this did not result in an increased treatment failure. Conclusions: Our preliminary response data demonstrate an overall response rate (81%) comparable or even better in other multicenter studies with a remarkable high proportion of complete responses (40%) for the entire group of patients. Regarding toxicity, there is a tendency to more adverse events in the MCV4 arm, esp. concerning hematopoeisis, however this has no impact on infections or dose intensity of chemotherapy. In the analysed cohort of patients we have had no severe infection related events following rituximab.

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STEM CELL TRANSPLANTATION (SCT) IN FOLLICULAR LYMPHOMA (FL): ANALYSIS OF 42 PROCEDURES IN A SINGLE INSTITUTION. S. Masecotto, A. Lopez-Granillo, E. Carrera, M. Rovera, A. Ferrer, F. Bosch, J. Esteve, P. Conventis, J. Blasco, D. Collado, B. Nonedre, E. Camps and E. Montserrat. Department of Hematology and Hematopoietic Unit, Hospital Clinic, IJDBAP, Barcelona, Spain

Objective: To analyze the outcome after SCT in patients with FL.

Patients and methods: From June 1993 to January 2001 42 SCT (35 autologous, 6 allogeneic, 1 syngeneic) were carried out in 39 patients (23 M/16 F; median age at transplantation 45 years). 7 (after histological transformation) in 24%, 3 lines. Pro-transplantation status was CR in 52% of the cases, PR in 40%, failure in 2% and untreated relapse in 5%. Peripheral blood was the source of stem cell progenitors in 93% of the transplantations. The conditioning regimen was BEAM in 74% of the cases, BEAM and Cy-TBI in 21%. Results: Toxicity was acceptable with III/IV neutropenia in 6 cases and III/IV infection in 9. Among the 6 allotransplanted patients, 3 had grade I acute GVHD and 4 chronic GVHD. One-year transplant related mortality was 8% in the whole series (3 all, 2 auto). Response at 3 months after SCT was CR in 79% of the cases, PR in 7% and failure in 4%. After a median follow-up of 2.7 years 11 patients died, with the 5-year survival after SCT being of 67% (95% CI: 51-84). Among 36 patients in CR or PR post-SCT 15 subsequently progressed, the 5-year failure-free survival after SCT being of 45% (95% CI: 15-60). In the subset of patients transplanted after second relapse of 5 years OS and PFS post-SCT were 65% (95% CI: 53), but has no impact on CR: 14-81%, respectively. As compared to the patients transplanted in FL lymphoma phase, those grafted after transformation into high-grade lymphoma presented shorter median OS (NR vs. 1.23 yrs; p=0.004) and DFS (NR vs. 0.81 yrs; p=0.01). Conclusions: Better response rates can be achieved after SCT in patients with follicular lymphoma, even in advanced phase. However, disease transformation prior to transplant precludes poor prognosis.

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AUTOLOGOUS PROGENITOR-CELL TRANSPLANT AFTER HISTOLOGIC TRANSFORMATION OF LOW-GRADENON-HODGKIN LYMPHOMA (NLH)

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Histologic transformation of 'low-grade' NHL to higher grade disease is well recognized, and is associated with a poor prognosis. In a large series of autologous progenitor cell transplant (Auto-CTP), patients were identified who had 26-month interval from date of diagnosis of low-grade disease until time of transformation, subsequently had undergone auto-CTP, and whose medical records were available for review. Patients with follicular lymphoma (grade 3) at original diagnosis or transformation and those who presented with de novo diffuse large cell lymphoma (DLCL) and subsequently relapsed with low-grade disease, were excluded. Patients were eligible for auto-CTP at the time of transformation or subsequent relapse if chemosensitivity to a salvage regimen was demonstrated. From April 1989 to December 2005, 26 patients who met the inclusion criteria were identified. At time of transformation, the median age was 55 years (range 34-69). All patients had DLCL, 13 (50%) patients had stage IV disease, 9 (33%) had bone marrow involvement and 8 had an elevated LDH. IPI was 0-1 in 15, 2-3 in 10 and 4-5 in 1 patient. The median time from original diagnosis to transformation was 3.3 years (range 9-56 months - 14.6 years) and from original diagnosis to auto-CTP was 4.9 years (range 1-17.7 years). 20 patients underwent auto-CTP after treatment for 1st episode of histologic transformation and 6 patients were in 2nd episode of transformation. The median number of chemotherapy regimens from time of transformation to auto-CTP was 1 (range 1-4). Median age at time of auto-CTP was 56 years (range 36-71 years). Conditioning regimen was BEAC in 14, BEAM in 9 and Cy-TBI in 3 patients. At a median follow-up of 1.4 years (range 2.4 months - 11.8 years) after auto-CTP, 11 have died, 9 of disease progression. There were no treatment-related deaths or cases of secondary myeloplasia/AML. 5-year OS and DFS from auto-CTP are 45% and 40%, respectively. Our survival data are comparable to previous reports of auto-CTP for transformed NHL and suggest that auto-CTP is associated with prolonged survival in selected patients with chemoresponse, transformed NHL.

AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) FOR RELAPSED/REFRACTORY FOLLICULAR LARGE CELL LYMPHOMA (FLCL).

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Introduction: The long-term outcome following ASCT for relapsed or refractory FLCL remains to be determined. Methods: We performed a retrospective analysis to investigate determinants of outcome of ASCT for FLCL versus diffuse large B cell lymphoma (DLBCL). Pathology was reviewed locally according to the REAL classification. High dose therapy consisted of etoposide 60mg/kg d-4 and melphalan 180mg/kg d-3 ± TBI (24%) supported by unmaintained BM or PB stem cells. Results: 57 patients with FLCL underwent ASCT between July 1988 and Jun 01. Median age was 50yrs and 65% were male. 19% were in 1st PR or had primary non-responsive disease, and 81% were in chemoresponse 1st relapse. Disease characteristics at diagnosis: stage 3/4 76%, extranodal involvement 62%, bulk >10cm 32%, CR with initial therapy 62% (median CR duration 20mths). At ASCT, 49% were in CR and 51% in PR after salvage chemotherapy. During the same period, 82 pts with chemoresponse relapsed or refractory DLBCL were transplanted: pt characteristics did not differ significantly between FLCL and DLBCL, except for more BM involvement at diagnosis (35% vs 12%, p=0.004), bulky disease at relapse (37% vs 18%, p=0.05) and BM involvement at ASCT (86% vs 53%, p=0.06). Median follow up is 46mths. Five year DFS for FLCL is 32% (95%CI, 17-49) and 5yr OS 50% (95%CI, 30-67), vs 44% (95%CI, 31-55) and 52% (95%CI, 35-64), respectively, for DLBCL. Predictors of OS by univariate analysis for pts with FLCL were marrow involvement at diagnosis (p=0.06), bulk at relapse (p=0.05), initial response (complete responder vs relapsed, p=0.01) and status at ASCT (CR vs PR, p=0.08). Multivariate analysis indicated that DFS and OS were not influenced by lymphoma subtype (p=0.57 and 0.79, respectively). There is no obvious plateau in the FLCL survival curve. Conclusions: From our analysis, long-term outcome of FLCL following ASCT is not superior to DLBCL and may be worse. Longer follow-up and prospective studies are required to clarify the role of ASCT in relapsed or refractory FLCL.
Withdrawn

SAFETY PROFILE OF BEXXAR® IN PATIENTS WITH REFRACTORY/RELAPSED NON-HODGKIN'S LYMPHOMA (NHL)

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Bexxar (bendamustine) and Iodine 131 I tositumomab) is a novel RIT agent developed for the treatment of CD 20 positive B-cell NHL. In order to achieve the best therapeutic index and to minimize toxicity, each patient's administered activity (mCi dose) was individualized to deliver the correct total body dose (TBD) (mCi) of radiation. The Bexxar regimen has been previously described [JCO 2001; 19:3918]. Between Aug 93 and Aug 05, 620 patients were prescribed TBD of either 65 or 75 mCi of iodine 131 tositumomab in 5 clinical studies, 4 single-patient studies and an Expanded Access Protocol. Patients were heavily pretreated (median of 3 prior therapies) and typically had other poor prognostic characteristics: age>60 (30 %), advanced disease (89%), elevated LDH (51%), and transformed histology (23%). The most common non-hematologic events were anemia (32%), nausea (29%), and fever (22%). The dose limiting toxicity was acute, reversible marrow suppression. Forty percent of patients experienced Grade III/IV neutropenia and 35% experienced Grade III/IV thrombocytopenia for a median of 30 days. The median ANC nadir was 1200 cells/mm3 at a median of 42 days; the median platelet nadir was 63,000 cells/mm3 at a median of 34 days. Twenty-six percent of patients received supportive care measures: 11% (G-CSF: 7% pts; 12% platelet 1x and 15% PRBC 1x. The incidence of grade III/IV bleeding complications [1%] and serious infections [5%] were within the acceptable range for cytotoxic therapy for advanced malignancies. Bexxar may be associated with delayed toxicities. The 4-yr cumulative incidence for developing hypothyroidism was 12.8%, for developing HAMA was 11%. Based on a masked, independent review, and excluding patients with short follow-up, the annualized incidence of MDS/AML was 2.3%/year (95% CI: 1.2%-3.9%); an additional 2% of patients experienced non-hematologic malignancies other than skin cancers. Deaths were attributed to causes other than progressive lymphoma in 38/620 patients. In summary, a single individualized treatment with Bexxar, based on the total body clearance of iodine 131 tositumomab, produces predictable toxicities which are comparable to other systemic therapies used to treat patients with relapsed/refractory NHL.

PRELIMINARY RESULTS OF A PHASE I STUDY OF TWO SEQUENTIAL DOSES OF YTTRIUM-90 ZEVALIN IN PATIENTS WITH LOW GRADE NON-HODGKINS LYMPHOMA

GA Wiseman, TE Witzig, DN Gansle, NL Tuinstra, JP Colgan, SM Ansell, I Micallef, DJ Inwards, LF Porotta, TM Habermann

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Introduction: Zevalin radioimmunotherapy (IDEC Pharmaceuticals, San Diego, CA, USA and Schering AG, Berlin, Germany) consists of a murine anti-CD20 monoclonal antibody chemically linked to the therapeutic beta-emitting radioisotope Yttrium-90 (Y-90). The treatment has previously been administered as a single dose IV for treatment of B-cell NHL and using a single dose of Y-90 Zevalin in patients with relapsed or refractory low grade NHL showed an ORR to be 80% with a 34% CR rate.

Methods: In an attempt to increase the response rates from Y-90 Zevalin we are conducting a Phase I trial evaluating the safety of giving two sequential doses of Y-90 Zevalin to patients with low-grade NHL 12-16 weeks apart. All patients receive the standard dose of 14.8 MBq (0.4 mCi)/kg with the dose escalation being on the second dose.

Results: Six patients have been enrolled and the first 3 patients have completed two doses of Y-90 Zevalin. Two had a CR and one had a PR after the first dose of 14.8 MBq (0.4 mCi)/kg. All received the second dose of 7.4 MBq (0.2 mCi)/kg with the only PR converting to a CR. The blood biologic clearance was longer in all patients on the second dose compared to the first (average first dose T 1/2 = 53.5 hr, second dose = 66.8 hr). One patient with extensive tumor and 20% marrow involvement had a CR after the first dose and blood clearance T1/2 was twice longer on the second (29.6 hr vs 61.3 hr). All patients had only transient blood count nadirs similar to the prior single dose trials and better after the second dose compared to the first. This may be from the lower second Y-90 Zevalin dose and lower marrow tumor.

Conclusions: Tumor binding of the Zevalin may be important in blood clearance of Zevalin with slower clearance in patients with low tumor burden. Preliminary results demonstrate a second dose of Y-90 Zevalin can be safely administered to patients with low grade NHL.

ADHESION MOLECULES EXPRESSION IN CELLS FROM CLL PATIENTS WITH PREDOMINANTLY SPLENIC MANIFESTATION (STAGE III(S)) COMPARED WITH PATIENTS IN STAGES 0 and I

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Cell surface expression of adhesion molecules by B-CLL cells may determine the pattern of tissue localization and dissemination. Patients with CLL morphology and phenotype but predominantly splenic manifestation (stage II(S)) constitute a subgroup of this disorder. Expression of surface adhesion molecules of the (g) superfamily (CD54), of the integrin family (β1-CD29, β2-CD18, β3-CD41, CD11a, CD11c, CD49h), of the selectin family (L-selectin-CD62L) and the lymphocyte homing receptor (CD44) was determined by flow cytometry on CD19+ CLL cells isolated from blood of 42 CLL patients: 14 stage 0, 5 stage I and 23 stage II(S).

A significant differences in the % of small VS large lymphocytes, between stage 0 and stage II(S) patients was found with the latter having increased number of small lymphocytes. The mean % of expression of the different adhesion molecules between patients with stage 0, stage I and those with stage II(S) disease is shown in the Table below. The % expression of CD11c, and CD44 was found to be significantly higher in CLL cells from patients with predominantly splenic manifestation (stage II(S)) disease. This may account for the tendency of lymphocytes in CLL stage II(S) to home to the spleen.

<table>
<thead>
<tr>
<th></th>
<th>Stage 0 (14 patients)</th>
<th>Stage I (19 patients)</th>
<th>Stage II(S) (23 patients)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small cells</td>
<td>16.8%</td>
<td>21.7%</td>
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<tr>
<td>Large cells</td>
<td>59.9%</td>
<td>32.1%</td>
<td>40.0%</td>
<td>*0.006</td>
</tr>
<tr>
<td>CD11a</td>
<td>13.8%</td>
<td>17.2%</td>
<td>23.5%</td>
<td>*0.047</td>
</tr>
<tr>
<td>CD11c</td>
<td>13.2%</td>
<td>8.8%</td>
<td>25.7%</td>
<td>*0.047</td>
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<tr>
<td>CD18</td>
<td>3.3%</td>
<td>4.3%</td>
<td>5.1%</td>
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<tr>
<td>CD49h</td>
<td>9.8%</td>
<td>12.1%</td>
<td>14.0%</td>
<td>N.S.</td>
</tr>
<tr>
<td>CD44</td>
<td>77.2%</td>
<td>80.5%</td>
<td>90.5%</td>
<td>*0.007</td>
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<td>CD49d</td>
<td>7.6%</td>
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</tr>
<tr>
<td>CD54</td>
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<td>3.1%</td>
<td>4.7%</td>
<td>&lt;0.001</td>
</tr>
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<td>CD61</td>
<td>3.8%</td>
<td>4.3%</td>
<td>7.8%</td>
<td>N.S.</td>
</tr>
<tr>
<td>CD62L</td>
<td>6.9%</td>
<td>7.4%</td>
<td>11.8%</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
AUTOIMMUNE HEMOLYTIC ANAEMIA IN B-CELL

M. Badea, Daniela Badea; University of Medicine and Pharmacy, Craiova 1100, Romania.

Introduction: Autoimmune hemolytic anemia (AHA) is a frequent event in Chronic Lymphocytic Leukemia (B-CLL) (10-20% of cases) and can appear at any time during the course of the disease. The mechanisms causing production of antibodies in B-CLL are not fully understood.

Methods: Between 1987 and 1999, AHA1 was observed at our institution in 25/165 (15.15%) patients with B-CLL (15 men, 10 women; median age 59 years, range 21-74). AHA1 was observed at times ranging from diagnostic to 68 months from diagnosis; in 24% patients before treatment and in 76% after. In the latter cases, 78.94% patients had received chemotherapy with chlorambucil and prednisone or CVP (cyclophosphamide, vincristine, and prednisone) and 21.06% had received fludarabine (FAMP).

Results: Response to treatment of B-CLL with AHA1 was classified as partial response in 50% cases, stable disease in 28% cases and progressive disease in 15% cases. The mean hemoglobin value observed at onset of AHA1 was 5.2 g/dL (range 4.2-8.6). The direct antiglobulin test was positive with anti IgG + anti C3d antibodies in 72% patients; in 4% patients with anti IgG + anti IgM + C3d antibodies, in 16% patients with anti C3d antibodies. In another two (8%) patients IgM cold agglutinins were present, in the one antibody was active up to 30°C. Only 24% cases required packed red blood cell transfusions. All patients were treated with corticosteroids (PND 1mg/kg/day for up to three weeks). Complete hematological response to AHA1 was observed in 80% cases, 16% patients had partial response and 1 (4%) patient died of hemorrhagic crisis. 20% of complete responders had AHA1 relapse. Splectomy is performed in 72% patients in two cases for multiple relapses after PND and chemotherapy and one for incomplete response. Another three patients had received PND in low doses for three to six months (between chemotherapy) for incomplete response of AHA1. Mean overall survival of the patients was 46 months from diagnosis of malignancy.

Conclusions: Our data suggest that AHA1 is more frequent in previously treated patients, with progressive B-CLL, than in untreated patients. AHA1 had a low incidence in patients treated with FAMP. Corticosteroids as standard doses are able to keep hemolysis under control in most cases, splenectomy being rarely indicated.

IN VITRO AND IN VIVO ACTIVITY OF SDX-101 (R-ETODOLAC), A PRO-APOPTOTIC COMPOUND FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA, MULTIPLE MYELOMA AND LYMPHOMA.

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We report here that SDX-101 (R-etodolac) a stereoisomer of the non-stereoidal anti-inflammatory drug etodolac which lacks cyclooxgyenase (COX) inhibitory activity, induces apoptosis of B-chronic lymphocytic leukemia (B-CLL) cells and primary multiple myeloma (MM) cells at concentrations that do not kill normal blood or marrow monoclonal cells. The mechanism of action of SDX-101 studied in primary CLL cells involves the down-regulation of the anti-apoptotic protein Bcl-2, the activation of the pexyosome proliferator-activated receptors, and the induction of NO1R, an orphan nuclear receptor that has been associated with apoptosis and is found over-expressed in curred diffuse large B-cell lymphoma. A phase I, open-label, dose escalation study in 37 healthy volunteers (mean age, 62 years, representative of the B-CLL patient population) demonstrated that SDX-101 is safe and well tolerated at single oral doses up to 2000 mg. Analysis of SDX-101 plasma levels demonstrated a comparable pharmacokinetic profile when equivalent doses of SDX-101 and RS-etodolac were administered. SDX-101 doses of 1200 mg, 1600 mg, 2000 mg resulted in mean peak plasma concentration of 235 μM, 340 μM, 432 μM, respectively. S-etodolac was not detected in plasma samples from subjects receiving SDX-101, demonstrating the lack of in vivo inverison. Phase II clinical trials in CLL are currently in progress. As such, R-etodolac represents a potential agent for treatment of B-CLL, MM, and other malignancies of the B cell lineage.

DOUBLING TIME AND BASELINE LEVEL OF SOLUBLE CD23 ARE BIOLOGICAL FACTORS FOR DISEASE PROGRESSION OF STAGE A B-CLL.


B-chronic lymphocytic leukemia (CLL) is a disease with prolonged natural history, primarily for stage A patients (pts). However, some pts will have a more severe and rapid evolution. Although, there are useful prognostic factors: clinical stage, lymphocyte doubling time, Ig somatic mutation, cytogenetics, we have few parameters to predict which pts diagnosed at stage A will have a more rapid progression. Recent reports suggest that soluble CD23 level is a prognostic factor for progression and survival. To further investigate the prognostic value of soluble CD23 in untreated stage A B-CLL, we prospectively followed the history of all pts referred or diagnosed in our institution, according to their soluble CD23 level. The median age was 62 years. There were 48 pts: 25 stages A (52%), 15 stages B (31%) and 8 stage C (17%). The median level of soluble CD23 was 142.7 U/ml for pts with stage A, 272.8 U/ml for stage B, 351.5 U/ml for stage C and 265 U/ml for the whole population. Normal range being ≤ 10 U/ml. The median follow-up was 1661 days for pts with stage A. Progression of upper stage of the disease for the whole population was significantly inferior when soluble CD23 was below the median (49%), compared to pts with dosage above to the median (74%, p<0.00067). For the pts diagnosed at stage A the value of soluble CD23 was a predictive factor for the evolution to stage C, 14% of pts in the group with dosage under the median value progressed to stage C and 81% of other pts progressed to stage C. When we take a cut off point of 50 U/ml, no pts progressed, 77% of progressive diseases was observed for the other pts (p=0.004). Doubling time of soluble CD23 at one year was also found to be a significant prognostic factor for progression to stage C. In the group of pts with a doubling time >1 year, 73% did not progress and when the doubling time of the level of CD23 was < 1 year all the pts were progressive (p=0.0034). In conclusion, our data confirm that soluble CD23 in the serum of B-CLL is an important prognostic biological factor for progression from stage A to stage C. We also demonstrate that soluble CD23 doubling time is significantly correlated with the aggressivity of the tumour.
PHASE-II STUDY OF A COMBINED IMMUNO-METHOTREXATE USING RITUXIMAB IN COMBINATION WITH FLUDARABINE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: This phase-II trial investigated the safety and efficacy of a new immunomethotrexate combination rituximab (R) and fludarabine (F) in patients (pts) with fludarabine and anthracyline-naive Chronic Lymphocytic Leukemia (CLL). The rationale for using R + F includes: single agent efficacy of both drugs, in vitro synergism of R and F, and no apparent overlapping toxicities.

Methods: 11 patients were treated in a single center. R (375 mg/m²) was given on days 1-5, 21-25, 57-61, 85-89, and rituximab (375 mg/m²) on days 57, 85, 113 and 151.

Results: 31 eligible B-CLL pts were enrolled. 20 were previously untreated and 11 relapsed or transformed. Median PFS was 16.4 months. 18 pts (58%) achieved a complete remission with a median duration of 11 months. Median OS was 25 months. 22% of the patients developed infusion-related symptoms (primarily fever, chills, and diarrhea).

Conclusion: This combination is effective and feasible in B-CLL.

LYMPHOPLASMACYTOID DIFFERENTIATION PREDICTS POOR SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: B-cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in adult patients. According to the KIEL classification, B-CLL and immunocytoma (IC) were described as separate entities. Further, these two neoplasms differ in their clinical course. The WHO proposed that B-CLL comprises a variant with plasmacytoid differentiation and separated lymphoplasmacytoid lymphoma (IC) as a distinct entity (lymphoplasmacytoid immunocytoma of the KIEL classification). The aim of this investigation was to test whether plasmacytoid differentiation in the B-CLL variant was of value as a prognostic factor. Secondly, we wanted to test the incidence of aberrations on Ch1, Ch2, Ch13 and Ch17 in patients with B-CLL and IC using metaphase cytogenetics and thirdly, we wanted to investigate whether cytogenetic findings can help in distinguishing B-CLL, plasmacytoid variant and lymphoplasmacytoid lymphoma.

Materials and methods: From 7/1992 to 1/2000 we analyzed 116 cases using metaphase cytogenetics. Lymph node biopsies were analyzed in 51 cases. Nine patients had a diploid karyotype, in five no mitoses were detectable. Of the whole group investigated cytogenetically, clinical data were available from 90 patients. Results: Table 1 shows cytogenetic findings according to histological subtype (WHO):

<table>
<thead>
<tr>
<th>Chromosome Arm</th>
<th>Ch1</th>
<th>Ch2</th>
<th>Ch13</th>
<th>Ch17</th>
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<tbody>
<tr>
<td>3q</td>
<td>9/13</td>
<td>3/12</td>
<td>23/45</td>
<td>14/17</td>
</tr>
<tr>
<td>11q</td>
<td>9/13</td>
<td>3/12</td>
<td>23/45</td>
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</table>
**T-CELL IMBALANCE IN HAIRY CELL LEUKEMIA IS RELATED TO THE BIAS OF T-CELL RECEPTOR VARIABLE REGION USAGE.**


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**Introduction:** Hairy cell leukemia (HCL) is a chronic B-cell malignancy clinically associated with severe T- and NK-cell dysfunction. Patients with HCL exhibit a defective NK-cell function and sometimes an imbalance of T-cell subsets.

Methods: In this study, using flow cytometry and molecular analysis, including RT-PCR, heteroduplex and sequencing, we evaluated the pattern of accumulation of T lymphocytes in the peripheral blood of 12 HCL patients at the time of diagnosis by investigating blood lymphocytes (PBL). The phenotype of PBL was characterized by flow cytometry analysis with antibodies recognizing different TCRB region families. Flow cytometry analysis was associated to the molecular evaluation performed by semiquantitative RT-PCR in order to determine the T-cell receptor (TCR) repertoire using primers recognizing 24 different TCRB families.

Furthermore, heteroduplex and sequencing analysis were performed.

**Results:** RT-PCR analysis revealed a limited TCR repertoire usage in the blood of patients with HCL, with some TCRB being more expressed and others appearing deleted. In all the patients the analysis by RT-PCR revealed the absence of 1 or more TCRB products. In some patients the semiquantitative RT-PCR demonstrated an increased expression of some TCRB gene families, respectively VB2, VB6, VB7, VB8. Heteroduplex analysis of RT-PCR products usually showed a smear, indicating a polyclonal expression. In some cases, homoduplex bands were observed, indicating the presence of oligoclonal T-cell subsets. The oligoclonal pattern of two TCRB gene families was definitively confirmed by sequencing the CD3 region.

**Conclusions:** The molecular results obtained in HCL patients revealed the presence of TCRB T-cell gene families expression and, less frequently, the absence of some gene products, thus indicating the selection of T-cell subsets in these patients. These data indicate that this disease is also characterized by the imbalance of T-cell subsets at molecular level. Whether this pattern is related to the malignant clone remains to be clarified.

**IGM MYELOMA: AN ANALYSIS OF FOUR CASES**

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**Introduction:** IgM myeloma is a rare disease, which accounts for approximately 0.5% of all multiple myelomas (MM). Here, four cases of IgM multiple myeloma are reported.

**Materials and results:** Two of the four patients with IgM MM were diagnosed in advanced clinical stages with multiple osteolytic lesions, leading to hypercalcemia in one patient. Bone marrow morphology showed a variable degree of infiltration with mainly mature plasma cells. An immunophenotypic analysis performed in one case showed expression of CD38 and monoclonal cytoplasmatic immunoglobulins. Interphase fluorescence in situ hybridization done in one case did not reveal any anomalies or deletions of the retinoblastoma, P16, and P53 tumor suppressor genes. While one patient with a Smithsonian IgM myeloma did not need specific therapy, the others received cytotoxic treatment based on standard chemotherapy for MM, including high dose melphalan chemotherapy with peripheral stem cell support in two cases. One stable disease, one sustained complete remission and one progressive disease were observed. All four patients were alive one year after diagnosis. One died due to progressive disease after 21 months.

**Conclusion:** We conclude that IgM myeloma shares clinical and histological features with other MM rather than with Waldenström's macroglobulinemia (WM), which is most commonly diagnosed in cases with IgM monoclonal gammapathy. Since MM and WM are different concerning prognosis and treatment strategies, both disease entities should be distinguished based on clinical criteria, bone marrow morphology and immunophenotypic analysis.

**INCREASED EXPRESSION OF CD55 AND CD59 ON BENIGN AND MALIGNANT PLASMA CELLS: COMPARISON WITH OTHER PROLIFERATIVE B-CELL DISORDERS.**

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1. University of Sherbrooke Faculty of Medicine, Sherbrooke (Quebec), Canada 2. Mayo Clinic and Mayo Foundation, Rochester, MN, USA.

The action of monoclonal antibodies on tumor cells is partly mediated by complement-dependent cytotoxicity (CDC). Since the reported activity of rituximab is lower in plasma cell disorders than in lymphomas, we examined the expression of 2 complement regulatory proteins, CD55 and CD59 in plasma cells and in lymphocytes, using blood and/or marrow samples of 102 subjects (see table 1).

**Surface expression of CD59 was higher in plasma cells (PC) than in lymphocytes. No difference was detected between benign and malignant plasma cells (p value<0.05).** In contrast, CD55 was expressed more weakly in malignant B lymphocytes than in their normal counterparts (p<0.05).

**Table 1. CD55 and CD59 cell surface expression in plasma cells: median channel intensity, with range given in parentheses.**

<table>
<thead>
<tr>
<th></th>
<th>Normal individuals (n=20)</th>
<th>Myeloma (n=43)</th>
<th>B-CLL (n=31)</th>
<th>Low-grade lymphomas (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD55</td>
<td>59.4 (581-824)</td>
<td>832 (687-1024)</td>
<td>614.5 (453-920)</td>
<td>839 (100-1258)</td>
</tr>
<tr>
<td>CD59</td>
<td>506 (487-550)</td>
<td>506 (473-550)</td>
<td>477 (354-731)</td>
<td>831 (613-1393)</td>
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<td>Immature PC</td>
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<tr>
<td>B cells</td>
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</table>

Our results document bright surface expression of CD55 and CD59 in plasma cells and a lower CD55 expression in B cells. We postulate that this may be a mechanism of resistance of plasma cells to complement action and, therefore, to plasma cell disorders to immunotherapy with rituximab.

**NON MYELOABLATIVE TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA: EXPERIENCE FROM THE SPANISH REGISTRY.**

Pérez-Simó LE, Martín R, Caballero D, Alegre A, Lóeen A, De la Serena J, Tomás JF, Mateos MV, Canav C, Sierra J, San Miguel JF.

**Introduction:** In order to decrease the high mortality rate in patients diagnosed with multiple myeloma undergoing allogeneic transplantation maintaining a graft versus myeloma effect, different protocols have been reported using non myeloablative conditioning prior to infusion of allogeneic peripheral blood stem cells (PBSCT).

**Patients and methods:** 24 patients diagnosed with MM received fludarabine at 30mg/m2 x 5 days followed by melphalan 140 mg/m2 divided in two days (n=18) or 70 mg/m2 in one day (n=6) prior to the infusion of PBSCT from matched related donors. Median age was 54 years (45-65). 18 (78%) had previously received an autologous transplantation. GVHD prophylaxis consisted of CSA + MTX. At transplant 2 patients (9%) were in CR, 12 (55%) in PR, 2 (9%) in SD and 6 (27%) patients were refractory or in progressive disease. A median of 4.89 x 10^6 CD34+ cells/kg and 4.2 x 10^6 CD34 + cells/kg were infused.

**Results:** The procedure was well tolerated with only 6% of patients developing grades 3-4 toxicity (4% grade 4 mucositis and 2% grade 4 pneumonia). Incidence of aGVHD was 52% (18% grades 3-4). Incidence of cGVHD was 73% (40% extensive). Median follow up was 340 days. Projected EFS at 2 years was 42%. Patients who developed cGVHD had a significantly better EFS as compared to those who did not develop it (67% vs 40% at 2 years, p<0.04). OS at 2 years was 68%. Disease status at transplant was the only variable which significantly influenced OS: 87% of patients undergoing transplantation in RC sensitive disease remain alive at 2 years vs 33% for patients in relapse / refractory disease (p=0.001).

**Conclusion:** Non myeloablative allogeneic transplant is feasible with a tolerable transplant related mortality in patients diagnosed with multiple myeloma. Chronic GVHD is related to a better disease control suggesting a graft versus myeloma effect. Prior therapy, such as autologous transplant, may be required in order to reach disease control since it has a favourable effect on survival.
CLINICAL EVOLUTION OF ASYMPTOMATIC IgM MONOCLONAL GAMMOPATHY IN 168 PATIENTS

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Introduction: The diagnostic definition of IgM MGUS is still a subject of debate. The aim of this study was to identify the variables most closely related to the evolution to Waldenström’s macroglobulinemia (WM) in a population of patients with asymptomatic IgM monoclonal gammopathy in an attempt to contribute towards a more precise diagnostic definition (and monitoring programme) of these indolent lymphoproliferative diseases.

Methods: The study involved a series of 168 patients (M/F 109/59; mean age 62 years; range 31-85) followed from 1981 to 2000 by the Italian Lymphoma Study Group (GISL), who were diagnosed as carriers of a serum IgM monoclonal component and, after a complete diagnostic work-up, did not require immediate treatment.

Results: Their main characteristics at diagnosis were: mean serum MC: 1.46 g/dl (0.2-3.2 g/dl); mean Hb: 13.8 g/dl (10.4-17.2 g/dl); high β2-microglobulin (33% of cases); high LDH levels (6.5%); light-chain proteinuria (10%); positive HCV antibody (4.8%) and cryoglobulinemia (2.4%); moderate splenomegaly (4.8%) and mild adenopathy (3%); and slight signs of peripheral neuropathy (13.7%). The mean percentages of bone marrow lymphocytes and plasma cells were 16.2% and 2.3%. 67% of the patients had a normal bone marrow histological pattern and the remaining a lymphoid infiltrate accounting for 5-80% of the cellular component. After a median follow-up of 74 mos (6-213 mos), 29/168 patients (17.3%) required chemotherapy for WM (22 pts), NHL (3 with low-grade immunocytoma and 1 with large B-cells), amyloidosis (2) or peripheral neuropathy (1). Overall survival at 3 and 5 years was 99.2% and 98.1% respectively, and evolution-free survival was 97.2% and 97.8%, respectively. In univariate analysis, the variables that correlated with an evolution to overt lymphoproliferative disease were: male gender (P=0.001), hemoglobin (<12 g/dl in males and <11 g/dl in females; P<0.0001); serum IgM MC (>1.5 g/dl; P=0.0016) and diffuse pattern of BM infiltrate (P<0.001).

Conclusions: In a population of patients with asymptomatic IgM monoclonal gammopathy, male gender, anemia, serum MC levels >1.5 g/dl, and the bone marrow histological pattern at diagnosis strongly correlated with an evolution to lymphoproliferative disease.