EVALUATION OF 18F-FDG-PET IN THE STAGING OF PATIENTS WITH INDOLENT NON-HODGKIN'S LYMPHOMA.
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Background: Staging and response assessment in indolent non-Hodgkin's lymphoma (NHL) are currently heavily reliant on structural imaging. Gallium scanning has a limited role, with reported sensitivity ranging from 40-70%. PET is an alternative functional scanning modality however its role in indolent NHL has not been established. The purpose of our study was to retrospectively assess the sensitivity and clinical impact of PET scan findings in patients (pts) with indolent NHL.

Method: The computerized database was searched for pts with indolent NHL who had PET scanning from May 1997 to January 2001. Individual case records were reviewed.

Results: A total of 156 patients were identified (89% of whom) PET scans were performed. 94% of pts had FDG avid disease. The histology of the PET negative scans included 2 follicular and one marginal zone NHL. For initial staging, PET and conventional assessment were concordant in 90% of cases. In the remaining 10% PET demonstrated more extensive disease than conventional assessment. For progression, PET and conventional assessment were discordant in 40% of cases. PET findings down staged 21% of pts and upstaged 19%. Correlation of individual disease sites was also performed. CT and PET identified 180 and 146 sites respectively. For 24 discordant PET results were reviewed. The PET was correct in 21 of these lesions (92%). The high accuracy of PET in assessing discordant lesions suggests a greater diagnostic utility compared with CT.

Conclusion: These findings demonstrate that 18FDG-PET has a high sensitivity for indolent NHL, and often leads to alteration of disease stage and management.

UTILITY OF FDG-PET IN LYMPHOMA BY WHO CLASSIFICATION
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Accurate staging of disease and follow up after therapy are critical issues in the care of patients with lymphoma. Positron emission tomography (PET) using 18fluoro-2-deoxyglucose (FDG) is a functional imaging modality based on the observation that many malignant cells metabolize glucose at a higher rate than normal tissues. FDG-PET scanning is increasingly used in the evaluation of lymphoma patients. However, individual cases of lymphoma are not uniformly detected by PET, with some tumors lacking FDG avidity. We hypothesized that biological differences between different histologic subtypes of lymphoma would result in variability in FDG avidity between these subtypes. We have retrospectively studied the results of PET scanning in patients with lymphoma subdivided by the World Health Organization (WHO) classification system.

Results: 156 patients had FDG PET scanning performed at diagnosis or relapse prior to anti-tumor therapy. Overall, 145, or 93%, of patients had disease detectable on FDG-PET scanning. Of these, 100% of large B cell lymphomas, mantle cell lymphoma, anaplastic large cell lymphoma, Burkitt lymphoma, and mycosis fungoides, and 97% of follicular lymphoma and Hodgkin's lymphoma were detectable. In contrast, 65% of marginal zone lymphoma, 50% of peripheral T cell lymphoma and 9% of cutaneous B cell lymphoma were detected. A site by site analysis comparing PET with CT was performed on subsets of patients with lymphomas reliably imaged by PET. All sites of disease detected by CT scan were also detected by FDG-PET. In addition, occasional sites of FDG avidity were noted on PET scanning that were not identified abnormal on CT.

Conclusion: FDG-PET is an informative test in lymphoma subtypes, with differences in reliability evident between specific WHO subtypes. In these subtypes in which PET scanning is reliably informative, PET detects all sites of disease noted by CT. PET may detect sites of disease not visualized by CT, improving sensitivity over conventional staging.

EARLY FDG-PET FOR THE PREDICTION OF OUTCOME AFTER PSCT IN CHEMOSENSITIVE RELAPSED LYMPHOMA
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Introduction: Recent studies have shown that 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) might be a better tool than computer tomography (CT) to predict long-term treatment outcome in primary disease. We performed a study to assess the predictive value of FDG-PET in relapsed chemosensitive lymphoma.

Method: Retrospective analysis of clinical and imaging data of patients with recurrent or persistent aggressive non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD), who were treated with 3 courses of 2-1-line induction chemotherapy (DHAP-VIM-DHAP), followed by ablative therapy (BEAM) and peripheral stem cell transplantation (PSCT) for chemoresponsive patients. FDG-PET was performed in parallel to conventional diagnosis and induction chemotherapy, 6 months prior to PSCT, and again 6 months after remission. The PET information of lesions, volume, and intensity of the largest abnormal lesion was compared with conventional diagnostic methods and correlated with PFS (log rank test).

Results: Between January 1999 and September 2001, 43 patients were included, of whom 34 (21 NHL and 13 HD) were transplanted. Median (range) follow up was 16.6 (4-35) months after PSCT for those in remission. After 2 courses of induction therapy, 25/54 (74%) patients who went on to PSCT still had abnormal FDG uptake in one or more sites previously shown to be involved by lymphoma. 13/26 (50%) of these PET-positive patients progressed. Of the PET-negative patients only 2/9 (22%) progressed. Computer tomography was less able to predict outcome: 9/19 (47%) CT-positive patients progressed vs. 10/18 (55%) CT-negative patients. PFS was significantly worse for PET-positive patients: 6 months for PET-positive patients vs. 22 months for PET-negative patients (p=0.013).

Conclusions: Early FDG-PET might be a reliable tool to predict long-term treatment outcome in patients with relapsed lymphoma. Disappearance of abnormal FDG-uptake after 2 courses of induction therapy correlates with favourable outcome.

UTILITY OF FDG-PET IN LYMPHOMA BY WHO CLASSIFICATION
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Positron Emission Tomography (PET) with 18F-fluorodeoxyglucose (FDG) Restaging as Prognostic Marker for Lymphoma Relapse/Progression

Introduction: One of the most powerful prognostic factors is the response to therapy. PET could be more informative than conventional methods in terms of disease activity than conventional examination methods.

Methods: Ninety-one newly diagnosed patients (pts) (30 with Hodgkin's lymphoma - HL, and 60 with Non-Hodgkin's lymphoma - NHL, 42 with DLCL, 15 with FCL) were studied. PET was performed early (after 2-3 cycles of chemotherapy) at the end of therapy and/or at the end of therapy. Thirteen pts out of 24 PET+ early converted to PET- at the end of therapy and 11 pts PET+ early remained PET+. Progression was observed in 5 out of 71 pts who achieved PET- compared to 12 out of 24 pts who remained PET+ (p<0.0001). Progression free survival (PFS) was significantly better (p<0.0001) in PET- pts (94% at 1 yr) compared to PET+ (51% at 1 yr). The results were highly significant in subgroup of pts with aggressive NHL (1 yr PFS was 93% in PET- compared to 45% in PET+, p<0.0001). The impact of PET+ in this subgroup were more pronounced in pts examined early (p<0.005) compared to examination at the end (p<0.08). Moreover PET+ significantly predicts relapses in patients with low (L) or low-intermediate (LI) risk IPI (p<0.005). The differences were of borderline significance in indolent NHL and in HL (probably due to low number of events).

Conclusion: PET examination as restaging of lymphoma presents a highly significant outcome predictor. It seems that in patients with aggressive NHL, it could be more informative when it is performed early compared to examination at the end of therapy and it could detect patients with high probability of early relapses, which could be of great importance mainly in patients with L or LI IPI.
1. Imaging

B-CELL NON-HODGKIN LYMPHOMA (NHL) IN CHILDREN AND ADOLESCENTS: CENTRAL PHENOTYPIC RESULTS FROM CHILDREN'S CANCER GROUP (CCG) STUDY CCG-5961 AND IMPLICATIONS FOR FUTURE TARGETED BIO-INFECTIVE THERAPY (TBT)
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Introduction: Targeted therapies in B-cell NHL are based on expression of specific cell surface antigens: CD19 (B225), CD79a, CD20 (B20), CD38, CD56, and CD22 (Egratuzumab). Methods: The patients analyzed in this report were derived from the CCG arm of an international study employing risk-adapted short intense multiagent chemotherapy for B-cell NHL. The types of B-cell NHL included: Burkitt's lymphoma (BL), high-grade B-cell lymphoma, Burkitt-like (BLl); diffuse large B-cell lymphoma (DLBCL). B-cell acute lymphoblastic leukemia (L5-B-ALL3). Diagnostic biopsies were obtained for central pathology review and immunophenotyping studies by paraffin immunohistochemistry (IHC). The antibodies employed were: CD15 (Becton Dickinson (BD) CD15; CD20; CD30; CD45RO (UCHL-1); CD3 (BDH); CD22 (FCC1); ALK-/NKL4 (phospho). Results: To date, 353 cases have completed central review with immunophenotyping. Age: range 1-20 yrs (median 14); M:F 3:1.1; Morph. Stage: I 20%, II 28%, III 38%, IV 35%; B-ALL L3 9%. FAB Group: A 20%, B 66% C 13%. The results of the immunophenotyping studies are listed in the Table. These results indicate that nearly (10%) of B-cell NHL in children and adolescents express CD56, ALK2, and CD22 expression was detected in nearly all of the cases tested. For cases of CD10 positive DLBCL, all cases were negative for ALK expression.

Conclusions: (see addition to aid proper diagnosis, these studies indicate that immunophenotyping of B-cell NHL in children and adolescents from archival (paraffin-embedded) samples can determine eligibility for bio-infected treatment approaches. These results show that nearly (10%) of the pediatric B-cell NHL addressed in this study would be candidates for TBT. Based on these results, the Children’s Oncology Group is planning future trials using chemotherapy with radiomunotherapy with anti-CD20 (COG ANH0111) and anti-CD22 (COG ADVL0113).

CHROMOSOME ABNORMALITIES IN B-CELL NON-HODGKIN LYMPHOMA (NHL) IN CHILDREN AND ADOLESCENTS: A REPORT FROM CHILDREN'S CANCER GROUP (CCG) STUDY CCG-5961
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Introduction: CCG has reported chromosome abnormalities associated with prognosis in childhood pre-B-cell lymphoblastic leukemia (ALL). However, few cytogenetic studies have been performed in childhood B-cell NHL. B-cell ALL L3, Burkitt lymphoma (BL), Burkitt-like lymphoma (BLl), and diffuse large B-cell lymphoma (DLBCL). Methods: The patients analyzed in this report were derived from the CCG arm of an international study of children and adolescents with B-cell NHL. Pathology and cytogenetic material at initial diagnosis underwent central review.

Results: 60 patients: median age 10 (2-19) yrs; male:female ratio 4:1. B-cell NHL types included: B-cell ALL L3 (16); BL (27); BLl (8); DLBCL (9); Morph. Stage: I (9); II (11); III (18); IV (6); B-cell ALL L3 (16). FAB Group Staging: A (7); B (3); C (19). (1) Chromosome abnormalities were seen in 2% of cases (93%). Translocations involving c-myc were present in 44 cases (73%) including: t(8;14)(q24;q32) in 41 (68%); t(8;22)(q24;q11) in 2 (3%); and t(4;11)q21p12 in 1 (2%). The frequency of c-myc translocation within B-cell NHL types was as follows: B-cell ALL L3 (94%); BL (81%); BLl (73%); DLBCL (11%). Translocations involving c-myc were the only abnormality in 14 cases (23%), while c-myc translocations were associated with additional abnormalities in 30 cases (50%). In 15 cases (7 B-cell ALL L3, 5 BL, 2 BLl, 1 DLBCL), there were additional copies of the lo(2q32-1q31) region, including 13 cases associated with c-myc translocation. Chromosome abnormalities were present in all 9 cases of DLBCL, but none involved 3q27 (pl0-1) or 18q11 (pl0-1). Chromosome abnormalities involving 11q23 (MLL) were present in 2 cases of DLBCL.

Conclusions: B-cell NHL in children and adolescents has a high frequency of chromosome abnormalities that most often involve c-myc translocations, frequently associated with additional chromosome abnormalities including t(q23-1q31) amplification. Pediatric BL usually has c-myc translocations suggesting genetic similarity to BL. The absence of abnormalities involving bcl-5 or bcl-2 in pediatric DLBCL suggests genetic differences from DLBC in adults. The analysis of the c-myc locus molecular breakpoints associated with the translocations and their recurrent genetic remains to be determined. Future analyses will include correlation of chromosome abnormalities with survival to identify specific chromosome abnormalities associated with prognosis.

LIMITED THERAPY FOR EARLY-STAGE NON BODGKIN'S LYMPHOMA (NHL) IN CHILDREN: THE EXPERIENCE OF ISTITUTO NAZIONALE TUMORI DI MILAN.
From the Department of Pediatric and Pathology, Istituto Nazionale Tumori, Milan, Italy.

Background. Children with early-stage NHL have an excellent outcome regardless of histologic type. Pediatric oncologist's attention is addressed to minimize acute and late toxicities.

Methods: From 1988 onwards, 32 children with Murphy's stage I-II NHL were treated; median age was 10 yrs (range 2-17) and M:F ratio 1:2. Histologic subtype was as follows: Burkitt 15, diffuse large B-cell (histiocytic 4, large cell lymphocytic 1-8) 8, precursor B-cell (B-lymphoblastic) 4, MALT-type 3, follicular center (centroblastic-centrocytic) 1, peripheral T (T-immunoblastic) 1. The Waldenström's ring was affected in 1 case in 11 cases, other primary sites included: peripheral node, ileum-ocrom 4, skin, stomach 4, ovary 1, bone 1. In 19 pts the surgical biopsy determined a complete absence of the disease ("erased" stage I). The chemotherapy (CT) program consisted of a 7-week induction with prednisone, VCR (1.4 mg/m², 9 doses), CHM (1 g/m², 4 courses), DDX (25 mg/m³, 3 courses), HDMTX (3 mg/m³, 1 course), variously associated on a weekly basis. Until 1998 a maintenance phase alternating 6M/MTX, i.m. DEX/VC/CR/PMP/STM was given to complete a total duration treatment of 8 mos. From 1998 onwards the induction was intensified by adding VP16 (500 mg/m³, 1 dose) and a further HDMTX dose, but abolishing maintenance until T-lymphoblastic histology. The scheduled duration of the ongoing CT is 9 weeks. The treatment strategy did not provide intrathecal CT nor radiotherapy.

Results. Twenty-nine out of 32 pts are alive in CCR or NED, 12/13 pts with evaluable disease had a prompt CR (all within the 3rd week of therapy); the child experiencing early Burkitt's progression shifted to the advanced-stage protocol and had been cured. Two pts died (at 3 and 5 mos); 1 died of lymphoma, 1 achieved cure with the advanced-stage protocol. At a median follow-up time of 8 yrs (range 0.15-14) EFS, PFS and OS rates at 5 yrs are 91% (±5%), 91% (±5%) and 95% (±5%), respectively. None of the 9 pts treated with the ongoing 9-regimen has relapsed at a median follow-up of 2.5 yrs (range 3-54). No serious toxicity occurred.

Conclusions. A 9-week CT regimen is as effective as the 6-mo therapy, maintaining the very high cure-rate in localized NHL, less morbidity.
SUCCESSFUL TREATMENT OF PEDIATRIC AND ADOLESCENT PATIENTS WITH PMLBL WITH SHORT-TERM B-CELL-LINKED TYPE CHEMOTHERAPY WITHOUT RADIOTHERAPY - RESULTS AND PROGNOSTIC FACTOR ANALYSIS FROM THE BFM-TRIALS


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Introduction: Primary mediastinal large B-cell lymphoma with sclerosis (PMLBL) is a rare entity of NHL arising from thymic mature B-cells. This study analyzes characteristics and outcome of pediatric patients with PMLBL, treated in therapy trials NHL-BFM.

Methods: Treatment was stratified by stage and tumor mass (serum-LDH). Patients received four, five, or six 5-day therapy courses based on MTX, Dexamethasone, VCR, L-asparaginase, Cyclophosphamide, Doxorubicin, Etoposide, Cyclophosphamide, Doxorubicin, Etoposide, and intrathecal therapy. Radiation was not part of the protocol.

Results: Of 1600 patients (pts) enrolled from April 1996 to September 1999, 30 pts with suffering from PMLBL. Median age was 14.3 years (range 1.4-16.7 yrs), 15 pts were male, 15 female. Tumor locations were: mediastinum only n=12, lung n=7, renal n=7, bone marrow or CNS n=2. Two pts died early during therapy (n=1), relapse n=1. 19 patients are in CR 1 yr after. A median observation time of 5 years (range 1-12 years; were two last to follow-up (EFS 5 yrs)). Age >14 yrs or lower tumor mass (LDH >500 U/L) at diagnosis were identified as risk factors for relapse in multivariate analysis. Residual mediastinal masses were present in all pts after 2 courses of therapy, and in 18 pts after the end of therapy. In 12 pts 2nd look biopsy was performed, showing avascular tumor in all these patients.

Conclusions: PMLBL mainly concerns adolescents. High-dose chemotherapy for B-cell NHL yields an EFS at 5 years of 70% (SE 0.08). Age at diagnosis and initial tumor mass (serum-LDH) are of prognostic value in pediatric patients with PMLBL.

PERSISTENCE OF ANTIBODY RESPONSE TO ALK PROTEINS IN PATIENTS WITH ANAPLACTIC LARGE CELL LYMPHOMA

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Introduction: Anaplastic large cell lymphomas (ALCL) comprise approximately 50-60% of paediatric large cell lymphomas. The neoplastic cells in 80% of ALCL express oncospecific anaplastic lymphoma kinase (ALK) proteins and constitute the tumour entity ALK-positive lymphomas. The majority of patients with ALK-positive lymphomas have a favourable prognostic outlook compared with ALK-negative ALCL, possibly as a result of immune recognition of the tumour-associated ALK proteins. In support of this we have previously identified a B cell response to ALK proteins at the time of diagnosis. We have now extended this study to investigate the duration of the antibody response in patients with ALK-positive lymphoma.

Methods: Blood samples were obtained from five ALK-positive lymphoma patients at varying times following diagnosis. Antibodies to ALK proteins were then identified using an immunoperoxidase staining technique on COS cells transfected with cDNA encoding for NPM-ALK and ALK proteins.

Results: High titres of IgG antibodies (in excess of 1:250) to ALK proteins were identified in three patients up to 26 months after diagnosis and commencement of treatment. All three of these patients had presented with high titres of antibodies to ALK at diagnosis. A low titre of antibodies (1:100) was still detected in another patient 13 months after diagnosis. All of these patients remain in complete remission. The fifth patient had no detectable antibodies to ALK 4 months after diagnosis. In contrast to the other subjects, this patient had only a low titre (1:100) of antibodies to ALK on initial testing and has progressive disease.

Conclusion: This study provides further evidence that high antibody levels to ALK detected at time of diagnosis may be relevant to outcome and may provide valuable information to enable those cases of ALK-positive lymphomas with a poor prognosis to be identified. This study also opens up the possibility of immunotherapy for those patients who do not respond to conventional treatment. Further studies are ongoing to investigate these possibilities.

 With withdrawn

ANAPLASTIC LARGE CELL LYMPHOMA (ALCL) IN CHILDREN AND YOUNG ADULTS: DIFFERENT PROGNOSIS FOR PATIENTS WITH ISOLATED CUTANEOUS VERSUS SYSTEMIC DISEASE

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Introduction. ALCL represents approximately 10% of childhood lymphomas, being characterized by frequent extra-nodal involvement of skin, soft tissues and viscera. ALCL with isolated skin involvement is extremely rare in childhood, where it may represent a different clinical entity. Within ALCL, clinical differences exist respectively between ALK+ and ALK- nodal ALCLs and between primary nodal (systemic) and primary extranodal (cutaneous) forms. The latter being part of the spectrum of primary cutaneous CCLD-related disorders. Currently, it is not clear whether subjects with isolated skin disease deserve systemic treatment or whether a "wait and see" policy could be appropriate.

Patients and methods. Nineteen pts with ALCL, 35 M and 55 F, median age 12 yrs (1-26), were evaluated. Median FIPG was 65 mos (1-250 mos). Eighteen pts (90%) were affected by systemic disease, with extra-nodal involvement in 53 (65%). The remaining 9 pts had isolated skin lesions. The evaluation of ALCL was available for 76% (64% and it resulted positive in 61 of them (80%) and negative in the remaining 13 subjects (20%). 73% of pts were treated according to a LSAAL-02-like chemotherapy protocol, 8 (9%) according to a BFM schedule, 9 with other regimens. Radiotherapy was added in 21 cases (39%) while in 1 pt it was the only therapy adopted. Seven cases, all with primary cutaneous lymphomas, did not receive any type of treatment.

Results. Overall, 66 pts (74%) are alive with no evidence of disease (53 of them in CR and 11 after a disease relapse) and 2 pts are AWD (2%). The remaining 22 pts (24%) died, 18 for their disease and 4 because of treatment complications. EFS for the entire study population is 56 ± 6%. Considering the 81 pts with systemic disease, 41% relapsed, at a median of 8 mos from the diagnosis, while only 1 of the 9 pts with isolated skin disease (11%), who was previously treated with chemotherapy, presented a cutaneous relapse at 1 yr (6 mos-2.08). The remaining 8 pts with isolated skin disease showed a spontaneous disease regression. None of the 7 untreated pts with isolated skin lesions progressed, ALCL negatively significantly correlated with isolated skin involvement, as 10 of the 39 subjects (26%) with isolated skin disease treated for ALK were negative, whereas ALCL was negative in only 8 out of the 69 subjects (12%) with systemic disease. ALCL, however, was not a favorable prognostic factor in pts with systemic disease, since 63% of ALK-negative pts relapsed, as compared to 39% of relapses observed in ALK-positive pts.

Conclusions. Our results suggest that pts with ALCL and isolated skin involvement have a better EFS when compared to pts with systemic disease. Clinical observation without chemotherapy treatment could represent an appropriate approach for this subgroup, considering that spontaneous regression occurred in the majority of cases. In this multi-institutional series ALK negativity was a constant feature of the form with isolated skin lesions and did not demonstrate a significant impact on the clinical outcome of pts with systemic disease.
TREATMENT RESULTS OF CHILDREN WITH NON-B LYMHOBLASTIC LYMPHOMA: REPORT OF POLISH PEDIATRIC LEUKAEMIA/LYMPHOMA STUDY GROUP (PPLLSG)

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Introduction: From 1993-2001 54 pts 0.9 to 17.4 yrs of age, with non B lymphoblastic lymphoma (NB-LL) were treated in 11 centers of PPLLSG according to National BFM-90 protocol. The purpose of the study was the evaluation of the treatment results and an attempt to identify prognostic factors in this group of patients.

Methods: Cases were classified according to the criteria of the updated Kiel Classification for NHLs. The immunophenotypic subgroups were defined according to the European Group for the Immunological Characterization of Leukemias criteria. Staging was performed using Multiparameter classification.

Results: According to the extent of primary disease, pts were sub-classified into 4 stages: stage I (1.9%); stage II (5.9%); stage III (55.5%); stage IV (33.1%). The clinical manifestations were as follows: mediastinum-32 (59.3%) peripheral lymph nodes-15 (27.8%); abdomen-3 (5.6%); skin and soft tissue-1; head-neck-1; CNS-1. Among 18 pts with stage IV, 11 had BM involvement, 3 had CNS involvement, 1 had bone involvement. Fourteen patients entered CR (87.5%). 3 PR (5.6%). After median follow-up of 2.9 years the estimated EFS for the whole group was stage I-60%, stage II-60%, stage III-63%, primary site of disease in mediastinum-60%, peripheral lymph nodes-78%, for pts with residual tumor less than 70% TR on day 33-66% vs. pts with complete tumor response in this trial-84%. Events were 1 toxic death during LDF (1.4%), 4 progression during therapy and 8 relapses during and after therapy. The most relapses were local and occurred up to 16 months from diagnosis. We didn't identify the significant prognostic factors, which may have an impact on outcome in this trial. Correctness: The most frequent form of failure in our study was the early local recurrence. None of the tested parameters was found to define a subgroup of pts with significantly superior or inferior EFS. Further studies are needed to determine prognostic factors useful for patient stratification. These data are necessary for the optimal treatment for NB-LL pts.

CNS INVOLVEMENT IN CHILDHOOD NON HODGKIN'S LYMPHOMA

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Introduction: We investigated the biological, clinical features, and treatment results in non-Hodgkin's lymphoma (NHL) involving the CNS in a large unselected cohort of children suffering from any form of NHL, including acute b-cell leukemia (B-ALL).

Methods: From 04/09 to 01/01, 1812 patients (pts) up to 18 years of age, diagnosed with any type of NHL were registered in NHL-BFM studies. Staging included clinical examination (CE), bone marrow (BM), and neuroimaging (CT or MRI). CNS involvement was diagnosed in case of CSF blasts, on and/or intracranial mass (ICM), on and/or cranial nerve palsies (CNPs). All cases were registered in NHL-BFM studies (including 145 patients with CNS involvement). The CNS therapy was integrated in the multicenter clinical trial and was based on vincristine, prednisone, methotrexate, cytarabine, etoposide, dexamethasone, MTX 5 g/m², and intrathecally applied fractionated chemotherapy without CR.

Results: CNS involvement and treatment outcome according to NHL subgroups were:

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of pts</th>
<th>CR (%)</th>
<th>TR (%)</th>
<th>Relapse (%)</th>
<th>Death (%)</th>
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CNS involvement in childhood B-cell malignancies: is it a bad prognostic factor per se? Experience of the SFPF LMB97-01 trial

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The prognostic value of initial CNS disease is controversial (Haddy, JCO 1991; Sandlund, JCO 2000; Guranurang, JCO 2000) and reported as rather related to tumor burden than to CNS disease itself. We reexamine data of the recently published SFPF LMB98 series (Blood 2001) to compare with others. Among 561 pts with B-cell lymphoma leukemia, 67 had CNS initial involvement, including blasts in CSF (37 cases), cranial nerve palsies (38 cases), intra cranial mass (13 cases) and cord compression (12 cases). Among them, 16 pts only had blasts in CSF, 16 only CNP, 3 only intracranial mass and 5 only cord compression. In 44 pts, it was associated with BM involvement (M1: 2; M2: 6; M3: 30). It was more frequent in head and neck primaries (24%) than in abdominal (5%), nodal (6%), thoracic (0%), or other (15%) sites, and in patients with B-ALL and no primary (40%). After centrally pathology review, 54 were Burkitt, 3 large B-cell, 8 unclassified and 2 non reviewed diagnosed as Burkitt. LHD was Nx2 in 16 pts, Nsx2 in 47 and unknown in 4. All pts were treated in the higher risk group (group C) with intensified CNS directed therapy: HD-MTX 8 g/m², HD-Ara-C, triple IT and 24 Gy cranial irradiation. There were 15 events: 5 no CR, 3 toxic deaths, 1 death in CR and 5 relapses (1 isolated CNS, 2 CNS + BM, 2 other site + BM); these 14 pts died. One pt developed a cerebral PNET 5 years after the B-cell ALL. The EFS is 76% (95% CI 68-87) at 3 years and 77% after exclusion of the 5 pts with only cord compression. In a multivariate analysis for EFS, including LHD (N=92), CNS involvement, age at diagnosis, pre-CRT (N=15), CNS events in the pre-CRT period (<20%) and BM involvement (≥25%), the 4 first are independent prognostic factors. In conclusion, CNS involvement in childhood B-cell malignancies is an independent prognostic factor. Treatment must be improved for these pts, knowing that 20% of the failures are toxic deaths.
3. Biology/Genetics

INHIBITION OF CONSTITUTIVE STAT3 ACTIVITY SENSITIZES RESISTANT B NON-HODGKIN’S LYMPHOMA AND MULTIPLE MYELOMA TO CHEMOTHERAPEUTIC DRUGS

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Introduction: Hematopoietic malignancies have been shown to depend on cytokine growth factor autocrine/paracrine loops for growth and differentiation. This results in the constitutive activation of cytokine-mediated transcriptional factors like STAT3 in non-Hodgkin’s lymphoma (NHL) and multiple myeloma (MM). Recent evidence demonstrates that cytokines such as IL-6 and IL-10 also contribute to a drug-resistant phenotype in many tumor cell types. We hypothesized that inhibitors of STAT3 would sensitize drug-resistant and endogenous cytokine-dependent NHL and MM tumor cells to the cytotoxic effects of chemotherapeutic drugs.

Methods: We examined an AIDS-related NHL (ARL) cell line, ZF, known to be dependent on IL-10 for survival and an MM cell line, U266, known to be dependent on IL-6 for survival. IL-10 and IL-6 signal the cells through the activation of JAK1 and JAK2, respectively. Thus, we investigated the effect of two chemical STAT3 inhibitors, namely, picetastat (JAK1/STAT3 inhibitor) and tyrphostin AG490 (JAK2/STAT3 inhibitor) on the tumor cells for sensitization to therapeutic drugs-mediated apoptosis.

Results: We demonstrate by phosphoroptokin tumor necrosis factor imaging and electrophoretic mobility shift analysis that picetstat and AG490 inhibit the constitutive activity of STAT3 in ZF7 and U266, respectively. Furthermore, picetstat and AG490 sensitize ZF7 and U266 cells, respectively, to apoptosis by a range of therapeutic drugs including cisplatin, fludarabine, adriamycin, and vincristine. The specificity of the inhibitors was corroborated in experiments showing that picetstat had no effect on U266 and, likewise, AG490 has no effect on ZF. The sensitization observed by these inhibitors correlated with the inhibition of p-B2 expression in ZF7 and p-B1 expression in U266.

Conclusions: Altogether, these results demonstrate that STAT3 inhibitors regulate the cytokine-mediated transcriptional regulation of anti-apoptotic gene products like Bcl2 and Bcl-xL. We propose that STAT3 inhibitors are a novel class of chemotherapeutic sensitizing agents capable of reversing the drug-resistant phenotype of certain cytokine-dependent tumor cells.

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ANGIOGENIC GROWTH FACTORS VEGF AND b-FGF AS PROGNOSTIC MARKERS IN HIGH- AND LOW-GRADE B-CELL LYMPHOMA

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Introduction: We and others have previously reported about the prognostic potential of angiogenic growth factors VEGF and b-FGF in B-cell non-Hodgkin’s lymphoma (NHL). Relevant expression of these growth factors has also been underlined in recent microarray studies. Here we investigate the prognostic value of these markers in a larger group of 110 NHL patients with a longer follow-up.

Methods: VEGF and b-FGF were evaluated by ELISA in frozen serum or plasma samples collected at diagnosis. Predominant NHL types were diffuse large B-cell (DLBCL, n=56), low-grade NHL (follicular-MALT, n=28) and mantle-cell (MCL, n=15). Median follow-up was 7 years (range 19-3). The statistical analysis was performed dividing patient populations according to median and quartile values for serum and plasma samples. A total of 20 samples from healthy controls were also evaluated.

Results: Circulating VEGF values were significantly higher in NHL patients compared to healthy controls. VEGF was 2-fold higher in DLBCL compared to low-grade and MCL NHL patients. b-FGF values were 2.3-fold higher than controls in DLBCL and MCL and similar to controls in low-grade NHL (p=0.01). Circulating VEGF above median values was associated with significantly inferior OS (p=0.04 by log-rank), and a b-FGF above median values was a slightly stronger marker of poor prognosis (p=0.02). Patients with both VEGF and b-FGF above median values had an even poorer OS (p=0.01). Overall, the worst OS was observed in patients with b-FGF levels above the 3rd quartile (p=0.0009), and VEGF levels above the 3rd quartile did not further add prognostic potential. Conclusions: The basal measurements of angiogenic growth factors VEGF and, particularly, b-FGF seem to have prognostic value in NHL. Thus, the longitudinal evaluation of these growth factors warrants further investigation.

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RECEPTORS FOR VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ARE EXPRESSED ON B-CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) CELLS.

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VEGF is a glycoprotein that induces the proliferation and migration of vascular endothelial cells. Until recently, VEGF receptors were thought to be exclusively expressed by adult endothelial cells and to exert their proangiogenic effect by way of paracrine mechanisms.

Recently, however, it was shown that these receptors are present on a subset of hematopoietic stem cells, and on acute leukemia cells and cell lines. Since, VEGF is also produced by leukemic cells including CLL cells, coinciding expression of VEGF receptors may result in the generation of an autocrine loop that supports the proliferation and survival of leukemic cells. We used the Fluorokine kit (R&D Systems Inc Minneapolis, Minnesota, USA) to determine the percentage of cells expressing VEGF receptors in samples from B-CLL patients. For this purpose, 5X10^6 cells were incubated with biotinylated VEGF and then with avidin-FITC together with CD19PE. B-lymphocytes expressing VEGF receptors were fluorescently stained, with the intensity of staining proportional to the density of the receptors. The percentage of VEGF receptors expressing B cells was determined by flow cytometry using FACsCalibur.

We determined VEGF receptors expression on cells isolated from blood of 13 CLL patients. All the samples expressed VEGF receptors with a mean of CD19+/VEGF+ expression of 76% (range 52-92). Thus, in CLL the leukemic cells express VEGF receptors which may promote leukemic cell survival through an autocrine loop.
CHEMOKINE RECEPTORS TARGET DIFFERENT B-CELL NON-HODGKIN’S LYMPHOMAS (NHL) AND MEDIATE CHEMOTAXIS. L. Trenini, A. Cabrello, I. Basso, M. Facco, D. Carollo, A. Tosoni, P. Pizzo, G. Binotto, L. Nicolardi, R. Zambello, F. Adamo, C. Agostini, G. Semenzato. Dept. Clinical & Experimental Medicine, Clinical Immunology Branch and Department of Experimental Biomedical Sciences, Padua University School of Medicine, 35128 Padova, Italy.

Introduction: Chemokines and their receptors represent a growing number of molecules involved in B and T lymphocyte recirculation and in lymphoid organ homeostasis. Little information is available on the expression and role of other chemokine receptors on malignant B cells.

Methods: We investigated the expression and functional role of several chemokine receptors (CCR1, CCR2, CCR3, CCR5, CCR6, CXCR1, CXCR2, CXCR4, CXCR5) on normal and malignant B lymphocytes recovered from patients with chronic lymphocytic leukemia (CLL, 18 patients), hairy cell leukemia (HCL, 12 patients), mantle cell lymphoma (MCL, 4 patients), lymphoblastic lymphoma (6 patients), marginal zone B cell lymphoma (MZL, 6 patients).

Results: Flow cytometry analysis demonstrated a heterogeneous distribution of these receptors in B cell malignancies with a homogeneous pattern inside the same patient. CCR1 was commonly expressed in B-CLL and some HCL patients and lacks on MCL and normal B cells. CCR2 was heterogeneously detected in different groups of patients and normal B lymphocytes. CCR3 was positive in all patients, while it is usually lacking on normal B cells. CCR5 seems to represent a hallmark in HCL patients (7/9) while it was usually negative on normal B lymphocytes and other B non-Hodgkin lymphomas. CXCR6 was almost ubiquitous in all NHL and normal B cells. CXCR2 was commonly absent on all types of normal and malignant B cells while CXCR4 and CXCR5 are ubiquitous in all B cells investigated. Malignant B cells were also investigated for their chemotactic activity following binding with different chemokines. The data obtained showed that different chemokines are able to trigger migration of malignant B lymphocytes.

Conclusions: The data herein reported indicate that different patterns of chemokine receptor expression permits to identify different malignant B cell subsets and that these receptors are fully functional and may play a role in the traffic of malignant B-cells and spreading of the disease.

POSSIBLE INVOLVEMENT OF EMMRIN, AN MMP INHIBITOR, IN LYMPHOMA CELL INVASION AND ITS INHIBITION BY SYNTHEX PEPTIDES CARRYING THE ACTIVE DOMAIN SEQUENCES. T. Sumiyama, K. Nabeshima, K. Ohashi, T. Samehima, H. Hojo, Y. Nakahara, B. Toda, K. Tanuma, and M. Kikutani. Dept. of Internal Medicine and Pathology, Fukuoika Univ., Fukuoika, Japan; Dept. of Pathology and Neurosurgery, Miyazaki Medical College, Miyazaki, Dept. of Industrial Chemistry, Tokai Univ., Kanagawa, Dept. of Anatomy and Cellular Biology, Tohoku Univ. School of Medicine, Busaton, MA, USA and Dept. of Pathology, Keio Univ., Tokyo, Japan.

Emmрин (CD147) is a tumor cell surface factor that stimulates tumor-associated fibroblasts to produce collagenase (MMP-1), stromelysin-1 (MMP-3), gelatine A (MMP-2) and also possibly MT-1-MMP. T-cell lymphomas frequently show dermal and epidermal invasion, but a role of tumor-fibroblast interactions in their invasion has not been investigated in detail. In this study, we show expression of emmрин and its possible role in MMP-2 production in human T-cell lymphomas, together with inhibition and substitution of emmрин function with synthetic peptides carrying its active domain sequences.

Immunohistochimical study demonstrated emmрин expression only in germinal center cells and some histiocites in non-neoplastic lymph nodes, whereas all T-cell lymphomas showed strong (9 of 10) or weak (1 of 10) expression. A syntenic gene, 2 Anaplastic large cell, 4 AILT expressed emmрин strongly and diffusely. In skin sections, fibroblasts near the infiltrating lymphoma cells expressed MMP-2. In vitro cocultures of emmрин-positive HTLV-1-transformed lymphocytes (MT-2) and emmрин-negative human fibroblasts enhanced the production of pro- and active MMP-2 compared with cultures of either cell type alone. Production of MT-1-MMP, an activator of proMMP-2, also increased in co-cultures. Moreover, this emmрин-dependent stimulation of MMP production in cocultured cells was effectively inhibited by the synthetic peptide carrying a sequence of the active domain of emmрин, which is the first extracellular Ig domain (EC1). This peptide contains a putative N-glycosylation site (Asn44). On the contrary, the whole EC1, substituted with sugars [α-chitoosane unit (GlcNAc)2], stimulated the production and activation of MMP-2 by fibroblasts.

These findings suggest that emmрин can enhance lymphoma cell invasion via its MMP-stimulatory effect and this effect can be inhibited by use of synthetic peptides carrying the active domain sequence of emmрин. Furthermore, the presence of sugars on the active domain appears to be important for its activity.

VISUALIZING DISTINCT P53 TUMOR SUPPRESSOR FUNCTIONS IN VIVO BY WHOLE BODY IMAGING.

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p53 is now known to act in cellular processes that influence cellular proliferation, survival, including several cell-cycle checkpoints, DNA repair, senescence, apoptosis, angiogenesis, and the surveillance of genomic integrity. Despite intensive efforts to understand p53 action, it remains unclear which p53 functions are essential for tumor suppression and, as a consequence, are targeted by cancer cells. Here we describe a novel approach using chimeric animals harboring genetically-modified primary lymphomas or hematopoietic stem cells to directly address this question during myeloid lymphomagenesis. Tumor progression was visualized spatially and temporally by whole body imaging of GFP labeled lymphoma cells. Using the anti-apoptotic gene bcl-2 or a dominant-negative caspase 9 (CD9), we show that disruption of apoptosis downstream of p53 confers a selective advantage during lymphoma expansion that phenotype p53 loss. Furthermore, disruption of apoptosis downstream of p53 completely alleviates selective pressure to inactivate p53 during lymphomagenesis, yet produces tumors that are identical to p53 null tumors with respect to their rapid onset and highly disseminated growth pattern. Despite their aggressive phenotype, apoptosis-defective lymphomas that retain intact p53 genes do not display the checkpoint defects and gross aneuploidy that are characteristic of p53 mutant tumors. These results demonstrate that apoptosis is the sole p53 function overridden during the development of disseminated lymphomas, whereas defective cell-cycle checkpoints and aneuploidy are simply byproducts of p53 loss.

A GENETICALLY MODIFIED FOLLICULAR DENDRITIC CELL LINE SUPPORTS CLONAL GROWTH OF FOLLICULAR LYMPHOMA.

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In follicles of normal lymph nodes, follicular dendritic cells (FDC) play essential roles in growth and maturation of germinal center B cells. However, little evidence is known whether FDC support the growth of follicular lymphomas (FL) cells. We have previously established a follicular lymphoma cell line FLK-1 that grows only in the presence of FDC like cell line HK, suggesting an important role in the follicular lymphoma cell growth. To clarify whether FDC support the growth of FL cells in general we cultured the lymph node cells of FL with the HK cells. However, most of the cases examined failed to grow continuously, indicating that additional growth signals are required for the cell growth. In an attempt to enhance the growth of FL cells in vitro, FL cells were activated with CD40 ligand which was expressed on the surface of HK cells (HK-40L). When lymph node cells of a FL case were cultured on HK, NIH3T3-CD40L or HK-40L, cell growth on HK-40L was superior to that on other two feeder cells. Therefore, two additional follicular lymphoma case were cultured with HK-40L to confirm growth support for FL cells on HK-40L. The results were quite similar to the first case. Lymphoma cells adhered to HK-40L, became large and proliferate vigorously within a few days. During proliferation, although a part of cells detached and die, cell number increased for more than 1 month. The clonal proliferation was examined by VH gene rearrangement with PCR method, and two cases demonstrated identical rearrangement band to the original lymphomas, while the other could not have clonal band. Next we examined the effect of interleukin(IL)-4 or IL-10 for this in vitro culture system. Lymph node cells of a FL case were cultured on HK-40L with or without IL-4 and/or IL-10. After 1 month, cell number of this FL case was 10^7 and cells cultured by HK-40L with IL-4 and IL-10. Clonal analysis by VH gene rearrangement suggested that the growing cells were derived from original lymphoma cells. These data indicate that HK-40L culture system is useful to expand FL cells in vitro.
ACTIVATION OF MYC BY CYTOGENETICALLY CRYPTIC CHROMOSOMAL TRANSLOCATION, t(6;17)(q21;p12), in mature-B cell malignancies. Most lymphomas with this translocation are indolent malignancies of marginal zone origin including MALT type and SLV/LMSZL, although the identical translocation may also be seen in myeloma. One case of aggressive diffuse large B cell lymphoma with t(6;17)(q21;p12), also exhibited a MYC rearrangement on Southern blot. Here, we report the molecular cloning of the MYC rearrangement and show that this represented a cytogenetically cryptic t(6;17)(q21;p12), resulting in MYC transcriptional activation. Results: Southern and FISH analysis indicated MYC translocation. The MYC translocation breakpoint was closed using long distance inverse-PCR (LDI-PCR) from the region 3’ of the MYC coding sequences. The MYC breakpoint fell 44bp upstream of exon 2 and fused to MYC sequences from chromosome 17q22. The 17q22 break was 600bp centromeric of a previously reported 17q22 break closed from a case of leukemic transformation of follicular lymphoma with a t(6;17)(q24;p11) and t(14;18)(q32;q21) (Gavazzy CE et al. DNAS 1989;86;8867; Northern blot showed high-level expression of a truncated MYC transcript. Conclusion: Chromosomal translocation t(6;17)(q21;p22) is a rare but recurrent event in B cell malignancies, it appears to be cytogenetically cryptic and may be a secondary event associated with transformation to high-grade disease. Breakpoints in both MYC and 17q22 appear to be clusterted. One consequence of the translocation is MYC activation, presumably due to promoter substitution by sequences from 17q22. Whether another gene from chromosome 17q22 is also concurrently deregulated is under investigation.


DNA-amplifications are considered to be an important mechanism of proto-oncogene activation. By comparative genomic hybridization (CGH), these genomic amplifications are detectable in 15-20% of all Non-Hodgkin Lymphomas (NHL). However, CGH diagnostics relies on allocation of altered chromosomal regions to cytogenetic bands and its resolution for amplification detection is restricted to approximately 2 Mbp. Using microarray technology, recently a more sensitive approach allowing marker-directed genome wide screening has been developed, termed matrix-CGH. In addition to a much higher spatial resolution (100-200 kbp), a fully automated evaluation of the genomic hybridizations is possible. To evaluate the CGH technique, lacking the detection of gene amplifications in primary NHL tissue samples, a B-cell lymphoma cell line containing 495 genomic PAC and BAC DNA fragments was designed. Targets were selected for proto-oncogenes, cell cycle control genes, tumor suppressor genes and chromosomal regions frequently altered in aggressive NHL (e.g. 2p14-16; 3q26-q28; 9p24, 12q13-14; 18q21 and Xq26-q28). DNA samples from 13 patients with diffuse large B-cell lymphomas (DLBCL) and 3 patients with Burkitt lymphomas were analyzed. A total of 14 high level amplifications were identified with matrix-CGH. The most frequently amplified chromosomal regions were 18q21 (n=4), 12q12-14 (n=3) and 9p12-q24 (n=3). Gene amplifications were identified for BCL2, REL, CCND1, CCND2, IκBα, P53 and the MDM2 gene. Two cases of DLBCL harbored small-sized amplified regions which were undetectable by chromosomal CGH but were confirmed with Fluorescence in Situ Hybridization. Our results underline the potential of matrix-CGH as a powerful tool for automated screening and fine mapping of gene amplifications in aggressive NHL. Currently, a larger series of tumors is being analyzed for the presence of gene amplifications of previously unknown pathogenic relevance and to verify whether the observed high frequency of amplifications can be confirmed.

FISH STUDIES IDENTIFIED TWO MOLECULAR SUBTYPES OF AID/P (3P6), A FREQUENT NHL-ASSOCIATED ABERRATION. J. Wodarczak, Ch. De Wolf-Peeters, A. Hagemeier. 1Center for Human Genetics and 2Division of Morphology and Molecular Pathology, University of Leuven, Leuven, Belgium.

Chromosome aberrations involving 3p6, particularly the add(1)(p36), have been observed in >10% of NHL cases. They occur frequently in follicular lymphomas and are considered as secondary chromosomal abnormalities. To better characterize these chromosomal aberrations and understand their role in lymphomagenesis we performed FISH analysis on series of NHL cases with the add(1)(p36). Initial studies aimed at rapid mapping of the 3p6 breakpoint(s) were performed in 29 cases. These investigations were further complemented by additional FISH (9 cases) and MFISH (10 cases) analyses in order to identify the additional material translocated to 3p6. FISH mapping of the 1p breakpoints using ten 1p33-35 DNA probes allowed us to subdivide the analyzed cases into 2 groups. The first group consisting of 10 cases was characterized by a breakpoint within the 1p telomeric region. The remaining 13 cases showed breakpoints spread within the 1p33-35 or more proximal bands. All these latter add(1)(p36) aberrations but one showed to be numerical translocations associated with loss of the 1p terminal sequences. The origin of additional material translocated to 1p33-35 determined in 19 cases showed involvement of various segments of different chromosomes including X, 1, 2, 3, 4, 5, 8, 9, 11, 12, 18 and 22. Except for 1q21 affected in 4 cases, no recurrent breakpoint could be found in partner chromosomes involved in the add(1)(p36). In summary, our FISH studies led to identification of two different molecular subtypes of the NHL-related add(1)(p36). The first type is represented by add(1)(p36) with breakpoint within the 1p telomeric area. These findings could suggest activation of an unknown NHL-associated oncogene located in the 1p subtelomeric region. The second type of add(1)(p36) is characterized by heterogeneous 1p breakpoints and a constant loss of the 1p terminal sequences. These aberrations could be associated with inactivation of a putative NHL-related tumor suppressor gene. Notably, loss of TP73, the candidate TSG mapped at 1p35.5, was found in all 13 lymphoma cases representing that subgroup of add(1)(p36).


Introduction: Mantle cell lymphoma (MCL) is a recently individualized entity characterized by the t(11;14)(q13;q32) resulting in cyclin D1 hyperexpression and a poor prognosis. MCL cases have been shown to progress to a more aggressive disease but the molecular events responsible of this phenomenon have not been determined. Materials and Methods: We have established from the pleural effusion of two patients with MCL, two cell lines we used for further cytogenetic characterization is better define the incidence and nature of secondary chromosome abnormalities using multiplex fluorescence in situ (M-FISH), whole chromosome paint and specific probes (CCND1, IκBα, MYC, MYC-NNMT, BCL3). Results: Both cell lines grew independently from growth factors. The patient UPNI was found with a marked t(11;14) by FISH/Fluorescence in situ hybridization) using CCND1/IGH specific probes. In the second patient (UPNA), the t(11;14) was cytogenetically evident. Numerous and complex chromosomal abnormalities were found in both cell lines including chromosome 2, 8, 13, 18, 22, X and Y. Further FISH analysis indicated that both cases presented a t(8;22) resulting a variant translocation of Burkitt's lymphoma. Similarly a loss of 8p was found caused either by unbalanced translocation involving 8p, X and Y in UPNI or by a 6q-monosomy in UPNA. These multiple and complex clonal recurrent chromosome abnormalities highlight the multiphenotypic character of MCL tumorigenesis and progression. When injected into NOD-SCID mice, the UPNA cells induced death of the animals in 15 days. At metachromasia, the neoplastic plasma cells were found to have spindles and lumen enlargement as well as multiple retroperitoneal lymph nodes. Pathological analysis showed, in the tissues of mice a typical MCL pattern with evidence of spleen lymph node, and CNS involvement. To follow the progression of the disease in mice we have also designed a quantitative PCR analysis using Tagners and IgH CyclinD1 primers showing a major amplification of cyclinD1 in both patients. Conclusions: Thus, we have established an aggressive MCL model which could be of major interest to determine molecular events involved in MCL progression, allowing isolation of involved genes and their functional characterization as well as to study the effects of new chemotherapy regimens in mouse models.
VALUE OF INTERPHASE FISH ON SECTIONS EVALUATED BY COMPARISON WITH RT-PCR, PCR, AND IMMUNOHISTOCHEMISTRY FOR THE DIAGNOSIS OF MANTLE CELL LYMPHOMAS
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Introduction: The features of mantle cell lymphoma (MCL) may overlap with other B-cell lymphomas and its diagnosis may require the detection of t(11;14)(q13;q32). Conventional cytogenetics is not always available. The purpose of our study was to compare interphase FISH for t(11;14) detection with other techniques: 1) PCR for amplification of t(11;14) genomic breakpoint; 2) RT-PCR and PCR for the detection of cyclin D1 transcripts overexpression and 3) Immunohistochemistry (IHC) for cyclin D1 protein detection.

Methods: The above techniques were evaluated for their applicability (% of samples with interpretable result) and sensitivity (% of positive cases) on a series of 35 MCL with characteristic features (CD5+, CD10+, CD20+, CD23-). Tissues from different origins were analyzed: lymph nodes (24), spleen (3), digestive biopsy (3), tonsil (3), skin (2).

Results: Interphase FISH was performed on touch preparations (11) and frozen sections (9) or paraffin sections (15). Interphase FISH detected t(11;14) in 34/35 cases (97%). DNA PCR was positive in only 13/35 cases (37%). RT-PCR was applicable in non-epithelial tissues (35) and cyclin D1 transcripts overexpression in all tested cases (27/27). Cyclin D1 protein was detected by IHC more easily on paraffin sections (21/23) than on frozen sections (3/12). Cyclin D1 immuno-detection was obtained in 24/25 cases (69%).

Conclusions: The applicability and sensitivity of interphase FISH exceeded those of other techniques. Double interphase FISH also allowed the detection of additional cytogenetic alterations, such as CCND1 amplification or p53 allele loss in t(11;14)+ nuclei of blastoid MCL.

GENOMIC HETEROGENEITY CHARACTERIZES TRANSFORMATION OF FOLLICULAR CENTER LYMPHOMA: A COMBINED GENOMIC SCANNING AND GENE EXPRESSION PROFILING USING MICROARRAYS
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We have developed a microarray-based comparative genomic hybridization (array CGH) technique, which assembles ~2400 BAC/P1 clones for quantitative measurement of gains and losses across the entire human genome. We investigated the pattern of genomic imbalances occurring in the higher-grade transformation and relapse of follicle center lymphoma (FCL). A series of paired FCL post-transformation and relapse biopsies were analyzed. A variable spectrum of genomic imbalances was observed in the post-diagnosis samples, including DNA over-representation on chromosomes 2p13 (REZ and BCL1A1 gene loci), 3q27 (BCL6 locus), 17p13-qter and 18q21 (BCL2 locus), and deletions of 1p36, 4q34-qter, 13q44-q13, 1q42, 9p21 and 13q13 gene loci. Changes present exclusively in post-transformed biopsies included gains of 12q (4 of 12 cases), 17p13-qter and trisomy of chromosome 7 (2 of 12 cases). The structural analysis of 12q gains in patients and eight cell lines harboring t(14;18)(q32;q21) was performed using array CGH. A complex pattern of discontinuous regions of gain and loss encompassing 12q12-q24 was detected. To investigate the genes that may be deregulated in 12q, expression analysis of genes localized at 12q was performed in the sequential samples by hybridization of labeled cDNA to lymphoma EST/gene microarrays. None of the previously identified oncogenes localized within 12q12-q24 demonstrated marked increase in their expression upon transformation to diffuse large B cell lymphoma. Only the methionyl-tRNA synthetase (METRS) gene was differentially slightly overexpressed in the transformation samples between transforming and without 12q gains.

Our results indicate that transformation of FCL is associated with a heterogeneous pattern of genomic imbalances, which includes trisomy of 12q in a subset of cases. These data demonstrate the ability of combined genome scanning and gene expression profiling to determine accurately aberrant genetic patterns in clinical material.

DELINEATION OF SECONDARY CHROMOSOMAL ABERRATIONS IN t(14;18) FOLLICULAR LYMPHOMA BY M-FISH ANALYSIS
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Introduction: Multicolor karyotyping (M-FISH) was performed to better delineate previously incomplete karyotypes by G-band analysis, to detect new recurrent chromosomal aberrations and to identify alternative karyotypic pathways in follicular lymphoma (FL).

Methods: The 55 t(14;18) positive FL cases analyzed consisted of specimens from 1991 to 2001 with complete cytogenetic, morphologic and clinical data available. All cases were studied at diagnosis and prior to any treatment. Most (45/55) showed complex karyotypes with marker, rings and additions not fully characterized by G-band analysis. The G-band and M-FISH karyotype data were assessed for gains and losses using the ISCN nomenclature and plotted against a 344-band profile created in an MS Excel format.

Results: Most of the marker and/or ring chromosomes were identified as material from chromosomes X, 1, 5, 17, 18 and 21. Most of the additions were identified as material from chromosomes 2, 6, 8, 17 and 18. Prominent unbalanced translocations involving chromosome 1 with various partner chromosomes such as 1, 2, 3, 6, 8, 13, 15, 17, Y and X were observed. Subsequent multicolor band (M-band) analysis for chromosome 1, undertaken to identify the subregions involved in these aberrations, revealed consistent breakpoints at 1p36.3 and 1q21.2 and prominent amplification of 1q21-q31.

Further study with BACs spanning these chromosome 1 breakpoints as well as M-band studies for chromosomes 17 and 18 are ongoing.

Conclusions: As the markers, rings and additions were resolved by M-FISH analysis, chromosomal gains were seen more often than deletions. Multicolor karyotyping and banding techniques substantially improved the precision of karyotype analysis of malignant cells in FL cases and led to a more precise assessment of the genotypic abnormalities in these cells. This type of data in correlation with expression data, will definitely lead to more accurate assessment of the genotypic-phenotypic correlation in FL.
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GENE EXPRESSION PROFILING IN AIDS-RELATED LYMPHOMAS (ARL): BURKITT LYMPHOMA (BL) AND DIFFUSE LARGE B-CELL LYMPHOMA - IMMUNOBlastic VARIANT (DLBCL-BL)

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Introduction: AIDS-related lymphomas are characterized by extreme clinical aggressiveness. The two main subgroups are Burkitt lymphomas (BL) and diffuse large B-cell lymphomas (DLBCL). Whereas BL show activation of c-MYC and inactivation of P53, DLBCL are heterogeneous without a single unifying genetic lesion.

Methods: To expand the understanding of the pathogenesis in ARL, we obtained oligonucleotide microarray data on a series of 7 BL, 5 DLBCL immunoblastic variant (DLBCL-BL), and for comparison 4 non-AIDS DLBCL using Affymetrix Hu532 chips. Expression data were analyzed using the Affymetrix Microarray Suite 4.0 and GeneSpring 4.0. The expression of selected genes is analyzed by quantitative real-time polymerase chain reaction (QRT-PCR) using RNA extracted from 10 BL, 10 DLBCL-BL, and for comparison 10 non-AIDS DLBCL. Selected genes are further confirmed on the protein level using immunohistochemistry (IH).

Results: With a non-parametric test (Kruskal-Wallis test), a p-value cutoff at 0.05, and multiple testing correction using the Benjamin and Hochberg False Discovery Rate, we identified 53 genes that really distinguished the 3 lymphoma subtypes. Additional gene lists were generated comparing non AIDS DLBCL with either DLBCL-BL or BL. Those lists were functionally classified and candidate genes are currently confirmed using QRT-PCR.

Conclusions: ARL are associated with a distinct gene expression profile that distinguishes them from non-AIDS DLBCL. These studies might help identify candidate genes in the oncogenesis of these aggressive lymphomas.

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GENE EXPRESSION PROFILING IN LASER MICRODISSECTED NORMAL FOLLICULAR MANTLE, MANTLE CELL Lymphoma (MCL) AND BLASTOID VARIANT OF MANTLE CELL LYMPHOMA (MCL-BV)

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Introduction: Cyclin D1 overexpression occurs in virtually all MCL. However, overexpression of cyclin D1 alone is not sufficient to promote the development of lymphoma in transgenic mice, and additional cooperating lesions are required for lymphomagenesis. Mechanisms of neoplastic transformation from normal mantle cells, and the relationship to the rare blastoid variant are poorly understood.

Methods: To expand the understanding of altered pathways in MCL, we performed oligonucleotide microarray analysis with the Affymetrix Hu532 chips using MCL (5 cases) as well as MCL-BV (4 cases). Expression data were analyzed using the Affymetrix Microarray Suite 4.0 and GeneSpring 4.0. The expression of selected genes was analyzed by quantitative real-time polymerase chain reaction (QRT-PCR) using RNA extracted from 12 cases of MCL, 4 MCL-BV, and multiple laser microdissected follicular mantle zones of 2 hyperplastic lymph nodes.

Results: Comparing MCL-BV to MCL, gene expression data showed a significant change in expression of 118 genes including tumor suppressor genes, transcription factors, protooncogenes, and genes associated with cell cycle, proliferation, chromatin assembly, and mitosis/spindle assembly. The QRT-PCR confirmation rate with statistically significant differential expression was 88% in the 17 genes tested. Of special interest are the confirmed increased expression of the seminal thymocyte kinase proteins PIM1, PIM2, RSK1, defender against cell death 1 (DAD1), and the transcription factor Y1.

Conclusions: Using gene expression profiling and QRT-PCR we identified aberrantly expressed candidate genes that might cooperate with cyclin D1 in the pathogenesis of MCL and in the progression to MCL-BV.

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THE NOVEL t(11;12;18)(q11.2;q12.1;q21) REPRESENTS A VARIANT TRANSLOCATIONS OF THE t(11;18)(q11.2;q21) ASSOCIATED WITH LOW GRADE MALT LYMPHOMA

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Introduction: The chromosomal translocation t(11;18)(q11.2;q21) characterized about one-third of extranodal marginal zone B-cell lymphomas of the mucosa associated lymphoid tissue (MALT) type. In this translocation, a fusion of the apoptosis inhibitor gene API2 on chromosome 11 and the novel MALT1 gene, which encodes the human paracaspase, on chromosome 18. The paracaspase deficient gene was identified in the derivative chromosome 11 and leads to the lack of several bcl2-like transcripts. We performed a genome-wide analysis of the t(11;18)(q11.2;q21) and the t(12;18)(q12.1;q21) translocations, which are associated with low-grade MALT lymphoma (lgML)

Methods and results: We herein describe the variant translocation of the t(11;18)(q11.2;q21) in a low-grade MALT lymphoma of the lung. Fluorescence in situ hybridization (FISH) with API2 and MALT1 specific probes showed a 5'API2-3'MALT1 fusion encoded on the derivative chromosome 11 as observed in the standard t(11;18). Further FISH analyses demonstrated a colocalization of genomic sequences derived immediately upstream of MALT1 and sequences of the chromosomal regions 12q12-13, 12q21, and 12q24, respectively. Reverse transcriptase polymerase chain reaction (RT-PCR) and DNA sequencing showed an in-frame fusion of exon 7 of API2 and exon 5 of MALT1. To identify the unknown gene on chromosome 12 fused to 3'API2 and 5'MALT1, respectively, 5' and 3'region amplified of DNA ends (RACE-PCR) was performed. However, exclusive wild type API2 and MALT1 sequences, respectively, could be detected.

Conclusion: We conclude that, similar to the standard t(11;18), the t(11;12;18) leads to a 5'API2-3'MALT1 fusion with linkage of the three bcl2-like transcripts (BRD) domains of API2 to the caryo-terminal region of MALT1 containing the cellular p20-like domain and that a small additional translocation event the expression of the reciprocal 3'MALT1-5'API2 is excluded.

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CHROMOSOMAL ABNORMALITIES IN SPLENIC MARGINAL ZONE LYMPHOMA DETECTED BY COMPARATIVE GENOMIC HYBRIDIZATION.

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Aim: To identify the characteristic chromosomal abnormalities of splenic marginal zone lymphoma (SMZL)/splenic lymphoma with villous lymphocytes (SLVL).

Materials and methods: Thirteen females and seven males with a median age of 66 years were studied. The diagnosis of SMZL/SLVL was based on peripheral blood morphology, bone marrow histology, immunophenotyping, and in five patients on splenomegaly. Comparative genomic hybridization was performed basically as described by A. Kallioniemi et al. (A. Kallioniemi et al. Science 238 (1992) p.318). Conventional cytogenetics and fluorescence in situ hybridization were performed in selected cases.

Results: In all patients but one villous lymphocytes were present in peripheral blood. A mature B-cell immunphenotype was found in all patients. Chromosomal abnormalities were identified in 70 % of the cases with chromosome 3 (20%), 6 (20%), 7 (30%), and 12 (20%) most frequently involved. Classical cytogenetics and FISH showed that chromosome 7 abnormalities were frequently translocations, but that neither the integration partner or the chromosome 7 breakpoints were consistent. From the copy number imbalances identified by CGH the most commonly involved regions were delineated, with respect to gains they were 3q25-qter and 12q13-15, and with respect to losses 6q23 and 7q31.

Conclusion: SMZL is cytogenetically characterised by gain of 3q25-qter and 12q13-15 and loss of 6q23 and 7q31. The presence of these characteristics is in agreement with the classification of SMZL as a distinct disease entity. The abnormalities described pinpoint regions that are likely to contain genes involved in the pathogenesis of the disease. A goal in the future will be to identify which of those that might contribute to the malignant clone.
INTERLEUKINE-FOUR INDUCED GENE 1 (F1G1): A NOVEL GENE EXPRESSED IN PRIMARY MEDIALAL LARGE B CELL LYMPHOMAS


Primary mediastinal large B-cell lymphomas (PMBLs) are recognized as a distinct lymphoma subtype among diffuse large B-cell lymphomas (DLBls). Recently, we demonstrated the recurrent and specific expression of the MAL gene in PMBL as compared to non- mediastinal large B-cell lymphomas (NM-DLBls). In order to extend PMBLs gene expression profile, we used representational difference analysis (RDA) to compare PMBLs and NM-DLBls transcripts. One of the differential DNA fragments isolated proved to be homologous to the mouse B-cell immediate-early I.4-inducible gene fgit (interleukine-Four induced Gene 1). Using Northern blot analysis, we showed that fgit mRNAs were highly expressed in 5 PMBLs and absent or expressed at a very low level in 5 NM-DLBls. Further studies demonstrated that 1) human fgit expression is mainly restricted to lymphoid tissues, 2) fgit transcripts are expressed at low level in thymus, spleen, tonsil and reactive lymph node but are highly upregulated in IL-4+CD40 activated tonsillar B-cells, 3) its expression in human B-cell lines is restricted to the PMBL derived MedB1 and Karpas 1106 cell lines. Using both Northern blot analysis and real time RT-PCR, we demonstrated that fgit mRNA levels were significantly higher in PMBLs (n=17) than in CDLBs (n=16) (Mann Whitney test, p<0.04). Moreover, high fgit mRNA expression was not observed in other low grade or high grade B-cell non-Hodgkin's lymphomas (n=12). Sequencing analysis revealed that fgit mRNA (1787 nt) encoded a 567 amino acid protein with a signal peptide and a large flavin-binding amino oxidase domain, 85% homologous to the mouse protein. This protein shares significant homology with two secreted l-amine-oxidases, the snake venom apoxin I and the fish derived ER-IAO. Both enzymes have been shown to induce apoptosis through HO2 production. These data suggest that F1G1 may present an LAO activity associated with pro-apoptotic properties or with emerging functions for ectoenzymes such as signaling and cell-adhesion, that may be of significant importance in PMBL lymphomagenesis. Furthermore, fgit expression in these lymphomas might reveal the activation of a cytokine signaling pathway.

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COMPARATIVE GENOMIC HYBRIDISATION AND GENOMIC MICROARRAY ANALYSES OF PRIMARY CUTANEOUS CD30+ ALCL AND NK/NK LIKE T-CELL LYMPHOMA.

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Introduction: Primary cutaneous CD30+ anaplastic large cell lymphoma (CD30+ ALCL) and NK/NK like T-cell lymphoma represent separate and distinct clinicopathologic variants of primary cutaneous lymphomas. The clinical relevance of underlying molecular pathogenesis remains unclear. The purposes of this study were to investigate and compare genetic alterations in these lymphomas.

Methods: We analysed 15 cases of primary cutaneous CD30+ ALCL and 8 cases of NK/NK-like T-cell lymphoma using comparative genomic hybridisation (CGH) and genomic-based microarray techniques.

Results: Chromosome imbalances (CI) were detected in 8 cases of CD30+ ALCL (53%) and in 6 cases of NK/NK-like T-cell lymphoma (75%) using CGH. The most frequent DNA copy number changes in CD30+ ALCL were gains of chromosomes 1 and 5 (4/8) and chromosomes 6, 7 and 19 (3/8). In contrast the most common CI in NK/NK-like T-cell lymphoma were losses of 9 and 13q (4/6). In addition, using microarray we identified copy number gains of the oncogenes FGFR1 in 3 of 6, and HRAS, MYCN, RAF1, MET, CTBS, FES and CBF2A in 2 of 6 CD30+ ALCL cases respectively. We also detected gains of REL, RAF1, TERC, MET, CTBS and STK15, and loss of ABL 1 in 2 of 4 NK/NK-like T-cell lymphomas.

Conclusion: We have identified different but consistent patterns of genetic abnormalities in CD30+ ALCL and NK/NK-like T-cell lymphoma, which are distinct from our previous results in mycosis fungoides and Sezary syndrome. These findings now provide the basis for further studies of the molecular pathogenesis of primary cutaneous lymphomas.

MOLECULAR SINGLE CELL ANALYSIS OF HODGKIN-REEDSTERNBERG CELLS OF LYMPHOCYTE-RICH CLASSICAL HODGKIN'S LYMPHOMA: REVELATION OF DIFFERENCES BETWEEN CLASSICAL AND LYMPHOCYTE-PREDOMINANCE HODGKIN'S LYMPHOMA.


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Introduction: Hodgkin-Reed-Sternberg (HRS) cells of classical (c) Hodgkin's lymphoma (HL) carry mutated Ig gene rearrangements and are likely derived from preplasmatic germinal center (GC) B cells while lymphocytic and histocytic (L&H) cells of HRS are derived from presumably selected GC B cells and often show ongoing somatic hypermutation. The recently identified lymphocyte-rich classical (lrc) HL is characterized by HRS cells with the immunophenotype of classical HRS cells but an infiltrate similar to lpc.

Methods: To determine the relationship of lrcHL to cHL and lpcHL we characterized five cases of lrcHL by immunohistochemistry and sequenced the rearranged Ig genes of single microdissected HRS cells.

Results: The expression patterns of BCL6, CD13, CD2 and BOB1 in HRS cells show differences to cHL and lpcHL. Analyses of rearranged Ig genes identified clonal HRS cell expansions carrying mutated Ig rearrangements without significant intraclonal diversity in all 5 cases and indicated stringent selection for functionality.

Conclusions: The mutation pattern of rearranged Ig genes of HRS cells in lrcHL does not support a derivation from preplasmatic, "crippled" GC B cells as suggested for HRS cells of cHL, and these cells are also clearly distinguishable from L&H cells by the silencing of hypermutation activity. Thus, lrcHL is different from both, cHL and lpcHL.
Molecular cytogenetic analysis of classical Hodgkin's disease and of Hodgkin cell lines
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We have performed a molecular cytogenetic study of classical Hodgkin's disease. Included were 40 cases of various subtypes (lymphocyte-rich n=6; mixed-cellularity n=16; nodular sclerosis n=16; lymphocyte depletion n=2) as well as four Hodgkin cell lines (KM-H2, L428, L1236, HDLM-2). Hodgkin and Reed-Sternberg cells were isolated by laser-based microdissection, DNAs were amplified by PCR using degenerated primers (DOP-PCR), and subsequently analysed by comparative genomic hybridization (CGH). A characteristic pattern of gains and losses emerged: Most frequent gains were detected on chromosomes 2p (n=19), 12q (n=13), 9p (n=11), 16p (n=11), 17 (n=9), and 5p (n=7). Losses were most frequent on 13q (n=12). A total of 5 high-level DNA amplifications were detected mapping to 2p, 4p, 4q, and in two cases to 5p. The four cell lines revealed complex CGH karyotypes including gains on 2p and 9p in KM-H2 and L2136. To test for a candidate gene three cases with gains on 2p were analysed by FISH with DNA probes for c-REL and BCL11A. All three cases revealed gains and/or amplifications of these sequences. The data indicate a highly characteristic pattern of gains and losses for classical Hodgkin's disease largely varying from data recently published for lymphocyte predominant Hodgkin's disease (Blood 2001:97:1845) and point at new critical genomic regions. Furthermore, cell line KM-H2 and L1236 fit well in this molecular cytogenetic pattern of classical Hodgkin's disease.

Molecular-cytogenetic subtyping of classical Hodgkin’s disease.
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Deutsches Krebsforschungszentrum Heidelberg, Germany; (2) Abteilung für Pathologie des Universitätsklinikums, Ulm, Germany; (3) Abt. Innere Medizin, Klinikum der Georg-August-Universität, Göttingen, Germany.

“Classical” Hodgkin’s lymphoma (cHL) is subdivided in lymphocytoid-rich (cHL-IR), nodular sclerosis (cHL-NS), mixed-cellularity (cHL-MC), and lymphocyte-depletion (cHL-DL). From previous cytogenetic studies it has remained uncertain, whether the malignant Hodgkin- and Reed-Sternberg (HRS) cells in these subtypes differ with regard to individual chromosomal aberrations. Using comparative genomic hybridization (CGH) we have performed a genome-wide screening for chromosomal imbalances in pools of 20-30 microdissected HRS cells per case. Characteristic gains and losses of individual chromosomal subregions were identified in 13 cHL-MC, 16 cHL-NS and 6 cHL-IR. In order to test whether the pattern of chromosomal imbalances reflect the histotypes, a hierarchical cluster analysis was performed. This revealed that cHL-NS has a pattern of chromosomal imbalances that is distinct of cHL-MC and cHL-IR suggesting a different pathogenetic pathway. Among cHL-MC two different subtypes were distinguished differing in the frequency of chromosome 17 gains as well as the age of tumor onset. This indicates that imbalances of chromosome 17 are associated with different age of tumor onset. The data presented show, that clusteranalysis based on CGH data is a promising tool to uncover genotype/phenotype relationships in human malignancies.
INDOLOLE NON FOLLICULAR LYMPHOMA: RETROSPECTIVE STUDY OF THE ITALIAN LYMPHOMA STUDY GROUP ON 560 CASES.
Background. Indolent non follicular lymphoma (NIFL) has been rarely considered for specific studies. The Italian Group (LFLS) (ILF) has started a retrospective study aimed to assess main features and prognost is to identify, if possible, a specific prognostic model. Pathogenesis and methods. Small lymphocytic, Immunocytoma/lymphoplasmacytotic and non extranodal marginal zone NHL patients, diagnosed from 1988 to 1997, were included. In addition, we considered CDS negative mature B cell leukemia with a phenotypic profile clearly excluding B-cell CLL/ and leukemia mantle cell NHL. Results. At January 2002, 334 cases, including 52 with CDS, NHL, reported by 4 cooperative Groups and 1 Independent center were considered evaluable. The analysis of the remaining 246 patients is ongoing and a centralized histologic review has already started for questionable cases. The main characteristics are: 175 males; median age 62 yrs (28-86); 9%; 7%, 8% and 76% had Stage I and IV, respectively; 39% splenomegaly; 17% B symptoms; 25% bulky disease; 32% anemia; 7% thrombocytopenia; 21% and 10% >5 >10 x10^9 PLt, respectively; 34% 1 > extranodal site; 24% elevated LDH and 33% ESR >30. Therapy: 94 patients were treated; 69% received chemotherapy, 27% were treated using a combination approach. A median follow-up of 57 months, 206 cases are alive, 14 lost and 114 dead, because of disease in 60%, toxicity in 4% and unrelated causes in 31% of patients. Median OS 151 months with 81% and 72% OS rate at 3 and 5 years, respectively, without statistical difference in OS between CDS NHL, the remaining NHL and the NHL NHL.
Conclusion. Accrual is ongoing in order to evaluate on the largest possible series of this NHL category the prognostic impact of clinic-hematological characteristics, whose results will be available for presentation.

NODAL VERSUS SPLENIC MARGINAL ZONE LYMPHOMA: DIFFERENT CLINICAL FEATURES AND OUTCOME
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Marginal zone B-cell lymphoma (MZL), without extranodal localization, encompasses two pathological entities recognized by the WHO classification: nodal marginal zone B-cell lymphoma and splenic marginal zone B-cell lymphoma. It is not clear, however, whether the presenting features and outcome define distinct clinical entities. We studied 30 pts with non-MALT MZL. All histologies were reviewed by two hematopathologists (M.P., E.B.): 12 were nodal and 18 splenic; 15 male and 15 female; median age 58 years (25-74); 5/12 nodal (42%) and 17/18 splenic (94%) had stage IV disease; 30% had B symptoms; 20 pts (30% of nodal and 90% of splenic) had bone marrow involvement, and 10 (1 nodal and 9 splenic) had blood involvement. Liver involvement was present in 10% of nodal and in 40% of splenic. Five cases (20%) had bulky disease. Only 2 (with nodal lymphoma) had a poor PS (ECOG s 2). Two autoimmune events occurred in 2 nodal cases (1 hemolytic anemia and 1 Sjogren disease). Four of 18 pts with splenic lymphoma (24%) had an M component (3 IgM, 1 IgG). Anemia and thrombocytopenia were present in 60% of splenic and in no case of nodal lymphoma. Two nodal and 6 splenic cases presented a HCV positive serology. Median F-U was 3 years (range 1-15). Of splenic MZL 81% had splenectomy. Chemotherapy (chlorambucil in 7, anthracycline-based CHT in 9) was administered to 16 pts (all nodal and 4 splenic); 30% obtained a CR and 35% a PR, with a median duration of 3 and 1.1 years. Median progression-free survival (PFS) was 1 year for the nodal type and not reached for the splenic type; median overall survival was not reached for both. This analysis indicates that in the contest of non-MALT MZL, nodal and splenic types have peculiar clinical features and outcomes: splenic MZL although presenting with stage IV disease has an indolent outcome, while the nodal type, less disseminated at onset, shows a more pronounced tendency to progression.

RELAPSE (REL) AND TRANSFORMATION (TF) OF FOLLICULAR LYMPHOMA: PROGNOSTIC FACTORS AND EFFECT ON SURVIVAL
University of M.D. Anderson Cancer Center, Houston, TX USA.
Introduction: Frequent Rel and TF to aggressive lymphoma characterize indolent follicular lymphoma. TF has been thought to carry a poor prognosis for survival. Delay or prevention of Rel and TF may change the disease course and prolong life. Therefore, identification of prognostic factors that predict Rel and TF may help to stratify treatment individually. Methods: We reviewed the records of 451 patients with follicular lymphomas sequentially registered from 1955 to 1992, treated at our center. Data included pathology at diagnosis (ds) and Rel, laboratory, and radiographic results. Pathologic TF was defined (1) follicular small cleaved or follicular mixed cell to large cell or pure diffuse lymphoma (2) follicular large to diffuse large cell or (3) any follicular to high-grade lymphoma. Features studied for impact on Rel and TF included attainment of CR with initial therapy, LDH, β2 microglobulin, age, hemoglobin, stage, albumin, B symptoms, and pathology at time of initial dx. Survival curves were drawn for patients with transformed and Indolent Rel. Results: With a minimum of 4.5 and a median of 10 yr of follow-up, 40% have died, 242 (53.4%) developed at least 1 Rel, 161 had dx biopsies at the time of first Rel, and 56 (12.4%) had TF at any Rel. Significant negative factors for Rel (p<0.02) included age >60, β2 microglobulin >3 mg/dL, LDH > upper normal limit, hemoglobin <12 (for women) or 14 (for men) g/dL, stage IV, and lack of CR. However, significant factors for TF (p<0.05) were lack of initial CR, elevated LDH, and presence of B symptoms at dx. Median times to TF or indolent histology (IH) at first Rel were 32 mo (range 5-114) and 38 mo (7-140). Five-year OS from dx for TF and IH at first Rel were 52% and 62%, but 10-year OS from dx were 46% and 53%. Median time to TF at any Rel (TFa) was 44 mo (range 5-138); median survival from TFa was 25 mo compared with 62 mo for IH at all Rel (IHa); 10-year OS for patients with TF from day of TFa was 29% compared with 42% for patients with IHa from day of first Rel (p<0.0005). Conclusions: prognostic factors for relapse include older age, elevated LDH and β2 microglobulin, low hemoglobin, stage IV, lack of CR. Only 4 factors for TF included lack of CR, elevated LDH, and β2 microglobulin, and presence of B symptoms. TF is associated with a higher risk of death than IH without TF at first and subsequent Rel; however, time to TF does not affect long-term survival.

INTERNATIONAL AND ITALIAN PROGNOSTIC INDEXES IN FOLLICULAR LYMPHOMA
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Barcelona, Spain.
Background: International Prognostic Index (IPI), initially designed for aggressive lymphomas, has been successfully used in patients with follicular lymphoma (FL). Recently the Italian Lymphoma Intergrupo (ILI) suggested a new specific prognostic index for FL. The aim of this study was to verify which of these indexes was more useful when applied to a large group of FL patients.
Methods: Available data were obtained to calculate both indexes in 249 patients diagnosed of FL. IPI was calculated as detailed by the International Non-Hodgkin's Lymphoma Prognostic Factors Project. The Italian prognostic index was calculated according to the ILI criteria. Overall survival (OS) and progression-free survival (PFS) figures were calculated according to the Kaplan-Meier method.
Results: The median follow-up of the 249 patients was 55 months (1-292). Concordance between the two indexes was 71% (60% in high risk groups). Results in intermediate-high risk and high risk groups of IPI were similar and we re-calculated data joining these two groups. Results of OS and PFS for each group of risk are summarized in the table.

<table>
<thead>
<tr>
<th>Prognostic Index</th>
<th>OS median in months (N)</th>
<th>PFS median in months (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPI Low risk</td>
<td>69 (61)</td>
<td>35 (50)</td>
</tr>
<tr>
<td>Intermediate-low risk</td>
<td>45 (31)</td>
<td>20 (31)</td>
</tr>
<tr>
<td>Intermediate-high</td>
<td>27 (29)</td>
<td>14 (26)</td>
</tr>
<tr>
<td>High risk</td>
<td>44 (60)</td>
<td>28 (57)</td>
</tr>
<tr>
<td>ILI Low risk</td>
<td>79 (64)</td>
<td>25 (48)</td>
</tr>
<tr>
<td>Intermediate-high risk</td>
<td>55 (66)</td>
<td>22 (62)</td>
</tr>
</tbody>
</table>

(1) Nc; non reached, 69% of patients alive at 16 months; P>0.001 in both cases.
Conclusions: Joining high risk groups, IPI defines a large group of patients (25%) with a very poor OS (14 months) and PFS (20 months), candidate to intensive therapies, and provides a better definition of lower risk groups than ILI index.

We prospectively evaluated in patients with follicular lymphoma (FL) and a low tumor burden the feasibility of delaying any treatment until clinically meaningful progression and published preliminary results of a randomized study showing no differences in EFS (Brice P et al, J Clin Oncol 1967). We further analyzed progression, cause of death in the same cohort with a median follow-up of 9 years. Patients and Methods: 193 newly diagnosed stage III/IV FL patients (mean age: 63±7) with a low tumor burden (GELA criteria) were randomly assigned to one of three arms: no treatment (n=64), prednimustine (n=63), or interferon-alpha (n=66) between 5/86 and 0/5/95. Death was attributed to the following causes: lymphoma (with or without histological transformation [HT]), salvage treatment-induced, second neoplasia or unrelated. Results: 17% of all patients experienced at least one progression HT in 44 cases at first (n=20), second (n=21) or third (n=3) progression. First release occurred at a median time of 33 months with high-tumor burden in 60% of cases. Patients were treated with monochemo-therapy (n=31), miniCHOP +/- interferon (n=57) or polychemotherapy and 22 patients received high-dose therapy with autologous stem cell transplantation. 80 patients died at the last follow-up evaluation (01/01): lymphoma (n=49) with HT in 28, toxicity (n=12); second neoplasia (n=9); unrelated (n=9) and unknown (n=1). Analysis of prognostic factors for deaths will be presented.

Conclusion: with a long follow-up, an initial watchful waiting policy is not deleterious for patients with FL and a low tumor burden. Although 2/3 of these FL patients with disseminated disease are alive 10 years after diagnosis, most deaths are related to the lymphoma, responses to salvage treatment are similar whether treatment was initially began or postponed until progression to a high-tumor burden.

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FLUDARABINE VERSUS CVP IN NEWLY DIAGNOSED PATIENTS WITH STAGES II-IV LOW GRADE NON-HODGKIN'S LYMPHOMA: FINAL ANALYSIS OF PROSPECTIVE RANDOMIZED PHASE III INTERGROUP STUDY


Introduction: We present the results of a prospective randomised trial comparing Fludarabine with CVP in 381 previously untreated patients with low grade NHL. Methods: Treatment was given immediately after diagnosis (n=248; ITx) or when patients required therapy (n=133, w/tx). Patients were randomised between 9 courses of Fludarabine (25 mg/m2 iv daily for 5 days) CVP (Cyclophosphamide 750 mg/m2 iv, Vincristine 1.4 mg/m2 iv day 1, with Prednisone 40 mg/m2 po days 1-5) or ITx. 29 patients became ineligible after central pathology review. Response rates and survival times are presented according to intention-to-treat analysis.

Results

<table>
<thead>
<tr>
<th>Treatment Arm</th>
<th>Response Rate % OR</th>
<th>CR</th>
<th>PR</th>
<th>TTF (mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fludarabine (ALL)</td>
<td>68</td>
<td>38</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>CVP (ALL)</td>
<td>51</td>
<td>15</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>ITx</td>
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Median overall survival (OS) has not been reached with no difference between the two arms at 5 years (68 vs 60%). Myelotoxicity was greater in the Fludarabine group but there was no difference in infection rates. Only LDH was significant for TTF (p<0.0001). Age (p=0.0001), LDH (p=0.0001) and liver involvement (p<0.0001) were significant for OS. 143 patients have died, mainly due to NHL.

Conclusion: response rates are significantly higher after Fludarabine but there is no increase in TTF or OS.

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FLUDARABINE, ADIANTAMINE AND Dexamethasone (FAD) IN NEWLY DIAGNOSED PATIENTS: A PHASE II STUDY BY THE BRITISH NATIONAL LYMPHOMA INVESTIGATION (BNLI). A McMillan1, D Cunningham2, B Hancock3, P Smith4, K MacLennan4 and D Linc5, Nottingham City Hospital, Nottingham, UK, 2 Royal Marsden Hospital, London, UK, 3 Weston Park Hospital, Sheffield,UK, 4 BNLI, London, UK, 5 Haematological Malignancy Diagnostic Service, Leeds, UK, 6 University College, London, UK

Fludarabine in combination with mitozantrone and dexamethasone has been reported to have achieved high response rates in a single centre report of patients with newly diagnosed Low Grade lymphoma. We have examined the efficacy of fludarabine with another anthracycline, adriamycin, in a multi-centre Phase II trial in newly diagnosed patients with Follicular Lymphoma (FL). The aims of the study were to confirm the findings of the original study in a multi-centre setting and to examine the effect of the substitution of the anthracycline component with respect to end point comparison. 35 patients were treated with a median age of 55yrs (20-76). All patients had stage III disease and required therapy (Progressive disease over 3 months, Critical organ involvement. Bone marrow failure or 3 symptoms). 2 patients had an ECOG performance status of 2 or more and 10 patients had an elevated Lactate dehydrogenase (LDH) value. All histopathology specimens were centrally reviewed and 2 patients were excluded after this review (1 mantle cell, 1 Diffuse Large B cell lymphoma.)

The overall response rate in evaluable patients was 100% (143/143 CR, 173/173 PR). Haematologic toxicity was acceptable 12/190 courses had grade 3/4 toxicity for WBC and only 1/190 had grade 3 toxicity for platelets. Non-haematopoietic toxicity was also mild. All patients received Septrin prophylaxis and there were no cases of proven Pneumocystis pneumonia. Currently, with a median follow up of 28 months there is a projected 3 year overall survival of 72% (53-85), a 3 year progression free survival (PFS) of 49% (32-67) and a median PFS of 23 months.

We conclude that FAD has achieved an excellent response rate in advanced stage FL. Phase 3 randomised studies will be required to establish whether this therapies represent an improvement over standard treatment schedules such as those using oral alkylating agents. It would appear that, in terms of response rate and toxicity, adriamycin is at least equivalent to mitozantrone in these schedules. Further large randomised trials would be required.
FLUDARABINE, CYCLOPHOSPHAMIDE AND MITOXANTRONE (FCM) REGIMEN AS FIRST-LINE THERAPY IN YOUNGER PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA (FL)


Departments of Hematology from the GELCAI (Grupo per l’Estudi d’Aixample de Catalunya i Baleares), Spain.

Objective: to analyze preliminary results in terms of response and toxicity of the FCM regimen as first-line treatment for younger patients with advanced stage FL.

Patients and methods: fifty-three patients (65 yrs (25/20), median age: 48 yrs), diagnosed with FL (grade I, 35 cases, grade II, 18) in stages III-IV from 10 GELCAI centers have been included in the trial between July 2003 and December 2001. FCM regimen consisted of 6 courses of fludarabine (25 mg/m^2/day, days 1-3), cyclophosphamide (200 mg/m^2/day, days 1-3), and mitoxantrone (6 mg/m^2, day 1). Continuation of prophylaxis was used until CD4 recovery >300 per μL. In addition to clinical studies, bel-2 clearance from peripheral blood (pb) and bone marrow (bm) was assessed by means of a real-time PCR technique.

Results: forty patients have finished the 6 courses of FCM, with 36 of them (90%) having received >200% of scheduled doses. After FCM, 30 patients (75%) achieved a CR, 8 (20%) a PR, whereas 2 (5%) were refractory to FCM. Eleven of 16 patients (69%) in whom bel-2 positive samples were available prior to FCM cleared bel-2 cells from both pb and bm after treatment. Overall, 267 courses of FCM have been administered. Grade III/IV neutropenia and thrombocytopenia was observed in 7% and 0.5% of the courses, respectively. Table gives grade III/IV infection in the different courses, including a progressive multifocal leucoencephalopathy (MPL) in one patient immediately after the 2nd FCM. After a median follow-up of 11 months, 3 patients have progressed, while an 8-month failure-free survival (FFS) of 90% (95% confidence interval: 79-100) has been reached. High serum beta-microglobulin and intermediate or high-risk international PCR index predicted a poor FFS. These patients have died during the follow-up due to FCM; in 1 case, and infection after exiting the FCM trial for relapse/progression in 2 cases.

Conclusion: In younger patients with FL, FCM produces a high CR rate, including bel-2 clearance from pb and bm, with manageable toxicity.

COMBINED IMMUNOCHEMOTHERAPY (R-FCM) IS SUPERIOR TO A FLUDARABINE-PHYOSAMIDE CONTAINING CHEMOTHERAPY (FCM) ALONE IN RECURRENT FOLLICULAR AND MANTLE CELL LYMPHOMA - RESULTS OF A PROSPECTIVE RANDOMIZED COMPARISON OF THE GERMAN LOW GRADE LYMOPHOMA STUDY GROUP

W. Heldemann, R. Forsterpoint, F. Fiedler, M. Gramatzki, B. Dörken, H. Illiger, M. Kiehl, M. Pleuendich, R. Paubl, P. Parwicz, M. Dreysing and M. Unterkircher for the GLSG.

Dept of Medicine III, University Hospital Grosshadern/LMU, Munich

Introduction: Rituximab has shown a high activity in relapsed follicular lymphomas when given alone. In addition, phase II studies indicate that its addition to chemotherapy may further improve the therapeutic impact. However, so far, prospective randomized studies have not been available. Methods: In 1998 the GLSG started a multicenter national trial in patients with relapsed or refractory follicular and mantle cell lymphoma. Since most patients had received a CHOP-like first-line treatment, a fludarabine-containing regimen (FCM) was chosen for salvage therapy: fludarabine 25 mg/m^2 d 1-3, cyclophosphamide 200 mg/m^2 d 1-3, mitoxantrone 6 mg/m^2 d 1-3, 28 days. A total of 4 courses were given. Patients were prospectively randomized for FCM alone or FCM plus Rituximab (375 mg/m^2 day on day 1, R-FCM).

Results: 80 of 147 randomized patients are currently evaluable for response. 43 patients had follicular, 27 patients mantle cell and 10 patients other indolent lymphomas. Statistical analysis was performed by survival testing which indicated a significant improvement in overall survival comparing R-FCM with FCM (p=0.001).

Conclusions: The results confirm the efficacy of Rituximab for untreated FL patients in terms of clinical and molecular response, and the role of Rituximab in improving molecular and clinical response when added to chemotherapy for first-line treatment of FL.

A RANDOMIZED TRIAL OF FLUDARABINE AND MITOXANTRONE PLUS RITUXIMAB VERSUS CHOP PLUS RITUXIMAB AS FIRST-LINE TREATMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA

P.L. Scarpellini on behalf of an Italian Cooperative Study Group on Lymphoma. Institute of Hematology and Medical Oncology "L. e A. Seraglio", University of Bologna, Bologna, Italy.

Introduction: Fludarabine and Mitoxantrone (FM) combination therapy is an effective strategy in follicular lymphoma (FL).

Methods: From October 1999, patients from 12 Italian centers patients were randomized in a prospective study, of FM versus CHOP chemotherapy, with the addition of Rituximab in selected cases. To be eligible, patients were required to have a histologically proven diagnosis of CD20 positive according to the REAL classification and a pt disease (P). For B2b disease, patients were age 15 to 70, stage II and an ECOG performance status of 0-2. After randomization, patients were allocated to the FM arm (Fludarabine 25 mg/m^2/2 times IV on days 1 to 3 and Mitoxantrone 10 mg/m^2 IV on day 1) or CHOP arm (Doxorubicin 50 mg/m^2 IV on day 1, Cyclophosphamide 750 mg/m^2 IV on day 1, Vincristine 1.4 mg/m^2 IV on day 1 and Prednisone 100 mg/day orally on days 1 to 5). In both arms, patients were assigned to receive 6 cycles of chemotherapy. Thereafter, to be eligible to Rituximab treatment, they had to have in partial or complete clinical response (PR) or CR and still be PCR positive in the BM and/or PB in two molecular analyses performed 4 and 6 weeks after the sixth cycle. These patients were entitled to receive 4 weekly IV of Rituximab (375 mg/m^2). Clinical response to treatment was defined according 3 categories: CR, PR and failure. Results: From 150 patients registered, 78 were evaluable for response after completion of chemotherapy. CR, PR and failure were, respectively, 89, 27, 3% in the FM arm (41 patients) versus 41, 51, 5% in the CHOP arm (37 patients) (p=0.06 for CR). The molecular evaluation documented the clearance of the B2b-2density in 32% and 15% for FM and CHOP regimen respectively (p=0.02). After Rituximab courses, the molecular evaluation documented the clearance in 61% and 41% for FM and CHOP subsets respectively, with an improvement of the clinical response in 50% of PR patients. Conclusions: These results confirm the efficacy of FM for untreated FL patients in terms of clinical and molecular response, and the role of Rituximab in improving molecular and clinical response when added to chemotherapy for first-line treatment of FL.
BENDAMUSTINE AND RITUXIMAB ACT SYNERGISTICALLY IN VITRO AND ARE EFFECTIVE IN THE TREATMENT OF INDOLENT AND MANTLE-CELL LYMPHOMAS PRETREATED WITH PURINE ANALOGS.


The cytotoxic agent bendamustine combines a purine-like benzimidazole and bifunctionally alkylating nitrogen mustard group. The drug is active in a variety of lymphoproliferative disorders without crossresistance to cyclophosphamide. We investigated simultaneous combination of rituximab with bendamustine and found synergistic effects of apoptosis in vitro on lymphoma cell lines (Daudi, 2-BUSW-NHL) as well as on ex vivo cells of patients with CLL (n=3). Apoptosis was determined by Annexin V and JC-1 (disruption of mitochondrial membrane potential). Rituximab was applied at a dose of 10µg/ml, where 80-95% of CD20-molecules per cell were sufficiently blocked. Noteworthy, the synergistic effects of rituximab in combination with bendamustine were even stronger when rituximab was applied at a dose, at which approximately half of the CD20 molecules per cell were saturated. We furthermore demonstrated that the chemosensitizing effect of rituximab was complement-independent and that this effect critically depends on caspase-3. Based on our in vitro results we initiated a phase-II study to examine efficacy and toxicity of bendamustine combined with rituximab as therapy of 1st, 2nd or 3rd relapse. All patients had previously received fludarabine or cladribine containing treatment. Treatment schedule: 6 cycles of rituximab 375 mg/m² day 1 combined with 4 cycles of bendamustine 90 mg/m² qd day 2-5, every four weeks. Included entities: immunocytomas, mantle cell, follicular lymphoma and marginal zone lymphomas. 21 patients entered the study, 14 are evaluable for response. 3 pts had 1 prior therapies, 2 pts 2 prior therapies, and 1 pt 3 prior BM.

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<td>Overall</td>
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The presented combination induced considerable low hematological toxicity with only 4% granyocytopenia grade 3 and 4. One patient with a mantle cell lymphoma being refractory to 2 prior treatments died due to lymphoma. 1 bacterial pneumonia has been observed. So far, it appears in this ongoing study that the combination of bendamustine and rituximab may be very effective in the treatment of relapse in low-grade NHL and mantle cell lymphomas reaching an overall response rate of 93% and a CR rate of 64%.

ANTIRRACYCLIN CONTAINING REGIMENS FOR THE TREATMENT OF FOLLICULAR LYMPHOMA: A RETROSPECTIVE ANALYSIS OF THE INTERGRUPPO ITALIANO LINFOMI (III).


Background: Follicular lymphomas often have a long survival in spite of their frequent relapses. The inability of chemotherapy and radiotherapy to eradicate this disease has led to radically divergent treatment approaches. We report a retrospective analysis of a large series of patients with historically confirmed diagnosis of follicular lymphomas enrolled on prospective trials in various Italian institutions.

Patient and methods: The III, performed a wide collection of patients treated in cooperative trials between 1985 and 1996; 633 patients were treated with an antirraacyclin chemotherapeutic containing regimens and a selected group of 128 patients treated without antirraacyclins. The two groups were stratified according to the clinical characteristics and resulted comparable for the major clinical characteristics and in particular no differences were observed according to IPI index and ILI index.

Results: Complete remission rate (CR) was 62.9% and 67.5% overall response rate was 92.5% and 85.4% respectively for patients treated with antirraacyclines and without antirraacyclines. After a median follow-up of 51 months, 54 months for patients still alive, we have observed a better OS in patients treated with antirraacyclines 90% in comparison to 65% of patients treated without antirraacyclines (p =0.004). Moreover FFS was significantly longer in patients treated with antirraacyclines (49% vs 34% p =0.006). A multivariate analysis including ILI score and therapy showed that both ILI and therapy had an independent prognostic impact on survival. In a survival analysis adjusted for age, which could be the main influencing feature at diagnosis, type of therapy retained its statistically significant relevance (p =0.01). According to ILI prognostic index patients with low or intermediate risk showed a better OS if treated with antirraacyclines (respectively p =0.001 and p =0.009). Patients in the high risk group showed a trend for a longer survival.

Conclusion: Our retrospective study shows that patients with follicular lymphoma treated with antirraacyclin containing regimens had a better outcome as compared to patients treated with CVP or CVP-like regimens in terms of response to therapy, overall survival and failure free survival. On the basis of these results antirraacyclin-containing regimens should be considered as the conventional treatment of choice in patients with advanced follicular lymphomas.

RITUXIMAB PLUS CHLORAMBUCIL IN LOW-GRADE NON Hodgkin's LYMPHOMAS (NHL): A PILOT STUDY.


European Institute of Oncology, Milan, Italy, and Oncology institute of Southern Switzerland, Bellinzona Switzerland.

Introduction: Monoclonal anti-CD20 antibody (Rituximab) has been worldwide evaluated as effective in treatment of low-grade NHLs. More recently the combination of chemotherapy plus Rituximab, evaluated in aggressive NHL patients, demonstrated better clinical results than those achieved with single modality of therapy. Chlorambucil (Chl) is considered the drug of choice for the treatment of low-grade NHL. No data are available about the combination of Chl plus Rituximab. Aim of this study is to define the feasibility, toxicity and efficacy of Rituximab plus Chlorambucil in low-grade NHL.

Methods: 14 patients (48 years median age) with biopsy proven CD20+ low-grade NHL (5 with de novo NHL and 9 with relapsed/refractory disease) were enrolled in the study since November 01 to January 02. Treatment plan consisted of Chl 5mg/m² daily administered for 6 consecutive weeks with standard 4-weekly Rituximab administration schedule. Patients responding to therapy received 2 weeks of Chl every month (4 cycles) plus Rituximab once/monthly. All patients were weekly monitored with WBC counts. Immunophenotype evaluation was performed at baseline, after the induction phase and then at the end of the treatment. Results: Therapy was well tolerated by all patients with no major (NCI 3-4) haematological toxicity, except 2 patients who developed an absolute lymphopenia (NCI 3). No patients had an imbalance in CD4+/CD8+/CD56+ ratio and no infectious morbidity was registered. All evaluable patients achieved a clinical response. Definitive results on toxicity and clinical activity will be available and presented in June.

Conclusion: Based on our preliminary data, combination of Rituximab plus Chlorambucil seems well tolerated and very active; its definitive efficacy will be evaluated in a subsequent randomized trial.

THE ADDITION OF AN ORAL ANTHRACYCLINE TO A CHLORAMBUCIL/DEXAMETHASONE COMBINATION HAS NO SIGNIFICANT IMPACT ON SURVIVAL IN LOW GRADE NHL: RESULT OF SNLH VIII TRIAL.

Authors: P Taylor, J White, B Angus, A Lessels, S Proctor on behalf of the Scotland and Newcastle Lymphoma Group, Dept PHS (R666), University of Edinburgh Medical School, Tiefed Place, Edinburgh, EH8 9AG.

Introduction: The optimal treatment of low grade B NHL (LBGNHL) remains problematic and in November 1993 we decided to investigate the addition of an oral anthracycline to our standard combination therapy.

Methods: A randomised controlled trial compared two oral treatment regimens: chlorambucil 20mg/m² daily for 3 days + dexamethasone 4mg/d for 5 days versus the same drugs with the addition of idarubicin 10mg/m² for 3 days. Cycles were repeated every 21 days for a total of 6 courses. Outcome was assessed using the SNLH prognostic index for low grade lymphoma (SNLH-P1) and the international prognostic index (IPI). Results - 200 patients entered the study, which closed in March 2000 (median follow up 42 months), 101 (50.5%) median age 56 (range 30-74) 11 patients were excluded at histology review, 2 patients refused treatment therefore 187 patients were eligible for assessment. 75% of patients had follicular lymphomas; 1 patient had stage 1, 20 patients stage 2, 45 patients stage 3 and 120 patients stage 4 disease.

Both arms were well tolerated with no TRM. 85% of cycles had no recorded toxicity.

There was no difference in overall survival (OS) in the 2 arms, median 75 vs 80 months (CD vs CID) (P=8). Time to treatment failure (TTTF) was significantly different, CD 18 months vs CID 26 months (P=0.1). When outcome was analysed using the IPI and the SNLH – PI risk groups there was no significant difference in time in TTTF or OS found.

Conclusion: The addition of an oral anthracycline to chlorambucil and dexamethasone prolonged time to treatment failure but failed to impact on overall survival in patients with low grade B NHL.
CLINICO-PROGNOSTIC IMPLICATIONS OF INCREASED BONE MARROW ANGIODENSIFERATION IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background and methods: In order to assess the merit of increased angiogenesis in predicting the clinical outcome of patients with early B-cell chronic lymphocytic leukemia (CLL) (i.e. Binet stage A) we evaluated the mirovessel bone marrow (BM) density by immunohistochemistry and computed assisted image analysis in 45 newly diagnosed such patients.

Results: Microvessel area and counts were significantly higher than those of patients suffering from other hematological non-neoplastic diseases (i.e., anemia due to iron or vitamin B12 deficiency). After setting a cutoff corresponding to the median microvessel area value (0.90 mm² ± 0.10 mm²) a correlation with main clinical parameters representative of tumor mass was attempted. Increased BM angiogenesis did not reflect advanced Rai stage (P=0.320, increased peripheral blood lymphocytosis (P=0.990), diffuse pattern of BM infiltration (P=0.507). In contrast, a positive correlation with either LDH (P=0.04) or β2-microglobulin (P=0.02) serum levels was found. BM angiogenesis did not parallel serum concentration of vascular endothelial growth factor (VEGFP=0.158), the same applied with circulating levels of basic fibroblast growth factor (bFGF=P=0.646), interleukin-8 (IL-8=P=0.511), CD3 (P=0.712), metalloproteinases-9 (MMP9=P=0.893). Interestingly, microvessel area correlated with the presence of BM tumor cells (P=0.93, Po=0.51), after a median follow-up time of 13 months (range, 2-40), 18 out of 45 (40%) patients progressed to a more advanced clinical stage. Curves of progression-free survival (PFS) obtained after stratifying survival analysis according to area values corresponding to 75% percentile (i.e., 0.90 mm² x 10²) differed significantly (P=0.03), hazard ratio (HR) 0.419, 95% confidence interval, 0.110-0.983).

Conclusion: Increased BM angiogenesis is a potential marker of disease-progression in early CLL which adds prognostic information to the Rai classification of Binet stage A.

PREDICTIVE FACTORS FOR INFECTIOUS COMPLICATIONS IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS TREATED WITH FLUDARABINE COMBINATIONS WITHOUT CORTICOSTEROIDS. JF Seymour, MM Wolf, AP Grigg, EH Januszewicz, MS Prince, WD Westerman

Department of Haematology, Peter MacCallum Cancer Institute, & Royal Melbourne Hospital, Melbourne, Australia.

Many studies have examined the risk factors for infections (i.e) with fludarabine (Fam) therapy (reviewed ICO 13:2431, 1995). It is established that the concomitant use of corticosteroids greatly increases the risk of opportunistic i.e. Fam-based combination therapy without corticosteroids appear highly effective and are increasingly used, but the risk factors for i.e with these regimens have not been explored. Metoprolol, we have treated 29 patients (35) with Fam / Metoprolol (n = 22), Fam / Metoprolol (n = 10), Fam / Metoprolol (n = 5) and Fam / Metoprolol (n = 2) with Fam / Metoprolol (n = 2) and Fam / Metoprolol (n = 2) with Fam / Metoprolol (n = 2) with Fam / Metoprolol (n = 2). The only significant factor for 314 cycles (median 4/6, range 1-2). The pts were aged 59 years (range 34-60) with 65% males, median 2 prior therapy (0-10). Overall, 12.4% of cycles were complicated by i.e (2 total, 2.3%) and 29% of pts suffered an i.e. Only 5 pts received PC prophylaxis, but there were no cases of PC seen. The factors examined were for their association with i.e., chemo regimen, gender, age (< 60 vs ≥ 60), i.e. performance status (< 2 vs ≥ 2). No. prior regimens (< 3 vs ≥ 3), prior Fam therapy (n = 13), diagnosis (CLL and variants vs indolent NHL), use of PC prophylaxis. Results: The risk of i.e was greatest with the first cycle (15%) with significantly decreasing risk with later cycles (Pfor trend = 0.05). On a per-cycle basis, only age ≥ 60 (19% vs 8%; P=0.038) and a prior therapy (21% vs 5%, P=0.037) were associated with an increased risk of i.e. Both age ≥ 60 and 3 prior therapies were also significant predictors of i.e on a per-patient basis (each P < 0.039). These factors were additive: pts with 6, 3 i.e risk factors had an i.e. risk of 14%, 38% and 60%, respectively (P=0.0017). On a per-cycle basis, i.e. Incidence was 6%, 16% and 21% for cycles with 0, 1, or 2 risk factors, respectively (P=0.0017). Conclusions: The overall i.e risk with these Fam-combinations without corticosteroids is moderate. Age ≥ 60 yrs and ≥ 3 prior regimens were significant risk factors, and pts with both risk factors had an i.e. risk of 60% and are appropriate targets for prophylaxis strategies.
FLUORADARINE VERSUS FLUARABINE PLUS EPRIBOCIN IN THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA—PRELIMINARY RESULTS OF A RANDOMIZED PHASE-III MULTICENTER STUDY


Haematology, Johann-Wolfgang-Goethe Universität Frankfurt, Ulm, Mainz, Darmstadt, Bad Friedrichshall, Trier Germany.

Background: In our previous phase-II study using the combination of fludarabine plus eprubicin in previously untreated patients with CLL we reached an overall response rate of 52% with a high rate of complete remissions of 40%. Aims: Based on these promising results we initiated a multicenter randomized phase-III study to compare efficacy and toxicity of the combination regimen fludarabine plus eprubicin versus monotherapy with fludarabine as first-line therapy or therapy of first relapse in addition. Cyto genetic aberrations will be investigated in all patients to evaluate, if special chromosomal abnormalities are associated with prognosis and response to fludarabine. Treatment schedule. Fludarabine 25 mg/m² qd days 1-5, eprubicin 25 mg/m² qd days 4-5. For a maximum of 5 cycles every four weeks. Results: 154 patients entered the study so far, 100 are evaluable for response. Cyto genetic aberrations are well balanced between both groups. The overall response rate (ORR) was 82%, with a CR-rate of 22%. 61 patients were previously untreated in which CR was achieved in 27% and PR in 54%. In 39 patients entering the study in their first relapse the overall response rate was 63% with a CR-rate of 14%. Patients in stage Binet B achieved a better CR rate with 30% in contrast to patients with Binet C with an CR rate of 11%. The ORR for patients treated with fludarabine and eprubicin was 88% compared to patients treated with fludarabine monotherapy reaching an ORR of 71%. This difference is not quite statistical significant with a p-value of 0.07. Rates of complete remissions are different in favor for combined treatment modality with a borderline significance of 0.05. Conclusions. From our preliminary results so far we can corroborate the high activity of fludarabine in the treatment of CLL, it is of particular interest if a higher response rate and a longer duration of achieved remissions will be observed for the combination therapy in this ongoing study.


University of Leipzig, Division of Hematology/Oncology, 04103 Leipzig, Germany.

Introduction: The 94BP01 protocol was designed to compare melphalan / prednisone (MP) and bendamustine / prednisone (BP) in multiple myeloma (MM). Between May 1994 and July 1998, 191 MM patients (stage II with or without stage III, n=11; stage III, n=125) were recruited by 31 hospitals in Germany. Five patients were not evaluable for response, 68 patients received BP (bendamustine 150 mg/m² day 1, 2, prednisone 60 mg/m² day 1-4) and 63 patients MP (melphalan 15 mg/m² day 1, prednisone 60 mg/m² day 1-4). Response to treatment was evaluated by SWOG criteria. Patient characteristics of both groups were similar and no significant differences were found with regard to age, stage, Ig isotypes, β2 microglobulin, and bone destruction. Results: After a median follow up of 48 months data were analyzed. Response rates were similar in both treatment groups (BP: 75 %, MP: 70 %). Complete remissions were significantly higher in the BP group (32 % versus 13 %, p<0.01). In responding patients remission was achieved after 6.8±3.4 cycles in the BP group compared to 8.0±4.7 cycles in the MP group. The duration of response was significantly longer in the BP treated patients with a median of 14 months compared with 10 months in the MP group (p=0.03). The 30 months probability of progression free survival was 28 % (BP) versus 8 % (MP). There was no significant difference in the overall survival in either group. The 60 months post-diagnosis probability of survival was 29 % in the BP arm versus 19 % in the MP arm (p=0.74). Minimally increased toxicity (leukopenia, gastrotoxicity, thrombocytopenia) was associated with BP therapy. Conclusion: We conclude that the BP regimen showed important advantages over standard MP as primary treatment for MM. For the BP treated patients a higher rate of complete remissions with a shorter duration of therapy is achieved and for most patients a longer therapy free interval until the first progress was noted. The latter represents an important improvement in the quality of life of patients.

NON MYELOABLATIVE TRANSPLANT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA: EXPERIENCE FROM THE SPANISH REGISTRY

M Cavallero1, R Martinez1, C Cegano1, A Urbano3, V Rubio1, J Odriozola2, C Cazalla2, MV Maito1, A Sure1, J Perez, JF San Miguel1

1H Valladolid, 2H Santa Cruz I San Pau, Barcelona, 3H Clinica de Valencia, 4H Clinica de Barcelona, 5H Hospital SAS de Jerez de la Frontera, Cadiz, 6H Ramon y Cajal, Madrid.

In September 1999, we started a prospective multicentre trial with non-myeloablative allo geneic transplant in hematological malignancies. Up to 31 December 2001, 121 patients have been registered in the program. From them, 38 had a chronic lymphoid malignancies: B chronic lymphocytic leukemia, 8 (21%), low grade lymphoma, 10 (26%), aggressive lymphomas, 9 (23%) and 11 (29%) Hodgkin's disease. The conditioning regimen consisted of fludarabine 30 mg/m² intravenously (IV) on days -8 to -4 followed by melphalan 100 mg/m² IV on days -3 and -2, and two patients received busulfan 1 mg/kg x 10 doses (days -6 to -4 , total 10 mg/kg), with plonovin given as anticonvulsant prophylaxis, instead of melphalan. Filgrastim-stimulated peripheral blood stem cells were infused on day 0. GVHD prophylaxis consisted of cyclosporine (A) (CsA) from day –7 plus short-course methotrexate (MTX) (10mg/m², days +1, +3, +6), followed by folinic acid rescue.

Median age at transplant was 65 years, (range: 19-67); 6 patients (16%) were in CR, 18 (47%) had sensitive disease (PR or stable disease) and 14 (36%) had refractory/progressive disease at transplant. 15 patients (39%) had failed a previous autologous transplant. All patients engrafted. Acute GVHD grade II-IV developed in 13 patients (34%) and extensive chronic GVHD in 9 of 22 (41%) patients at risk. With a median follow-up of 228 days (range: 9-660), 22 patients are alive and 18 of them are disease free. 16 patients have died (42%): 4 (25%) due to progression and 12 (75%) due to related transplant mortality. 14/20 (70%) patients transplanted with sensitive disease are alive whereas 6/12 (50%) refractory have died. Disease free survival (DFS) is 78% and event free survival (EFS) is 47%. Concerning relationship between (GVHD and relapse rate, one out of 12 patients developing acute GVHD progressed (6%) as compared to seven progressions out of 22 patients (32%) without acute GVHD (p=0.03). Also, only one out of 15 patients developing GVHD progressed as compared to two out of seven patients without oGVHD (6% vs 28.5%, p=0.1). Comments: This non-myeloablative program was associated with good engraftment and it seems to be effective in these high risk patients. Longer follow-up is required.

IMPORTANCE OF ESOPHAGOGASTRODUODENOSCOPY (EGD) IN EVALUATION OF NON-GASTROINTESTINAL MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA


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Introduction: Mucosa-associated lymphoid tissue (MALT) lymphoma is an extranodal low-grade B-cell lymphoma that most commonly presents in the gastrointestinal (GI) tract. However, it can present in non-GI primary sites. This study was conducted to determine the incidence of gastric involvement in non-GI presentation of MALT lymphoma.

Methods: We retrospectively reviewed the medical records of 36 consecutive patients who presented with non-GI MALT lymphoma and had EGD as a part of the initial staging work-up between 1992 and 2001.

Results: The median age was 62 years (range 27-82 years); 27 were female patients. The frequency of the primary site was: salivary gland (n=14), ocular adnexa (n=7), lung (n=3), oral cavity (n=2), skin (n=3), thyroid (n=2), nasopharynx (n=2), hypopharynx (n=1). Twelve patients were found to have gastric involvement; 2 additional patients progressed in the stomach 5 and 6 months later, respectively.

Conclusion: Routine evaluation of the stomach should be a part of the initial staging work-up of non-GI MALT lymphoma.