ABSTRACTS

POSTER PRESENTATIONS
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The German pediatric study group for Hodgkin's disease (HD) initiated a phase II trial for salvage therapy (PTPIII). The aim of this trial was to investigate the effectiveness of combination HD with rituximab (Rituxan) and cyclophosphamide, doxorubicin, vincristine, procarbazine, methotrexate, and prednisone (COPP) as an alternative treatment. The study group included 2 groups: Group A received COPP plus rituximab (R), and Group B received COPP plus rituximab (R). The study was conducted at 47 participating centers.

Results: 31 patients (77% females) were enrolled, and 27 patients were evaluable for response. The overall response rate was 70%, with 17 complete responses (54%) and 10 partial responses (30%). The median time to progression was 20 months, and the median progression-free survival was 22 months. The 2-year overall survival rate was 89%, and the 2-year progression-free survival rate was 83%.

Conclusions: This study demonstrates the feasibility and effectiveness of rituximab in combination with COPP as salvage therapy for relapsed HD. Further studies are needed to confirm these findings and to explore the role of rituximab in other pediatric malignancies.
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We analyzed the long-term results of the Children’s Cancer Group study CCG-551, a randomized trial comparing a 4-drug regimen, COMP, to a 10-drug regimen, LSAQp, for the treatment of childhood non-Hodgkin’s lymphoma. The initial results have been previously reported (NEJM 308: 559, 1988). In particular, we have assessed the likelihood of late relapse or deaths without progression after a median of 8 years of follow-up.

A total of 434 patients were treated, 68 (11 lymphoblastic [LB]), 11 large cell [LC] and 46 undifferentiated (UH)] with localized disease [LD] and 366 (168 LB, 61 LC and 137 UDL) with disseminated disease (DD).

Event-free survival (EFS) of patients with LD was 83% for COMP and 85% for LSAQp at 5 years (p<0.05). Two LB pts relapsed beyond 2 years on study; both were successfully retreated. Results for DD pts depended on histologic subtype: LB pts did better when treated with LSAQp (5-year EFS of 66% versus 33% for COMP [p<0.0001]). COMP was better for UDL (5-year EFS of 50% versus 29% for LSAQp [p<0.01]). Results were similar for LC (5-year EFS of 50% for COMP versus 43% for LSAQp [p=0.22]). Five percent of patients died of treatment-related complications while on therapy (primarily infections). Only 4 deaths without progression have been observed: off-therapy (2 from restrictive lung disease, 1 from an acute asthma attack, 1 from colon cancer). Survival of patients post diagnosis was poor without long-term survival after recurrence established at 11% at 5 years.

Long-term follow-up of pts treated on this study demonstrates that treatment success cannot be expected in all patients. For DD pts, treatment success can be expected in 66% of those with LB and 50% of those with LC or UDL. Late adverse events to date have been rare.


1/ The experience with LMB 84 randomized protocol (PHILIP et al., Blood, 1993) in progress and further experience with non-randomized patients showed a relapse rate of 33/265 (13%). The relapse protocol with CYVE (high dose Cytarabine-etoposide) obtained a CR2 in 7/177 pts versus 5/13 for other rescues. A massive therapy obtained a cure in 6/11 patients in CR2, 0/4 in PR2, 1/5 in resistant disease. Only one of the 15 patients who did not receive massive therapy was alive.

2/ From 07/89 to 11/92, 225 patients from 41 centers were evaluated for survival in the LMB 89 protocol. This protocol included 3 arms (A, B, C): arm B and C are used for patients with advanced disease respectively, without and with CNS and/or massive bone marrow invasion. Arm C received prolonged and more aggressive treatment (including 2 courses of CYVE). There was 14/225 (6%) relapses 10 stage III, 4 stage IV (2 CNS, 1 minor and 1 massive marrow invasion). Ten had received LMB 89 arm B, and 4 arm C (2 CNS, 1 massive marrow invasion, 1 resistant to COP). Median delay from diagnosis to relapse was 5 months (3 to 15). Height patients relapsed on 6 of chemotherapy. The site of relapse was lob-regional (8 cases of which 4 isolated), CNS (6 cases, 2 isolated), marrow (7 cases, 2 isolated).

Relapse protocol included CYVE for all 10 arm B patients: 7 patients were in CR after one (2) or 2 courses (5 patients), and 3 progressed. For arm C, 2 patient were in CR (1 after a MME-like therapy by VENOMID, 1 after high dose MTX with IV mercaptopurine and radiotherapy, but both had cleared their CSF with intrathecal therapy before chemotherapy), and 2 progressed.

Consolidation: Eleven patients received a massive therapy with bone marrow rescue. Conditioning included BEAM (10 autologous) or TAM (all autologous transplant). Mean survival was 33 months in CR (11, 12, 35 months), 5 progressed. One graft failed in partial, and one in progressive disease. Overall, 33 patients with isolated lobo-regional (all off therapy), and 1 patient with isolated CNS disease (post-hypothy) survived. The 3 patients who could not receive massive therapy (for progression) are dead.

3/ CONCLUSION We thus confirm that 1/ Salvage therapy by CYVE obtains a CR in 14/27(51%) patients. For those who received CYVE before their relapse, alternative treatment should be found. 2/ Patients in CR may be cured by aggressive management.

P 7 FAMILIAL AGGREGATION OF HEMATOLOGICAL NEOPLASMS AMONG PATIENTS WITH HODGKIN’S DISEASE AND NON-HODGKIN’S LYMPHOMA. O. Shinberg, M. Modan, B. Molan and B. Ramot. Institute of Hematology, The Chaim Sheba Medical Center, Tel-HaShomer, 52621, Israel.

Familial aggregation of hematological neoplasms (HN) was compared in 22 families of patients with Hodgkin’s disease (HD) and 57 families of patients with non-Hodgkin’s lymphoma (NHL) with two control groups of 28 families with non-malignant hematological disorders (NHD) and 33 families of patients with type II diabetes mellitus (DM). A self-administered questionnaire was completed by each family member, including a list of all relatives and their vital status, current age or age at death and their chronic diseases. The HD group included 701 relatives in whom 8 (1.1%) were reported to have HD and the NHL group included 1148 relatives in whom 13 (1.1%) reported to have HD. Those rates of familial aggregation were significantly higher (p<0.02 and 0.03) than those of the NHD and DM groups, respectively. The familial aggregation of HD in both study groups was not disease-specific: only one relative had HD in the HD group, while the other seven had other HD. Three had defined HD, 2 NHL, 1 multiple myelomas and 1 leukemia. In the NHL group only 2/13 relatives with NHL had NHL, while the others had various types of NHL. Four had undifferentiated leukemia, 2 acute leukemia, 1 multiple myelomas, 1 chronic lymphocytic leukemia and 1 myeloproliferative disorder. These data are consistent with the hypothesis that there is a genetic predisposition to HD and NHL that may be associated with a defect in the pluripotent hematopoietic stem cell.

P 8 MALIGANT LYMHPHOMAS AND SUBCUTANEOUS Lymphocytosis. ANALYSIS OF 962 CASES OF NON-HODGKIN LYMHPHOMAS. A. CORON, C. IVERI, E. MORRA, A. LIVRAGHI, A. SANTAGOSTINO, C. BORNANO, G. CASTELLI, M. LAPIERRE, C. BERTOLUCCI. University of Pavia, Division of Hematology, Policlinico S. Matteo IRCCS, Pavia, Italy.

Exposure to mutagenic agents such as organic solvents, herbicides or insecticides have been postulated as possible risk factors for the development of NHL. We analyzed 962 cases of NHL, including 100 cases of NHL with lymphocytosis (LY) caused by contact with industrial solvents. The cases of NHL with lymphocytosis were compared with the total NHL cases (p>0.001). Lymphocytosis (LY) could be a causative factor in an increased incidence of this hematopoietic malignancy. We reviewed retrospectively, 962 cases of NHL diagnosed at our department and followed consecutively from 1970 to 1992. Three groups could be identified with respect to exposure to the cancer risk factor in CR: nil exposure (1,12,16,33), low exposure (exposure to any known mutagenic agent; group II) was composed of 84 cases occupationally exposed to chemicals; group III comprised 33 pts previously submitted to Rx for radiodiagnostic examinations or radiotherapy. The age at diagnosis was slightly lower for pts exposed to chemicals (median 53 yrs) than for the 1 and 111 groups (median 56 and 58 yrs respectively). In group II 77 pts (92%) were males, a higher ratio than the other two groups (p<0.001). A variety of occupations was associated with exposure to solvents with prevalence of farmers (22%) and joiners (17%) and the duration of exposure was 3-30 (median 30) yrs. The majority of pts of group III had been submitted to excessive repeated radiodiagnostic examinations for tuberculosis. For these pts the latency time to NHL was 1-51 (median 15) yrs. There were no significant differences in histopathology among the three groups, nor in clinical presentation at onset, except for a higher incidence of bulky disease in pts exposed to chemicals, and an absence of mediastinum involvement in pts exposed to Rx. We did not notice the slight difference in the incidence of histopathological and initial supradiaphragmatic location of the lymphomas, reported in the literature. Response to therapy and survival were unequally distributed in the three major prognostic groups of NHL. Among low-grade lymphomas pts group II pts had a worse prognosis than the other two groups. Among the intermediate-grade pts the high-grade pts had the best prognosis, whilst for the high-grade pts the prognosis was similar in the three groups. In conclusion: 1) pts exposed to chemicals and most frequently farmers at diagnosis have a higher incidence of bulky disease and show the worst prognosis among the low-grade NHL; 2) pts exposed to chemicals had mediastinum involvement at diagnosis; 3) among the intermediate grade NHL exposed pts had a worse prognosis than non-exposed ones.
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P 9 Evidence for increased incidence of non Hodgkin's lymphomas in Burgundy (France) over a 10-year period (1980-1989). CARLI P.M., BOUTRON M.C., MAZOLLE M, CARLOT G. - Service des Hémopathies et Immunothérapie, Hôpital de Dour, (équipe associée INSERM-DG) - Laboratoire d'Hématologie, Hôpital du Bocage, 21034 DIJON, FRANCE.

Increasing incidence of NHL has been reported in several areas of the world and it has been often attributed to new AIDS-related lymphoma cases. Nevertheless, little population-based data is available for the 1980s period which corresponds to the arrival of the AIDS epidemic.

A Registry of hematopoietic malignancies was created in January 1980. It registers all HM occurring in subjects living in the department of Côte d'Or enabling us to present detailed time trends for all HM in particular NHL.

All NHL, both nodal and extranodal, were considered. Between 1980 and 1989, 380 new cases of NHL were diagnosed in the Côte d'Or in 21,000 and 16,000 women. Cases were classified according to their histological type in three groups: low, intermediate and high grade as defined by the Working Formulation.

There was an overall 10.9 % (8.5 - 15.0) annual increase in NHL incidence (p = 0.001).

This significant increase was observed not only in men and women (respectively + 11.2% ; + 10.5% ; p = 0.01) but the non-disproportion in the three age groups: although, it tended to be more important in the youngest age group (respectively + 19.6% ; p = 0.01 and + 8.1% ; p = 0.01).

The urban to rural ratio in incidence was 4.8 in 1980 and decreased progressively to 1.1 in 1989. As for histological type, increase in incidence was statistically significant in all three groups.

The most dramatic increase was, however, observed for high-grade lymphomas (+ 20.0 pc; p = 0.05). High grade lymphomas were the less common lymphomas until 1984 to become by 1989 the most common together with intermediate grade lymphomas.

In this series, only one case was associated with an HIV infection. These data indicate that although a significant increase in NHL incidence related to the AIDS epidemic might be expected in the near future, there is an independent dramatic trend which started earlier than the AIDS problem. The reasons for the present changes in NHL in the Western World are largely unknown. Such data should prompt antilogical research.

P 11 OCCURRENCE OF EBV GENOME IN NON-HODGKIN'S LYMPHOMA SUBTYPES: HIGHEST FREQUENCY IN T-CELL LYMPHOMAS

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Epstein-Barr virus (EBV) is strongly suspected of playing a pathogenetic role in endemic Burkitt's lymphoma and in immunodeficiency-related B-cell malignancies. However, nosocomial EBV associated non-Hodgkin's lymphomas (NHL) arising in apparently immunocompetent patients has also been suggested for this reason, an in situ hybridisation (ISH) survey, based on the archival histologic material of a Danish population-based NHL registry (LYFO), was performed. Carriers were screened by non-isotopic ISH for EBV-encoded small nuclear RNA's (EBER) and for abundant immediate early mRNA's (BHLF). So far 135 patients have been screened. They had a median age of 50 yrs (range: 2-91 yrs) and a M/F ratio of 1.8. Sixty-two cases were of B-cell and 66 of T-cell phenotype (B/T ratio = 0.94). 7 were non-B, non-T. Specific histologic subtypes were: centroblastic/centrocytic (CB/Cc) follicular (n=14), mycosis fungoides (LMF) (n=6), CB diffuse (n=26), peripheral T-cell (PTC) (n=31), i.e., 26 high grade and 5 low-grade), lymphoiblastic (LB) (n=33, i.e. 15 B, 16 T, and 2 non-B, non-T), Ki-1+ anaplastic large-cell (ALC) (n=18, i.e. 8 B, 5 T, and 5 non-B, non-T), other high-grade histologies (n=7, i.e. 2 B and 5 T). Of the 135 cases, 22 (16.3%) contained EBV genomes (21 EBER-positive, 1 both EBER- and BHLF-positive). Fourteen of the EBV-positive cases were of T-cell and 7 of B-cell phenotype, 1 case was non-B, non-T. The proportion of T-cell lymphomas was significantly higher among EBV-positive than EBV-negative cases (B/T ratio: 0.50 vs 1.06; p=0.02). With regard to T-cell histologic subtypes EBV genomes were found predominantly in high-grade cases. Other EBV-positive cases were 1 case of MF, 1 of angioimmunoblastic lymphadenopathy, 1 of Lennert's lymphoma and 1 of ALC type. The latter, together with an additional EBV-positive ALC (non-B, non-T), accounted for 11.9% of the cases. Among B-cell subtypes, LB had the highest frequency of EBV positivity (20% of cases). Interestingly, no EBV was found in any of the 16 T-cell derived LB lymphomas. Although rare, a scattered EBV-positivity, restricted to a low number of cells, was also seen in follicle-centre derived lymphomas (CB/Cc 3 cases; CB 1 case). Among the 22 EBV-positive cases, at least 2 distinctive infection patterns were observed: (i) few infected small lymphoid cells, with or without a component of positive histiocyte-like and/or immunoblastic cells, (ii) infection of the vast majority of the neoplastic cell population.

P 10 HIGH PERCENTAGE OF KIDNEY DIAGNOSED MALIGNANT LYMPHOMAS IS HIV-ASSOCIATED. A. Dietterle1, J. Tchorz1, T. Cerny2 for the lymphoma group of the Swiss working group for clinical cancer research (SASK)1 and the association of Swiss cancer registries (ASCR)2

In January 1991, the SASK activated a prospective registration study (SASK 91/90) for all newly diagnosed malignant lymphomas in the age group from 16 to 65 years seen at the regional SASK centers. Completeness of the registration was controlled in collaboration with the ASCR. The study aimed on information about the incidence of HIV associated lymphomas within the SASK centers and the ascertainment of the ASCR.

As of November 1992, 315 eligible patients were registered, of which full information is available in 310. By crosschecking with the cancer register data it was confirmed, that registration was nearly complete. Within the region covered by the cancer registries 37% of all eligible lymphomas were seen at the SASK centers. In 39 patients no HIV-test was performed. In 273 HIV-tested patients 39 (14%) HIV-associated lymphomas (23 Non Hodgkin Lymphomas, 7 Hodgkin disease, 9 other lymphomas) were observed. Surprisingly, one non eligible patient aged 77 was found to be HIV positive. Evaluation of the distribution of histology and stage, in PC and non PC, will be presented. From this preliminary data we conclude that a high percentage of the newly diagnosed malignant lymphomas at the SASK centers within the studied age group is HIV associated.

P 12 EPSTEIN-BARR VIRUS DNA IN CASTLEMAN'S DISEASE.
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Castleman's disease (CD) is an uncommon lymphoproliferative disorder with an enigmatic pathogenesis and variable biological behavior. Two variants have been described: a hyaline-vascular (HV) and a plasma cell type (PC). A common component of CD are autoimmune phenomena and immunological abnormalities. In some cases of CD monoclonal B-cell populations have occurred and other cases have been complicated by malignant lymphoma. In a recent survey, two of eight CD cases were reported to contain Epstein-Barr virus (EBV) specific sequences.

In the present study we have investigated the occurrence of EBV in 19 cases of CD. 9 of the HV, 3 of the PC-type and 7 of an intermediate type. Paraffin blocks and fresh frozen material (two cases) were collected from the four institutions. All cases were reviewed by four of us, immunohistologically evaluated and searched for EBV by three different methods: immunohistology for the latent membrane protein (LMP), in situ hybridisation with EBER-probes for EBV-specific RNAs, and PCR for both general and subtype-specific-sequences of viral DNA.

No LMP-positive cells were found in any of the CD cases. EBV-positive cells were present in 18 cases. All samples were subjected to PCR for beta-globin as a test for the availability of DNA and gave positive results. General sequences of EBV, as shown by PCR for up 220, were detected in twelve cases. All PC and intermediate type CD were EBV-positive, while only 2/9 HC-variant of CD presented with EBV-sequences.

We found more than half of the CD cases of our study to be EBV-positive. 10/19 PC and the intermediate variant of CD were more prone to EBV-association than the HV-type. We conclude that CD is an EBV-associated disease. This phenomenon may be related to immune dysfunctions in CD, especially of the B-cell system.
A definite diagnosis of adult T-cell leukemia/lymphoma (ATL) is made by documenting the presence of HTLV-I proviral DNA in the DNA of leukemic or lymphoma cells (Takatsuki K et al, GANN Monograph on Cancer Research 39, 1982). The HTLV-I proviral DNA pX Tax region can act as a kind of oncogene (Proc Natl Acad Sci USA 87, 1990). It is well known that the most peripheral T-cell lymphomas in patients with HTLV-I infection show pleomorphic and/or large atypical appearance. However, a direct evidence of HTLV-I infection in neoplastic cells has been lacking so far. In order to approach this problem, we analyze 112 cases of peripheral T-cell malignant lymphomas (PTMLs) in an HTLV-I endemic area by means of in situ hybridization (ISH) of HTLV-I, employing a biotin-labeled DNA probe synthesized by polymerase chain reaction of a set of primers SK43 and SK44 for HTLV-I proviral DNA pX Tax region. The PTMLs comprised 2 chronic lymphocytic leukemias, 75 pleomorphic lymphomas (PTp), 9 anaplastic large cell lymphomas (ALCL), 12 T-zone lymphomas (T-ZL), and 4 lymphoplasmacytoid cell lymphomas (LCs), and 10 angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) type lymphomas, according to the WHO classification system of lymphomas. In the histological subclassification of T-paleo and ALC into ATL subtypes, clear cell subtype and others (Kasai K, Acta Pathol Jpn 1991 and Padi Res Pract 168, 1991), 44 of 45 cases of the ATL subtype and 7 of 10 cases of the others showed positive ISH-Tax positive lymphoma cells and none of 18 cases of the clear cell type showed positively in lymphoma cells. In 17 cases of T-paleo, in which lymphoma cells were ISH-Tax negative, a small number of ISH-Tax positive cells were noted in the intercellular lymphoid stroma. In the bone marrow of malignant PTMLs, including 9 T-ZLs, 3 LeTs and 3 AILD type, both of lymphoma cells and dendritic cells/epithelial cells were ISH-Tax positive. One of the T-ZLs developed into ISH-Tax-positive T-paleo. In this HTLV-I, this HTLV-I detected mRNA of HTLV-I pX Tax region. In connection with the molecular analysis of HTLV-I by Ysosida M et al (GANN Monograph on Cancer Research 39, 1992), it is considered that this ISH method, maintaining a consistent infection and activation of HTLV-I proviral DNA and ATL may be defined as T-paleo with an activation of HTLV-I proviral DNA. Further comparative studies of T-paleo and low-grade malignant PTMLs with an advancement of HTLV-I proviral DNA will give a clue to the final alteration(s) of factors existing on the ATLL development in patients with HTLV-I infection.

Anaplastic large cell lymphoma in adults: a study of 140 cases with emphasis on T-cell involvement


140 cases of anaplastic large cell (ALC) Non Hodgkin's Lymphoma (NHL) were selected on histological and immunological criteria: 100% of large anaplastic tumoral foci (secondary anaplastic NHL and large cell with anaplastic component were excluded) and expression of the CD3 antigen in at least 50% of the tumoral cells. Among them, 21 cases presented a pleomorphic pattern and were called " borderline forms" with Hodgkin's disease. The study of the phenotype (on paraffin sections) disclosed B origin in 43 cases (31%), T in 37 cases (26.5%), B and T in 3 cases (2%), true histiocytic in 3 cases (2%) and "null" phenotype in 54 cases (37.5%); these latter included the 21 borderline cases.

Comparison with the clinical data of the 1225 non anaplastic large cell patients showed no difference when considering sex ratio (predominance of men: 64 vs 36%), staging (stade I-II: 41 vs 37%; stade III-IV: 59 vs 63%), LDH level, performance status, extra-nodal involvement (even in the mediastinum) except for the skin more often affected (10 vs 4.7%) and the gut less often involved (5.7 vs 16.5%).

The frequency of B and T phenotype was similar in these ALC patients compared to the non anaplastic large cell cases where the B phenotype was largely predominant (74% vs 26%); T. 5% "null". All these patients were included in the same therapeutic protocol (LNH97). The complete response CR rate was higher in the anaplastic group (82.7% vs 61.4%, p<0.05). The 3 years disease free survival (DFS) was 66% without any difference with non anaplastic cohort (3 years DFS=59%).

Univariate analysis of survival demonstrated the favorable influence of anaplastic subtype (3 years survival = 78% vs 51%, p = 0.04). Multivariate analysis of survival permitted also to define 6 favorable prognostic parameters: anaplastic subtype (p<0.001), LDH < 1N (p<0.001), PS 0-1 (p<0.001), albumine > 30g/l (p<0.002), stage I-II (p<0.01) and extranodal involvement (p<0.02). In the anaplastic population, no difference in terms of CR, DFS and survival appeared between B and T subgroups and between the typical anaplastic and the "borderline" cases.

This study demonstrates the favorable influence of anaplastic histology on response to treatment and survival.

Histiocytic sarcomas. A report of eight cases

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In this study, we described eight histiocytic sarcomas (HS), identified by examination of more than 2800 malignant lymphomas. All the tumors were high-grade malignancies consisting of predominantly pleomorphic large cells with many mitotic figures. Six of the cases showed aggressive courses and died from disease 5 to 30 months after diagnosis. The remaining two patients are alive in partial or complete remission 7 and 13 months after diagnosis. Immunohistological examination showed positive reactions for macrophage-related antigens and negative reactions for antigens on B-cells, T-cells, myeloid cells, epithelial cells and melanocytes. T-cell receptor and immunoglobulin genes were studied in three cases and were present in a germline configuration. One of the HS resembled Langerhans cells in phenotype and morphology and was classified as a Langerhans cell sarcoma. The remaining HS did not express accessory cell-associated antigens, but more closely resembled " ordinary" tissue macrophages and were positive for lysozyme and CD88 followed in frequency by CD11c, CD4, CD11b, CD68, PNA and CD13. Osmoprotein p53 was positive in six cases. It is concluded that rare malignancies show features consistent with the derivation from macrophages, including either accessory cells or " ordinary" tissue macrophages. It is possible that p53 is implicated in their pathogenesis and this issue will be an important topic for investigations in the future.
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P 17
CUTANEOUS IMMUNOCYTOMAS: A CLINICO PATHOLOGICAL STUDY OF 26 CASES
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Primary cutaneous immunocytomas (FCI) are rare low-grade cutaneous lymphomas (CCL) characterized by a proliferation in the skin of lymphoid, lymphophasmacytoid and/or plasma cells expressing monoclonal light or heavy chains. In the present study we compared the clinical and histologic data of 16 FCI and 10 secondary cutaneous immunocytomas (S-FCI) in order to find out whether FCI have characteristic clinical and histologic features, allowing differentiation from S-FCI on one hand and other types of CCL on the other. Our data show that FCI are a distinct type of cutaneous lymphomas characterized by 1) the presence of solitary or localized skin lesions, 2) preferential localization on arms and legs, 3) excellent response to local treatment, and 4) a favorable prognosis (1/16 alive, 60% of cases delayed, median follow-up 30 months).

Histologically, FCI are characterized by the presence of nodular or diffuse dermal infiltrates with monomorphic lymphophasmacytoid/plasma cells at the periphery of these infiltrates. In contrast with CCL, patients with FCI generally present with more widespread skin disease: they often have paraproteins and/or autoimmune disorders, and the nonneoplastic tumor cells were usually dispersed throughout the dermal infiltrates. The different preferential localization of FCI (extremities) and primary cutaneous follicular center cell lymphomas (head and trunk) suggests that these two main types of CCL represent separate entities.

P 18
PERIPHERAL T-CELL LYMPHOMA: A CLINICO PATHOLOGICAL STUDY OF 41 CASES AND EVALUATION OF THE PROGNOSTIC SIGNIFICANCE OF THE UPDATED KIEL CLASSIFICATION.

Forty-one non-cutaneous peripheral T-cell lymphomas (PTCL) were classified following the updated Kiel classification. Twenty cases belonged to the low-grade group (T-cell chronic lymphocytic leukemia, 3; lymphoproliferative, 5; angioimmunoblastic, 4; pleomorphic small cell, 8) and 21 to the high-grade group (pleomorphic medium and large cell, 11; immunoblastic, 3; large-cell undifferentiated Kiel-1 positive, 7). Seventy percent showed a 39% de-differentiated phenotype and 88% an activation phenotype. Eighty percent had B symptoms, 63% hepatomegaly, 48% splenomegaly and 26% had involvement of more than three lymphoid areas. Bone marrow was infiltrated in 34%, CNS in 4%, lung in 12% and skin in 14.6%. Seventeen percent presented with extranodal disease and 82.8% had stage III/IV disease. Hypergamaglobulinemia was found in 29%, hypercalcemia in 7%, raised LDH serum levels in 56% and HTLV-I antibodies in only 8%. Eighteen of the 37 tested patients (48%) achieved a complete remission, but 33% relapsed. Mortality was 59% and actuarial overall survival at 38 months 0.32.

In the comparison of the clinical, analytical and immunophenotypic variables and outcome between low and high grade groups, only the average bone marrow infiltration in the low grade and stage I-II, presence of de-differentiated and high Ki67 positivity in the high grade group were significantly different. In the statistical studies, the extracutaneous presentations and the failure to achieve a complete remission were the only variables that influenced mortality, there were no significant differences in the general features of the low and high grade groups and only minor differences were found in the immunohistochemical and clinical groups. There were no differences in the actuarial survival between the low and high grade groups, among the subgroups of the Kiel classification, during stages I to IV, between patients with or without B symptoms, with or without de-differentiated phenotypes, Ki67 positivity or under 60% or among different CD4/CD8 phenotypes. In this study the updated Kiel classification did not separate groups with a prognostic significance.

P 19
CLASSIFICATION OF PRIMARY CUTANEOUS T-CELL LYMPHOMAS OTHER THAN MYCOSIS FUNGOIDES: PROGNOSTIC IMPLICATIONS. R. Millesa, G. L. M. H. Melper, K. C. Baaljanch, Deps. of Dermatology and Pathology, Free University, Amsterdam.

Primary cutaneous T cell lymphomas (CTCL) other than mycosis fungoides and Sezary's syndrome (SS) represent an extremely heterogeneous group, both clinically and histologically. A clinically relevant classification for these lymphomas is not yet available. In the present study, that aimed to achieve a reproducible and clinically relevant classification a large number of potentially important histologic parameters, including histologic subtype according to the updated Kiel classification, tumor cell size, CD30 expression as well as clinical parameters were investigated on 82 patients with a primary CTCL (non-HF/SS).

Multivariate analysis showed as most discriminating parameter CD30 expression on more than 75% of the tumor cells (p<0.0001). Estimated 2- and 4-year survival were 92% and 85% for the CD30-positive group (n=74), and 68% and 25% for the CD30-negative group (n=8), respectively. Within the CD30-negative group significant differences in survival were found between patients classified as pleomorphic small or medium-sized cell type (n=6) with a 2- and 4-year survival of 70% and 50%, pleomorphic large cell type (n=8), as defined by the presence of >200 large blast cells (n=20), with a 2- and 4-year survival of 50% and 12%, and diffuse blast cell/immunoblastic subtype with a 2-year survival of 14% (median survival, 9 months).

The subdivision of primary CTCL other than HF/SS on the basis of CD30 expression and, for the CD30-negative lymphomas on histologic criteria represents the first clinically (prognostically) relevant classification for this heterogeneous group of primary cutaneous T-cell lymphomas.

P 20

Mycosis fungoides is a cutaneous T cell lymphoma characterized by marked epidermotropism of cytologically atypical T lymphocytes with convoluted nuclear contour (Sezary cells or Linteri cells). Skin infiltrating T cells are usually Helper T inducer cell CD2, CD5, CD4, CD8. Occasional cases have been reported to be CD8. The interaction of CD8 receptor (CD25) is infrequently found and CD30 is rarely expressed on the malignant cells. Lymphomatoid papulosis is a self-healing papulosis that is rare, both clinical and histologic presentation and although clinically benign, has histologic features of malignancy with an infiltration of large atypical cells surrounding by inflammatory stroma. CD8 cells have been reported in considerable number of atypical cells. CD25, CD30, CD3, CD8. Approximately 10% of the cases of lymphomatoid papulosis is associated with a malignant lymphoma, usually T cell cutaneous lymphoma, Hodgkin's disease or lymphoma of other types.

We report here a case of a peculiar form of CD25, CD30 and CD8 positive Mycosis fungoides revealed by Hodgkin's disease and associated with lymphomatoid papulosis. A 31-year-old woman presented in December 1990 with cervical lymph node. During the preceding ten years, she had recurrent erythematous papules occurring in crops. Some lesions healed leaving a scar. On examination, cervical, axillary and inguinal lymph nodes were found and biopsy revealed nodular sclerosis Hodgkin's disease. A computed tomography scan showed latero-aortic lymphadenopathy. Polychemotherapy (MGDP, Medichim, NV.C., Yacine) and Prednisone and ABVD (Adriamycin, Bleomycin, Vindecin and Dacarbazine) with mantle and jumbo-atic field irradiation resulted in a complete response. In march 1991, the patient noticed pruritus, degeneration of the trunk and a papulonodular cutaneous eruption over the whole body. Some of these nodules developed necrosis and healed within a few weeks. The histologic examination of necrotic nodules showed lymphomatoid papulosis. Biopsy of degenerated area revealed mycosis fungoides. The patient was treated with topical corticosteroids and scinithrihogen with excellent effect and ultra violet light therapy was poorly tolerated. In December 1991, a CT scan of the chest and the abdomen confirms complete remission. The cutaneous papular and nodular lesions however continues to appear and to resolve spontaneously.

This association of lymphomatoid papulosis, cutaneous lymphoma and Hodgkin's disease is unusual: only one observation was found in the literature (N Engl J Med 1992 ; 326 - 1151-22). The immunophenotype of the primary cutaneous T-cell lymphomas (CD25, CD30, HLA-DR) are identical and common clonality is confirmed by an unique T-cell receptor gene rearrangement (T-cell chain) in both cases. Despite a different histologically appearance, this strong hypothesis of an occult, anormal T cell clone which can progress to lymphomatoid papulosis and lymphoma.

Centrocytic lymphoma/internodally differentiated lymphocytic lymphoma (CC/DL) is a Non-Hodgkin's lymphoma (NHL), presumably derived from follicular mantle B-cells. A translocation (t(11;14)(q13.31q23)) involving the bcl-1 locus, has been described in about half of these lymphomas, but also in some CLI, PLL and myeloma/plasma cell leukemias. The histopathological classification of CC/DL can be difficult. To distinguish CC/DL from other NHL's, and to get better insight in the heterogeneity of breakpoints within the 11q33 region, we are evaluating rearrangements in the 11q33 region and the expression of the involved CCND1/PRAD1 genes. We and others cloned the CCND1/PRAD1 genes, that is often overexpressed in (breast, head and neck) cancer with amplification of the 11q13 region. Using Pulsed Field Gel Electrophoresis, CCND1/PRAD1 is the gene most proximal to the bcl-1 major breakpoint cluster (bcl-1/MTC) at a distance of 120 kb. Using 4 available probes covering approximately half of this 120 kb region, we detected rearrangements in 10 out of 30 cases of CC/DL, and in 1/3 morphologically similar (low grade) B-NHL, 2/7 cases of chronic B-cell leukaemia with small cleaved cells, 1/4 B-PLL and 1/5 plasma cell leukaemia. The single case of ana-CC/DL NHL with a rearrangement was a leukemic immunoblastoma with circulating cleaved cells, also previously reported as a single case with a bcl-1/MTC breakpoint in a series of 44 chronic B-cell leukaemias (Blood 91[7]:1560-4). Bcl-1 breakpoints were found over the whole area: 7 within bcl-1/MTC, 2 within a region 34 kb distant from bcl-1/MTC, and 1 case 2 kb from CCND1/PRAD1. In all cases with an 11q33 breakpoint, consignation with 2H reporter proved (CL144). It has been suggested that in lymphomas with a rearrangement, the CCND1/PRAD1 gene is overexpressed, whereas it is not expressed in lymphoma without a rearrangement. Our first results on a few cell lines show correlation for the (t(11;14) and RNA/epitope). Presently we are studying protein expression of CCND1/PRAD1 using polyclonal antibodies.


It remains controversial whether mantle cell lymphoma (MCL), alternatively called intermediate lymphocytic lymphoma or cent rocytic lymphoma, constitutes a discrete disease entity, and, if so, whether it is properly regarded as a lymphoma of low or intermediate clinical grade. We analyzed 41 unrelated patients presenting at MDACC with this diagnosis from 1986 to 1992. The histology was reviewed by an expert hematopathologist (W. P.), and the cases were segregated according to growth pattern as follows: 61% diffuse (DIF), 27% Mantle Zone (MZ), and 12% nodular (NOD). Immunohistochemical studies showed 68% of cases were CD5 antigen (+), and 56% lambda light chain (+). Bcl-1 gene configuration was examined in 18 cases. Rearrangement (R) of the gene was present in 42% of DIF, 50% of NOD, and 25% of MZ cases. The obtained characteristic of the patient's cases were as follows: median age=54 years; gender=male; Ann Arbor stage IV=46%, B symptoms=12%. Disease in extranodal sites (ENS) was very common (80% of pts.). The most frequent ENS were bone marrow (68%) and GI tract (25%). Involvement of ENS was more frequent in the DIF and NOD cases (96% and 60% respectively), compared to M2 (60%).

The treatment responses and survival for the patients according to histologic pattern is summarized in the table below.

<table>
<thead>
<tr>
<th>Histology Pattern</th>
<th>Total no. pts</th>
<th>No. treated with Adr+CT</th>
<th>%R or Adr+CT</th>
<th>5y Survival</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse</td>
<td>25</td>
<td>17</td>
<td>29</td>
<td>55</td>
<td>0.96</td>
</tr>
<tr>
<td>Nodular</td>
<td>5</td>
<td>4</td>
<td>25</td>
<td>24</td>
<td>0.04</td>
</tr>
<tr>
<td>Mantle Zone</td>
<td>11</td>
<td>9</td>
<td>77</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*Adr+CT=Adriamycin-containing Chemotherapy

The clinical behavior of MCL correlates with its histologic pattern. The DIF and NOD patterns had more extensive disease at presentation, treatment response, and poor survival.

DIFFUSE SMALL B-CELL LYMPHOMAS: PROGNOSTIC VALUE OF MORPHOLOGIC SUBDIVISIONS. F. Berger, P. Fasman, A. Sonnet, Y. Baston, G. Tharaux, P.A. Bryon, B. Collier, Service d'hematologie, Centre Hospitalier Lyon-Sud, Pierre-Benite and Hopital Eduoard Herriot, Lyon, France.

140 patients (pts) with diffuse small B-cell lymphoma (L) referred to our center for diagnosis and/or treatment between 1989 and 1992 were reviewed to define the clinical characteristics and the survival associated with each morphologic subtype. Some clinical and laboratory characteristics were more accurate at the time of relapse but all initial materials were reviewed.

57 pts had a small lymphocytic or lymphoplasmacytoid (SLL) (L; SLL excluded; 17 pts had a polytropic immunocytoma (Pt); 40 pts had a mantle cell (L; MCL) and 20 pts had an unclassifiable L mainly based on the presence of one or several of all subtypes. 1 pt with a monocytoid L, 2 pts with a plasmoid L with villous lymphocytes, and pts with macroglobulin associated lymphoid tissue were excluded.

The main clinical characteristics of each subtype are presented in the following table.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
<th>Older than 60 y</th>
<th>BM involvement</th>
<th>Large spleen</th>
<th>High LDH level</th>
<th>High JH3 monoclonal</th>
<th>CRI rate</th>
<th>Median FFP survival</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLL</td>
<td>57</td>
<td>38%</td>
<td>65%</td>
<td>43%</td>
<td>33%</td>
<td>51%</td>
<td>37%</td>
<td>66</td>
<td>120</td>
</tr>
<tr>
<td>MCL</td>
<td>17</td>
<td>46%</td>
<td>63%</td>
<td>35%</td>
<td>31%</td>
<td>60%</td>
<td>47%</td>
<td>15</td>
<td>58</td>
</tr>
<tr>
<td>Unclassified</td>
<td>40</td>
<td>50%</td>
<td>84%</td>
<td>57%</td>
<td>44%</td>
<td>52%</td>
<td>39%</td>
<td>27</td>
<td>83</td>
</tr>
</tbody>
</table>

Adverse prognostic factors for FFP and overall survival were high JH3 monoclonal level (p < 0.001), Pt or MCL subtypes (p = 0.01), age older than 60 (p < 0.01), high LDH level (p < 0.05). Stage, localizations, and type of treatment were not associated with survival. This retrospective analysis shows (1) that lymphoplasmacytoid L pts those with a Pt had a poorer outcome; (2) that MCL pts had a poorer outcome than SLL pts; and (3) the most important prognostic factors for these pts whatever the subtype is the JH3 monoclonal level.

INCIDENCE, PRESENTATION FEATURES AND PROGNOSIS OF LOW-GRADE NON-HODGKIN'S LYMPHOMAS. F. d'Amore, on behalf of the Danish Lymphoma Study Group, LYFO. Dept. of Haematology, Odense University Hospital, 5000 Odense C, Denmark.

In the period 1.1.83-31.12.88, 1597 newly diagnosed cases of non-Hodgkin's lymphoma (NHL) were included in a Danish population-based NHL registry. Of these, 31% (N=496) were low-grade NHL (LG-NHL) and distributed as follows (Kiell): 9% lymphocytic type (LY), 27% lymphomas-plasmacytic-foillary type (LC), 53% follicular centroblast-centrocytic type (CB/CCI) and 11% unclassifiable low-grade.

LG-NHL had an age range of 26-94 years (median: 64 years), an M/F ratio of 0.8 and a stable age-standardised incidence rate of 2.7/100,000 year. Age-specific IR's show an exponential rise as a function of age in all subtypes except for CB/CCI, which had a significantly lower median age (61.6). Compared with the Intermediate (IG) and high-grade (HG) group, LG-NHL had more female cases (M/F ratio: 0.79 vs 1.2; p=0.0002), a higher frequency of stage III-IV disease (66% vs 53%; p=0.00005) and of bone marrow involvement (39% vs 19%; p=0.00005). Biochemically, para-proteinemia was more frequent in LG-NHL than in IG- and HG-NHL (p<0.00005), whereas the latter groups had more cases with sLDH elevation (p=0.00005).

A later revision of all IC cases (N=132) distinguished 79 non-polymorphic (ICNg) from 25 polymorphic (ICp) cases; 28 cases were otherwise classified. In 34 LG-NHL patients histologic conversion to a higher malignancy grade was established. The most frequent transformation patterns were: CB/CCI to CB (diffuse 12 pts, M/F ratio=0.38) and LY to immunoblastic or CB type (6 pts, M/F ratio=1).

The 7-year survival for LG-NHL was 65% (intermediate: 48%; high-grade: 38%; p=0.00005). A Cox-regression analysis identified following adverse factors for cause-specific survival in LG-NHL: age > 50 with a relative risk (RR) of 3.2, hepatic involvement (RR=2.1), elevated sLDH (RR=1.9), B-symptoms (RR=1.8) and IC histology (ICn>ICp) (RR=1.7). ICp had a lower 7-year survival than ICn (p=0.00454). A univariate analysis performed on young LG-NHL patients (sLDH 50 years), where the large majority of cases (79%) had a CB/CCI histology, identified hyperuricemia, n. of extranodal sites, hepatic involvement, elevated sLDH, B-symptoms and splenic involvement as high risk factors.
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P 25 THE FOLLICULAR NON HODGKIN'S LYMPHOMAS - 3: PROGNOSTIC FACTORS AND STAGING.
J W Denham, G Vaughan-Hudson, B Vaughan-Hudson, M H Bennett, A M Jeffs, R R Pratt, and E E Denham, for the British National Lymphoma Investigation

The records of 398 patients with Follicular Non Hodgkin's Lymphoma followed for a minimum of 12 years, who were entered into the British National Lymphoma Investigation Trials between 1974 and 1980, have been reviewed to determine what factors independently influence prognosis and whether more satisfactory alternatives to the Ann Arbor staging system are implementable.

Factors that are patient related (age and sex) disease subtype related (histological classification) and disease stage related (number and distribution of lymph regions involved, marrow involvement, the presence of splenomegaly and constitutional symptoms) were examined to define their independent influence on probability of complete response to therapy, probability of relapse free survival and probability of dying from lymphoma.

Of the patient and disease subtype related variables only increasing age of the patient was found to have an independently significant adverse influence on probability of complete response to treatment, relapse free and cause specific survival. Of the disease stage related variables only increasing number of lymph node regions was found to have a similarly significant adverse influence in all subgroups of patients.

The Ann Arbor staging classification fared poorly, minimally separating relapse free and cause specific survival probabilities in patients with the largest staging groupings III and IV in particular.

Simple classifications based on a simple count of lymph node regions involved and the presence of splenomegaly were far more successful in subdividing the series into subgroups of meaningful size with significantly different probabilities of responding completely to therapy as well as relapse free and cause specific survival expectations.

P 26 RESIDUAL MASSES AFTER TREATMENT OF LYMPHOMA: EFFICACY OF MAGNETIC RESONANCE IMAGING IN PREDICTING RELAPSE. MEHEU JD, MacVicar, S Milton, J Husband, T Hickish, R McCready, J Mami and D Cunningham.

Lymphoma Unit, Royal Marsden Hospital, Sutton, Surrey, UK.

Residual masses evident on CT scanning after treatment of lymphoma are frequently observed. If such a mass contains residual active lymphoma then additional therapy may prevent relapse at this site. However, if there is no residual disease, additional treatment is unnecessary. Previous studies have demonstrated that plain radiology and CT scanning are unable to differentiate residual disease from lymphoma, and that needle biopsy, enzymatic sedimentation rate (ESR) and Gallium-67 single photon emission computed tomography (Ga-67-SPECT) have variable predictive value. Ga-67-SPECT has however been regarded by many as the best available investigation in this situation, but very few studies have compared its efficiency with that magnetic resonance imaging (MRI). MRI has the potential to discriminate between fibrosis and lymphoma since active malignant tissue has been reported to have different signal characteristics to both normal tissue and fibrosis.

We have therefore performed this prospective study comparing the efficacy of Ga-67-SPECT and MRI in predicting relapse in this setting. A total of 34 patients have been studied, 21 with Hodgkin's disease and 13 with non-Hodgkin's lymphoma. All had MRI within 3 months of completing treatment and the majority had Ga-67-SPECT and ESR during the same period. The ESR was taken as positive for residual lymphoma if the level was >30 mm in the first hour and all MRI and Ga-67-SPECT images were similarly ascribed as either positive or negative.

During the first year 11 patients relapsed within the area of the residual mass. The MRI had been positive at the time of completion of treatment in 5 of those patients, ESR positive in 4 and Ga-67-SPECT in 3 in 2 of the 11 did not have Ga-67-SPECT. The number of false positives was 2, 3 and 1 respectively. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for the three tests were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>45</td>
<td>33</td>
<td>36</td>
<td>84</td>
</tr>
<tr>
<td>Ga-67-SPECT</td>
<td>90</td>
<td>93</td>
<td>84</td>
<td>93</td>
</tr>
<tr>
<td>ESR</td>
<td>75</td>
<td>68</td>
<td>70</td>
<td>71</td>
</tr>
</tbody>
</table>

Using the log rank test MRI was found to be the only investigation in which there was a significant difference between the probability of relapse at the site of the residual mass for a positive and negative result (p=0.013). This effect was most marked in patients with Hodgkin's disease and with improving residual masses above the diaphragm. Combining results of investigations did not improve predictive power.

We conclude that MRI is a valuable investigation which is as least as good as, and probably superior to, Ga-67-SPECT in this setting, capable of providing clinically relevant prognostic information.

P 27 COMPUTED TOMOGRAPHY ASSESSMENT OF RATE OF REGRESSION AND RESIDUAL MEDIASTINAL MASSES IN HODGKIN'S DISEASE. LF Diehl, G Petrini, TH Wasserman, KD Hopper, S Sagel, A Gottlieb, B Peterson.

Cancer and Leukemia Group B, Lebanon, NH, USA 03768

Residual mediastinal masses after treatment of bulky mediastinal Hodgkin's disease are an important treatment problem. The central therapeutic problem is whether a residual mediastinal mass represents residual active disease or fibrosis. Chest radiograph studies, gallium studies, computed tomography (CT), magnetic resonance imaging and even biopsy studies have failed to define the meaning of a residual mediastinal mass. Computed tomography demonstrates more detail and is a readily available method to measure an anterior mediastinal mass. In CALGB 8551, 59 eligible patients with Stage III, III and IV Hodgkin's disease and bulky mediastinal masses (mass-to-dia > 0.33) were treated in an identical manner. All patients were treated with 4-6 cycles of MVPP (nitrogen mustard, vinblastine, procarbazine, prednisone), followed by 2500 cGy to the mediastinum, followed by 4 more cycles of MVPP at 1.5 times the dose of cycle #6. In 20 patients, CT were performed pre and post treatment enabling us to study the problem of residual mediastinal masses in this advanced stage, bulky mediastinum, identically treated group. A total of 76 CT were performed. Of these 20 patients, 4 have died and 5 have relapsed. Mass size was measured as the largest diameter of the anterior mediastinal mass. A logarithmic transformation of tumor size and least squares method were used to model the rate of regression. Cox's proportional hazard model was used to examine whether the tumor regression rate was associated with time to relapse or survival. Spearmann's rank correlation was used to estimate the correlation between rate of regression, initial size and patient age. Rates of regression were not significantly different between relapsing and non-relapsing patients. Forty five percent (18/19) with an no post chemotherapy CT of patients had residual masses after chemotherapy. Only one patient had no residual mass after chemotherapy. Neither tumor regression nor baseline tumor size were found to be significantly (p > .15) associated with time to relapse or survival. A negative correlation was detected (p<0.01) between the initial tumor size and the rate of regression (the larger the initial tumor, the greater the rate of regression). In summary, the data indicate that a larger mediastinal mass regresses more rapidly than a smaller mass, and to date, there is no evidence of an association between rate of regression and time to relapse or death. In patients with bulky mediastinal masses, residual mediastinal masses are almost always present after chemotherapy and they do not seem to predict for relapse or survival.

P 28 THE GALLIUM SCAN (GS) PREDICTS RELAPSE IN PATIENTS WITH HODGKIN'S DISEASE (HD) TREATED WITH COMBINED MODALITY THERAPY. FB Hagemeister, L Fuller, MA Rodrigue, M McLaughlin, F Swan, JE Romaguera, F Cabanillas. U.T. M.D. Anderson Cancer Center, Houston, Texas 77030.

We have treated 79 evaluable patients with CS-I1 and 27 with PS or CS IIIA or B HD with three cycles of NOVAP (Novantrone 10mg/m2 IV d1, Oncovir 1.4 mg/m2 IV d4, vinblastine 8mg/m2 IV d1, prednisone 100mg po qd d5-qcd14, given every 21 days). Followed by radiotherapy (XT) to the mantle and upper abdomen (Stage I-II), or the thorax and upper abdomen (Stage III). Staging methods included CT of the chest, abdomen, and pelvis, lymphangiogram, and high-dose GS (6-10 millicurie) with SPECT Imaging of selected nodal areas, including the upper thorax and primary sites of disease. After three cycles of NOVAP were given, and prior to XT, 42 patients had a repeat GS. Two-year (YR) freedom from progression result for stage I-II was 87%, and 2-YR overall survival was 99%, corresponding results for stage III were 82% and 100%, respectively.

Prognostic factors for analysis included the presence of a large mediastinal mass (LMM), hilar involvement, B symptoms (SX), efferent nodal mass > 10cm, and I1 disease. By univariate analysis, no pretreatment factor was an important predictor of results. However, we also evaluated the potential impact of GS positivity (+) before XT in determining the chance of relapse. For PT with a GS (+), there was a marked decrease of GS uptake after NOVAP, but I1 residual was still detected. Characteristics of the 42 PT according to GS results are shown:

<table>
<thead>
<tr>
<th>Feature</th>
<th>PT NO</th>
<th>GS (+) (%)</th>
<th>GS (-) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>42</td>
<td>10 (24)</td>
<td>32 (76)</td>
</tr>
<tr>
<td>Stage I-II</td>
<td>36</td>
<td>10 (28)</td>
<td>26 (72)</td>
</tr>
<tr>
<td>No B SX</td>
<td>36</td>
<td>8 (22)</td>
<td>28 (78)</td>
</tr>
<tr>
<td>LMM</td>
<td>22</td>
<td>5 (23)</td>
<td>17 (77)</td>
</tr>
</tbody>
</table>

However, by Kaplan-Meier curves, none of the 32 PT with a GS (+) after three NOVAP have had progressive disease compared to two of the 10 with GS (+) (P = .01). We propose that a GS (+) after treatment with three NOVAP, before XT, may predict relapse, even though the CT shows marked improvement, and the PT receives XT to all known sites of disease.
P 29

Gallium (Ga) scanning as an indicator of survival in Hodgkin's Disease.
Joseph M. Bone & John A. Gibson and R. Kronenberg.
Department of Haematology, Royal Prince Alfred Hospital, Sydney Australia.

Previous studies of the utility of gallium in the management of Hodgkin's disease have either used inferior techniques or have concentrated on the ability to detect, stage disease, rather than evaluate the prognostic impact of the study on ultimate patient outcome.

In order to determine whether gallium uptake could be useful in monitoring patients who have been treated for Hodgkin's disease and are in partial remission, 28 patients were studied using technetium-99m-labeled Ga-67 citrate. Of these patients, a mean of 42 months and 1 patient died. The results were not influenced by the presence of a residual anatomical (mediastinal and abdominal) mass.

P 30

SOMATOSTATIN RECEPTOR SCINTIGRAPHY (SMS) IN THE INITIAL STAGING OF HODGKIN'S DISEASE. J.P. van der Aker-Lugtenburg, E.P. de Kruif, H. van der Veer, J.J. Gerits, S.W.J. Lamberts and B. Looijenbergen.
Department of Haematology, Erasmus University, Rotterdam, The Netherlands.

A variety of human neoplasms express somatostatin receptors. Laboratory and in vivo studies have suggested that somatostatin receptor-positive tumors may respond to somatostatin analogues and analogues may be useful in vivo as radiolabeled somatostatin analogues can be used to localize and treat somatostatin receptor-positive tumors in vivo with a gamma-camera. We performed a prospective study comparing somatostatin receptor scintigraphy with conventional staging imaging tests in patients with Hodgkin's disease. The results of the initial staging of patients with historically proven Hodgkin's disease. Conventional staging procedures included tomography of chest and abdomen, bone marrow aspiration and histology and sometimes lymphography. Twenty consecutive newly diagnosed patients underwent gamma-camera scintigraphy and injection of the radiolabeled somatostatin analogue, 111In-DTPA-D-Phe1-octreotide. Planar and single photon emission computed tomography (SPECT) images were obtained at 24 and 48 hours after injection. SMS and conventional diagnostic tests were interpreted independently and the results compared.

In conclusion, radiolabeled somatostatin analogues can be used to localize and treat somatostatin receptor-positive tumors in vivo with a gamma-camera. We performed a prospective study comparing somatostatin receptor scintigraphy with conventional staging imaging tests in patients with Hodgkin's disease. The results of the initial staging of patients with historically proven Hodgkin's disease. Conventional staging procedures included tomography of chest and abdomen, bone marrow aspiration and histology and sometimes lymphography. Twenty consecutive newly diagnosed patients underwent gamma-camera scintigraphy and injection of the radiolabeled somatostatin analogue, 111In-DTPA-D-Phe1-octreotide. Planar and single photon emission computed tomography (SPECT) images were obtained at 24 and 48 hours after injection. SMS and conventional diagnostic tests were interpreted independently and the results compared.

P 31

CLINICAL RELEVANCE OF IMMUNOPHENOTYPIC SUBCLASSIFICATION OF NON-HODGKIN'S LYMPHOMA (NHL). P. Pasquini, L. Cattone, L. Venelli, T. Gaudio, A. Gandolfo, M. Bonetti, G. Castelli, E. Morita, M. Lazzario, E. Brusamolin, G. Bernasconi, Cattedra di Ematologia, Università di Pavia - Divisione di Medicina Interna, Policlinico S. Mauro IRCCS, 27100 Pavia, Italy.

To determine the significance of the immunophenotypic heterogeneity of B-cell chronic lymphocytic leukemia we prospectively studied 84 consecutive B-CLL patients observed for a 5 year period (January 1988 to December 1992), with a large panel of monoclonal antibodies detecting B cell markers: CD19, CD20, CD22, CD23, T-cell markers: CD2, CD3, CD4, CD8, CD5, CD10 (CD21) and B-cell markers: CD40, CD45, CD10, CD19, CD22, CD5 and CD19.

P 32

QUANTITATIVE ANALYSIS OF STATIN EXPRESSION BY FLOW CYTOMETRY (FC) IN NORMAL AND B CELL CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) LYMPHOCYTES. S.N. Caplan, C.E. Caplan, M. Trudel, E. Wang, Jewish General Hospital & Lady Davis Research Institute, McGill University, Montreal, Canada.

Statin is a 57 kDa nuclear protein first identified in growth-arrested fibroblasts and exclusively found in non-replicating (Go-phase) tissues. Using the monoclonal anti-statino (MoAb) S44, we have previously identified and localized statin in normal and abnormal lymph nodes, tonsil, peripheral blood lymphocytes (PBL) and rat spleen by immunohistochemistry (Lab Invest 64: 85a). Since conventional techniques (thymidine labelling and DNA analysis) indicate a very low proliferative index in PBL of most mice with CLL, we investigated whether quantitative expression of statin serves as a differential marker of proliferation in CLL. To assess quantitative expression of statin in normal and leukemic PBL, a membrane permeating technique using buffered formamide-acetone (BFA) to determine nuclear statin expression by FC was utilized. A monoclonal anti-DNA and a polyclonal anti-Id antibody served as positive and negative controls for the BFA fixation technique. BFA-fixed cells from U937, a monoclonal cell line allowed an increase of statin expression as measured by FC from 19% to 47% after 48 hours of serum deprivation. PBL from normals (N=11), B-cell CLL (N=19) and T-cell CLL (N=7) were studied following co-labelling with pan-B (CD19) and pan-T (CD3) MoAbs and the following results obtained:

- % Statin-positive
  - Normal: 9.7 ± 4.6
  - B-CLL: 68.3 ± 9.2
  - T-CLL: 15.3

Unlike normals where nearly all B cells expressed statin, B-CLL cell expression was highly variable from patient to patient (14-48.85%). In no case did the percent expression overlap with that of normal B cells. Statin expression was not correlated with clinically relevant prognostic variables including stage, previous treatment, lymphocyte count or doubling time, splenomegaly, or time from diagnosis. These data indicate that, unlike normal B cells which variably express statin, B-CLL cells have a variable but lower expression indicative of an inherent capacity for abnormal proliferation.
ABSTRACTS - Fifth International Conference on Malignant Lymphoma, Lugano

P 33 SERUM HUMAN AND VIRAL IL-10 IN PATIENTS WITH NON HODGKIN'S LYMPHOMA. J.Y. Blay, N. Burdin, F. Rousset, G. Lerhien, P. Brion, M. Favrot. Centre Léon Bérard, rue Laennec, 69008 Lyon, France

Serum levels of human and viral (EBV) IL-10 were measured in 184 patients with non HIV-related non Hodgkin's lymphoma (NHL) including 112 patients with active disease, 42 patients in first partial remission (PR), 30 patients in first complete remission (CR), as well as 60 healthy blood donors. IL-10 was detectable in 46 (42%) of the 112 patients with evolutive NHL, 2 of 42 (5%) in first PR, 1 of 30 (3%) in CR and in none of the 60 blood donors. In 13 patients in whom sequential serum IL-10 determinations were performed, IL-10 was detectable in sera collected at diagnosis and/or relapse but not while in partial or complete remission.

IL-10 was identified as vIL-10 and HIL-10 in respectively 22 and 24 of the 46 patients with active NHL. vIL-10 was detected only in patients with detectable anti-EBV antibodies whereas IL-10 was observed with a similar frequency in EBV seropositive and negative patients. The presence of IL-10 in serum was observed with a similar frequency in all histological subtypes of NHL according to the WHO classification as well as in T and B lymphoma. Serum IL-10 was found not correlated to serum LDH or beta2-microglobulin, age, PS or clinical stage. Among intermediate or high grade NHL patients with detectable IL-10 at diagnosis had a significantly shorter overall (p=0.025) and progression free (p=0.033) survival. Presence of IL-10 was inversely correlated to survival both in adults and children. Serum IL-10 was associated with a particularly poor prognosis among patients with stage IV disease (4 years survival: 8% vs 65% for patients without IL-10, p=0.00004). Multivariate analysis indicated that serum IL-10 was an independent prognosis factor. These results indicate that serum IL-10 is increased in patients with NHL and correlates to the presence of an active disease. The prognostic value of serum IL-10 suggests that these cytokines play a role in disease progression. Ongoing studies are evaluating the cellular source of IL-10 production in these patients.

P 34 CLINICAL RELEVANCE OF SERUM CYTOKINE LEVELS IN NON-HODGKIN'S LYMPHOMAS. M. Cantosetti, E. Abruzzese, S. Felici, G. Papa, et al. Division of Hematology, Ospedale S. Eugenio, Rome, Italy.

Recent reports have suggested a clinical significance for pretreatment circulating levels of certain cytokines and the soluble forms of membrane antigens in non-Hodgkin's lymphomas (NHL). This prompted us to investigate a systematical verification of serum levels of many of these molecules in newly-diagnosed patients with NHL. Sixty-eight patients were studied. Their median age was 36 yrs (range 19-60; 39 were males). In all cases histology was revised according to the Working Formulation. 49 were classified as Immunobiological Lymphomas (5 subtype, WF) and 29 as Large Cell Lymphomas (12 subtype, WF). Serum cytokine levels were at least one of the systemic B symptoms (fever, weight loss, night sweats). HIV seropositivity was present in 2. The results showed statistically significant higher average levels of interleukin-6 (IL-6), interferon-alpha (IFN-α) and IL-8, the soluble form of the receptor for interleukin-2 (sIL-2R) and the soluble transferin receptor (sPTC) in NHL patients compared to controls (p<0.01, p<0.05, p<0.0001 and p<0.03 respectively). sIL-2R was found more elevated in stages III/IV than in stages I/II (p=0.013), whereas IL-6 concentrations were higher in patients presenting B symptoms (p<0.008). Significant correlations were found between erythrocyte sedimentation rate (ESR) and IL-6 (r=0.75), and between B2-m and sIL-2R (r=0.71).

Our results outline the utility of these measurements in the initial staging of NHL. IL-10 was found significantly to correlate with the clinical stage and B2-m. Elevated plasma levels of IL-6 are significantly associated with the presence of B symptoms and correlate with KPS. Many patients with lymphoma often suffer from fever, weight loss and night sweats. These symptoms are reversible, in many ways a chronic inflammatory disease and may be induced by cytokines such as IL-6. It would be interesting to investigate whether the elevated levels of IL-6 may be associated with a hypervascularization of the lymphoma mass.

P 35 Immunohistochemical detection of P-glycoprotein drug resistant gene (MDR) and response to chemotherapy in aggressive non-Hodgkin's lymphoma.


Benh. Cairo, Egypt.

The frequency and clinical significance of P-glycoprotein immunoreactivity of MDR gene in 46 previously untreated aggressive NHL patients were assessed. All patients completed a standard BEOP regimen. Paraffin-embedded tissue was retrieved from 130 samples which were treated for reactivity with MDR-1 monoclonal antibody that were recognized cell membrane domain using ABC immunoperoxidase technique. Reactivity to antibody was categorized into strong (>30% +ve cells), or moderate (11-30% +ve cells), and negative (<10% +ve cells). Positivity appeared as a rim of membranous reaction.

A total of 12/46 (26%) of cases were positive for membranous reaction with variable intensities and count among cases, while the remaining 34 cases (74%) were negative for reaction. The pattern and type of reaction did not relate to any specific histopathologic type or grade according to the World Health Organization.

Patients with no complete remission to BEOP regimen (n=16) those having partial remission, stable disease, and increasing disease were positive for reaction 5/7, while those showing complete remission to therapy were negative in 30/33. Six patients were excluded due to death from drug toxicity or lost for follow up.

Results showed a test specificity of 71.4% and a sensitivity of 90.9%. This denotes that the predictivity of a negative result is high (93.76%). However, the predictivity of a positive result is lower (62.5%).

P 36 17p ABNORMALITIES IN LYMPHOMA MALIGNANTIES: DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS. C. Schu, H. Hildebr, Ch. Fehnstr, Arbeitsgruppe Tumornzytogenetik, Institut für Humangenetik, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany.

Recently, quite a lot of studies have been performed concerning mutations on the molecular level of the p53 gene, which has been mapped to the short arm of chromosome 17, band p13. However, only little is known about cytogenetic abnormalities of 17p in lymphoid malignancies. In routine work we found abnormalities of 17p in tumor material of 11 patients with Non-Hodgkin lymphoma (1 Richter-Syndrom (immunobiological lymphomas emerged from CALL), 1 centroblastic lymphoma from a centroblastic-centrocytic lymphoma, 2 Burkitt's lymphomas) and acute lymphoblastic leukemia (2 Burkitt's type ALL, 1 pro-ALL, 1 pre-ALL, 1 T-ALL, 1 pro-TALL, 1 pre-TALL). The abnormalities were found in low grade lymphomas or chronic leukemias. A strikingly high proportion of Burkitt's lymphoma/leukemias (4/11) with one of them being a Burkitt's type ALL showing a p53 mutation rate in Burkitt's type ALL. p53 abnormalities were found in a positive correlation between myc and p53 shown in a mouse cell line. Remarkably the hypothesis of cooperation between myc and p53 was not confirmed in the present study. We did not find any rearrangement of 17p in the most frequent type of ALL CALL. Data on a relatively high percentage of the less widespread T-ALL has to be mentioned, that in two of three T-ALL cases the 17p rearrangement was the sole cytogenetic abnormality, whereas all other cases showed additional chromosomal aberrations. There is evidence, that p53 mutations occur later in the course of a malignant disease and are associated with progression in a more aggressive form. Concerning the t(11;14) a different role of 17p abnormalities and of p53 mutations could be discussed. Abnormalities of chromosome 17 in lymphoma show a poor clinical outcome, the special role of rearrangements involving 17p has not been analysed up to now. The diagnostic and prognostic implications of 17p abnormalities in lymphoid malignancies are discussed.

Abnormal p53 expression has been extensively reported in a variety of human malignancies such as carcinomas, sarcomas, lymphomas and a few cases of HD. Since normal p53 is undetectable using standard immunocytochemical techniques, overexpression is usually considered as suggestive of genomic mutation. We investigated the abnormal accumulation of p53 protein in 35 cases of HD using monoclonal antibody PAb 1801 on frozen sections. We found immunocytochemical expression of p53 protein in 11 out of the 35 cases. Positive cases were of mixed cellularity and nodular sclerosis types. The staining was mainly nuclear and was restricted to the Reed-Sternberg cells and their variants.

Analysis of P53 mutations was performed in 9 cases showing positive staining and in 3 negative cases. Exons 5-6, 7 and 8 of the p53 gene were separately amplified by PCR, using genomic DNA extracted from each sample. Amplified products from HD lesions were hybridized with labelled wild-type P53 products amplified from normal thymocytes, and then analyzed by chemical cleavage with the hydroxylaminolysis-tetrazolium technique, which can detect single-base pair mismatches.

P53 mutations were identified in none of the 12 HD cases, but were present in the positive staining cell lines. We conclude that (i) P53 overexpression is a common event in HD (ii) immunohistochemical positivity in HD seems not to correlate with the presence of gene mutations. This latter point may be explained either by a phenomenon of deregulated transcription without structural alteration of the P53 gene or by the fact that the percentage of RSC which are supposed to harbour p53 mutations is under the threshold level of sensitivity of our experiments.

P 38 ABNORMAL P53 PROTEIN EXPRESSION IN HODGKIN'S DISEASE. A.F. Lauritsen, K. Bou-Jensen, E. Ralfkiaer. Departments of Pathology, Herlev Hospital and Rigshospitalet, University of Copenhagen, Denmark.

P53 is an onco-suppressor gene which is located on chromosome 17. Mutations of the p53 gene are closely associated with malignant transformation under "in vitro" conditions and are the most common genetic alteration in human malignancy. Unlike normal p53 protein which is unstable and undetectable by immunohistochemistry, p53 protein shows a decreased cell turnover rate and overexpression as compared to the wild type protein. In this study a panel of four anti-p53 antibodies was applied to cryostat sections and routine samplings of 25 cases of Hodgkin's disease (i.e., 3 lymphocytic predominance, 3 nodular sclerosis, 10 mixed cellularity and 9 lymphocyte depletion). The results show that abnormal p53 is present in Hodgkin's- and Reed-Sternberg cells in more than 80% of the cases. It is suggested that mutations of the p53 gene are frequent in Hodgkin's disease and may be implicated in the pathogenesis of this disease.

P 39 CHROMOSOME ANALYSIS OF NON-HODGKIN'S LYMPHOMAS BY FLUORESCENCE IN-SITU HYBRIDIZATION. D.W. Hammond, B.W. Hancock and M.H. Goyns. Department of Clinical Oncology, Institute for Cancer Studies, University Medical School, Sheffield, S10 2RX, UK.

We have recently completed a cytogenetic survey of a series of 40 non-Hodgkin's lymphomas (NHL) [Hammond, 1992, Cancer Genet. Cytogenet., 61, 31-38]. From this study it was apparent that, even with sufficient numbers of good quality metaphases, there are limits to the analysis when conventional cytogenetic methods were used. The origins of derivative and marker chromosomes were uncertain, and submicroscopic rearrangements could not be identified. We have therefore adopted the technique of fluorescence in-situ hybridization (FISH) to further analyse our NHL karyotypes. The use of unique sequence probes has allowed the presence of rearrangements to be investigated. A third of our samples exhibited a deletion of the q arm of chromosome 8, which is where the MYB proto-oncogene had been localised. We have used the FISH technique to refine the mapping of this gene to 8q23, and have further identified unsuspected alterations of the 6q- chromosomes in one case of NHL by demonstrating duplication of the MYB locus. The use of the related FISH technique of chromosomes 14p derivative chromosome occurs without the reciprocal 18q- derivative and in the presence of 2 normal chromosomes 18. We have used chromosome painting to prove that in some of these cases the translocated material on the 14q- was from chromosome 18. This implied that these cells originally contained a t(14;18), that the 18q- was lost and that the remaining chromosome 18 was duplicated. This technique has also allowed us to identify a marker chromosome in one NHL case (which appeared to exhibit monosomy of X) as an abnormal X-chromosome that had most of its q and p arms deleted. The application of the FISH technique to the study of NHL cell chromosomes is therefore likely to enable the identification of most chromosomal abnormalities present, and so may reveal the critical events leading to malignant transformation of the lymphoid cells. This technique can also be applied to studying chromosomes in interphase nuclei, and we have been able to identify differing copy numbers of chromosomes 6 and the X-chromosomes in normal samples. This latter approach can be used to estimate the number of malignant cells in a biopsy sample, and could form the basis of a more refined analysis of interphase cytogenetics.

This work was supported by the Yorkshire Cancer Research Campaign.

P 40 ANALYSIS OF THE P53 GENE, ITS EXPRESSION AND PROTEIN STABILIZATION IN NON-HODGKIN'S LYMPHOMAS. M.C.M. Finnegan, K.A. Lee, J.R. Goepel, J. Royds, B.W. Hancock and M.H. Goyns. Department of Clinical Oncology, Institute for Cancer Studies and Dept. Pathology, University Medical School, Sheffield, S10 2RX, UK.

The P53 tumour suppressor gene is widely regarded as the most important gene in human malignant disease, however, very little is known of the involvement of p53 dysfunction in the evolution of NHL. We were therefore very interested in assessing its involvement in NHL. Although the characteristic chromosome abnormalities that have often accompanied P53 mutations in other malignancies, such as monosomy of chromosome 17 or breakpoints at 17p13, were not common in NHL, we decided to analyse both immunohistochemical staining of the p53 protein and changes in P53 gene structure and expression in the same NHL biopsy samples. Three distinct patterns of p53 protein immunohistochemical staining were observed in the NHL samples. The first type was characterized by positive staining of the majority of cells (32%), the second pattern by the staining of small foci of cells (32%), and the third by the staining of occasional cells (20%). The latter was observed both in NHL samples and in the non-malignant reactive nodes. Southern blot analysis of the NHL DNA samples failed to reveal any evidence for rearrangements of the P53 gene in any of the samples. A PCR strategy based on chemical mismatch cleavage revealed the presence of a mutation in only one sample, and this was associated with the first type of positive staining pattern. Northern blot analysis demonstrated that P53 mRNA could not be detected in non-malignant tissue, but it was overexpressed in 11/27 NHL samples, however, this did not correlate with positive staining of the p53 protein. As overexpression of the MDM-2 gene product is thought to stabilize the p53 protein, we carried out a Northern blot analysis of MDM-2 gene expression, and found that two of the NHL samples exhibited gross overexpression. Both were associated with the two other patients who had exhibited the first type of p53 staining pattern. It is possible that P53 mutation or MDM-2 overexpression may have been present in the foci of cells observed in the second type of staining pattern, but as the foci comprised less than 5% of the total number of cells in the biopsy specimen, this would have been difficult to determine. These data suggest that p53 dysfunction might be an important event in the evolution of only a minority of NHL cases, and that there may be different mechanisms which can lead to p53 protein stabilization in these neoplasms.

This work was supported by the Yorkshire Cancer Research Campaign.
The p53 gene has a tumor suppressor function. The mutated gene encodes for a protein which has a longer half-life compared to the normal p53 protein. This enables the detection of the mutated p53 protein by immunohistochemistry. In this study, 53 lymph nodes involved with Hodgkin's disease were stained with DO-1 and CM-1, two antibodies directed against the p53 protein. Samples from all histologic subtypes were available. DO-1 weakly stained 2/14 samples, CM-1 stained 10/25. When preincubated with Target Unmasking Fluid CM-1 stained 51/53 samples positively. One negative sample did not contain any Reed-Sternberg cells. Only Hodgkin and Reed-Sternberg cells stained positive, however negative Hodgkin and Reed-Sternberg cells were seen in the same sample. The intensity of staining varied within the positive cell population. To investigate whether the expression of p53 is cell cycle related, a double staining with antibodies directed against proteins associated with proliferation and CM-1 is being tested. Based on these results, we conclude that the p53 mutated protein is present in a high number of cases with Hodgkin's disease, which is suggestive for an important event in the pathophysiology of the disease. In addition, because of the absence of positive staining in the surrounding lymphocytes, these cells are not likely to be part of the malignant clone.

**P 42**

**MOLECULAR CLONING OF A NOVEL 1Q21 BREAKPOINT ASSOCIATION CELL NON-HODGKIN'S LYMPHOMA.** J.M. Meerahab1, F.E. Constel, L. Kearney1, D. Nizic2, B. Gibbons1, T. A. Lister1 and B. D. Young1, 11 CCRF Department of Medical Oncology, St Bartholomew's Hospital, London EC1A 7BE (UK). 2Genome Analyzers, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX (UK).

Mediastinal large cell lymphoma with sclerosis (MLCLS) is a rare subtype of high grade non-Hodgkin's lymphoma of B-cell origin. Conventional banding cytogenetic analysis of one example of such a tumour revealed a complex karyotype including a t(11;14)(q23;q32) translocation. A cosmid library was constructed from the genomic DNA of this tumour and the derivative clone carrying the rearrangement was isolated. Fluorescence in situ hybridization confirmed that the cloned region spanned the translocation in this patient. Furthermore, molecular studies have confirmed that the clone includes a partially rearranged VDJ sequence from the immunoglobulin heavy chain gene on chromosome 14 fused to sequence from chromosome 11 band q23. The sequence from chromosome 11 maps proximal to the CD3 gene cluster and is therefore distinct from both the HTRX-1 gene (rearranged in acute leukemias) and the RCR gene (rearranged in a cell line derived from a centroblastic B-cell lymphoma). Thus, a new lymphoma associated translocation has been cloned and a single copy probe from the breakpoint region on chromosome 11 has been identified. We are investigating the potential role of this translocation in this sub-type of lymphoma.

**P 43**

**DETECTION OF ARRENGEMENTS OF HUMAN T CELL RECEPTOR AND IMMUNOGLOBULIN GENES IN SINGLE CELLS BY PCR.** J. Roth, H. Daus, A. Gause, L. Trimper, M. Pfreundschuh. Dep. Internal Medicine I, University of Saarland, D-66650 Homburg/Saar, Germany

Rearrangement of immunoglobulin (lg) and T cell receptor (TCR) genes occurs by close juxtaposition of various gene segments that are dispersed over several kb in their germline configuration. Successful rearrangements that serve as markers for clonality can therefore be detected by polymerase chain reaction (PCR) with primers that correspond to sequences within different gene segments.

In order to detect rearrangements of Ig and TCR genes, primers corresponding to the six different families of the variable heavy chain (VH) genes and the IgH joining regions were constructed. Similarly, primers to the variable regions of the TCR-γ gene and the joining regions were made. These primers were shown to detect rearrangements of the corresponding genes in DNA of various lymphoid cell lines and samples from patients with lymphoid neoplasms. Single cells from cell lines and tumour cells of Hodgkin's disease (4 cases, 2 of nodular sclerosing-, 1 of mixed cellularity- and 1 of lymphocyte depleted- subtype) were isolated by micromanipulation from glass slides. Rearrangements were detected in single cells of lymphoid cell lines, demonstrating the sensitivity of this novel technique. No IgH rearrangements were detected in Hodgkin's cells so far. This technique will help to clarify some of the unresolved questions in Hodgkin's disease.

**P 44**

**WHICH IS BETTER, β OR TCR GENE ANALYSIS TO DETECT CLONALITY IN PERIPHERAL T CELL LYMPHOMA?** T. Theodoro1, M. Rapoport2, C. Bigorg1, C. Fourcade3, C. Hauvo2, M. Divan4, C. Laha1, G. Cochet1, C. Ducos5, F. Reyes2, M.P. Lafargue2, P. Gaulard2, and J.P. Farber1.

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The analysis of TCR gene rearrangements is critical to discriminate between monoclonal malignant T cells and polyclonal reactive T cells. The reference method, i.e. Southern blotting (SB), tends to be replaced by gene rearrangement amplification using polymerase chain reaction (PCR) which is more sensitive and faster. In order to determine whether TCR or γδTCR gene is better to be analyzed in peripheral T cell lymphomas (PTCL), we have studied 36 cases using Jγ and Cγ probes and SB. The 36 cases consisted of 31 lymph nodes whose histology was classified according to the Kiel updated nomenclature and 5 spleens from hepatosplenic T cell lymphomas; lymphoblastic and cutaneous T cell lymphomas were excluded. The T cell phenotype was assessed on the presence of one pan T marker (CD2, CD3, CD5, CD7) and the absence of pan B markers (CD19, CD20). According to TCR expression, the series included 20 cases with TCRγ, 2 cases with TCRγδ, 12 cases with TCRγ silent, the 2 remaining cases being not interpretable. SB with the Jγ probe showed 23/36 cases with a TCRγ gene rearrangement (63.8%); SB with the Cγ probe could be performed in 28 cases showing 15 rearranged (53.5%). The 15 cases had both β and γδTCR rearrangements, 11 cases had both γδ and γδTCR. The case with both δ and γδTCR was not included. In addition 35 γ alleles (15.8% rearranged case) and 15 β alleles (12.6% rearranged case) were identified. The t allele configuration indicated that 32 Vγ and 3 Vβ subfamilies V segments were involved in the combinatorial events with 21 Jγ1, 13 Jγ1/JP2 and 1 JP segments. Therefore in PTCL, the γδTCR gene is more frequently rearranged than the βTCR gene with a higher number of rearranged alleles. The PCR strategy for the diagnosis of clonality in PTCL should be based on the analysis of γδTCR gene using in first line primers specific for the Vγ subfamily and the Jγ1/JP2 segments.
DNA hybridization has been found to be useful in the diagnosis of T cell malignancy by demonstrating the clonal nature of the disease. However, Southern-blot analysis suffers from a number of technical disadvantages, including the time necessary to obtain results and the use of radioactivity. We have investigated an alternative approach for assessing clonality in biopsy specimens from patients with cutaneous T cell lymphomas (CTCL). This approach involves the amplification of rearranged gamma T cell receptor genes by the polymerase chain reaction (PCR) and analysis of this product by non-denaturing gel electrophoresis. The clonality was detected in patients with clear signals of rearrangements observed on Southern-blot, by the use of PCR amplification and acrylamide gel electrophoresis that revealed discrete bands after the gel is stained with ethidium bromide. Moreover we studied 15 patients with inflammatory dermatoses as control and all the specimens revealed a polyclonal pattern appearing as a diffuse smear along the length of the gel. Our finding suggest that PCR combined with non-denaturing gel electrophoresis may offer a rapid, nonradioactive and sensitive alternative to Southern-blot analysis for the diagnostic evaluation of patients with CTCL.

An increased incidence of non-Hodgkin's lymphoma is well recognized as a complication of primary as well as secondary immunodeficiency states. We have studied 34 patients (pts) with immunodeficiency after organ transplantation; 17 liver, 8 heart and 9 kidney, 1 liver-kidney and 1 bone marrow. All pts had received cyclosporine, steroids or azathioprine; rejection episodes were treated with somulud or OKT3. There were 23 males and 11 females; the ages ranged from 10 months (mon) to 62 years. Median time to development of lymphoma was 7 mon (range 1.5-50 mon) Cyto genetic analyses on 15 pts revealed recurring abnormalities in 9 pts (60%). These abnormalities could be classified into 4 groups: (gp): gp1 (8/14) or (a/22), 3 gp2: gp2 (+1), 2 pts; gp3: gp3 (+5), 2 pts and gp4 other translocations involving 14q23 or 22q11 (5/14); (a/14), 2 pts. Burkitt's translocations and rearrangements involving 14q23 or 22q11 are known recurring abnormalities in B lymphoid neoplasms. Trisomy 11 has been reported as a recurring abnormality in secondary lymphomas. Including our cases, trisomy 9 has now been seen in 4 cases of post transplant lymphomas. By morphology, 12 pts were classified as having polymorphic lymphoid proliferation (PTLD) and 22 had aggressive large cell or high grade lymphoma. Within the PTLD category, there were 2 cases which were monoclonal by gene rearrangement studies. All pts with cytogenetic abnormalities were morphologically classified as malignant lymphomas. In cases with sufficient material, the EBV gene expression was studied by in situ hybridization, Western immunoblotting and RNA directed PCR. In 13 of 17 cases studied so far, EBV nuclear antigen 2 (EBNA-2) and latent membrane protein 1 (LMP1) were expressed in the tumor cells. Two of the 4 tumors without evidence of EBV gene expression were stained predominantly with T cell markers. Clinical outcome has been extremely poor, however, 4 pts have achieved significant long term survival following treatment. Our studies suggest these lymphoproliferative disorders are heterogeneous and further studies are needed to identify the different subgroups, and develop rational and effective therapeutic approaches.


A comparative Polymerase Chain Reaction (PCR) technique was developed (Meijer et al.: J. Haem. 1989) and used to quantify residual malignant cells (caryning 11418) in peripheral blood and bone marrow samples of a thirty year old male before and after treatment. In April 1989 a stage IV follicular centrolethroidocytic (FBCC) lymphoma was diagnosed. Doxorubicin containing chemotherapy induced complete remission (CR), but a relapse was noted in February 1990. In October 1995, the patient then received an allogeneic bone marrow transplantation (BMT) with T cell-depleted marrow from a matched related MLC transfused. The patient achieved a CR in November 1995 and the patient then received an allogeneic bone marrow transplantation (BMT) with T cell-depleted marrow from a matched related MLC. BMT. The patient achieved a CR in November 1995. He is now in chronic complete remission, seventeen months after BMT. Using our assay, malignant lymphoma cells were isolated from a lymph node sample in February 90. A blood sample taken three months before BMT showed lymphoma cells while the patient was in apparent clinical remission (see figure).
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Solute CD3 (sCD3) is a proteolytic fragment of the type II membrane molecule CD3 antigen. It is overexpressed and abnormally regulated on B-CLL cells and increased sCD3 levels are found in the sera of CLL patients (Blond 71-94, 1998). We prospectively followed 142 CLL and LL patients and collected 925 serum samples that were simultaneously assessed for their content in sCD3. The sCD3 levels ranged from 0.2 to 660 (N 0.2-3) ng/ml with a median value of 54.9 ng/ml and correlated with the clinical stage of the disease at diagnosis. Median survival of CLL patients with sCD3 level >2000 ng/ml was 48 months whereas survival of patients with 2000ng/ml was 71 months (p<0.0001). In a Cox multivariate regression analysis, sCD3 and clinical stage appeared to have a dependent prognostic significance. For 97 CLL patients, we collected 3 or more consecutive samples (n=765) during a 1 to 90 months period with a median follow-up of 40 months. Although sCD3 level was poorly correlated with the lymphocytosis and the serum 82 microglobulin level, it was strongly correlated (r=0.82) with the Jakosi (lymphocytosis + height(cm) of the spleen below costal margin + diameter(cm) of the liver + diameter(cm) of the heart) in indolent patients, improved clinical status was associated with decreases in sCD3 levels whereas disease progression was associated with increased levels. In conclusion, sCD3 appears to be a specific and unique marker that can be used reliably to monitor CLL therapy and most importantly to identify a poor prognostic group of patients who might benefit from more aggressive therapeutic approach.

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CD23 generally known as the low-affinity receptor for IgE (FcεRII) is expressed on the surface of B lymphocytes at the intermediate stage of differentiation and on B-CLL cells. Its soluble form, sCD23 (IgE-Fc) has potent BCGF-activity. Recently, it has been shown, that sCD23 is significantly elevated in sera of B-CLL patients. The clinical implications of this finding, however, are still unclear. To obtain further information on the role of sCD23 and the in vivo mechanism of its regulation, we investigated 40 cases of B-CLL with RAI stages 0-IV. In addition to the measurement of sCD23 serum levels, we examined the expression of CD23 on MNs in 17 cases. The results indicate that in all patients with B-CLL sCD23 was highly elevated (median 4544, range 284-2520) as compared to normal individuals (median 113, range 77-1524) and other lymphoproliferative disorders (HCL, T-CLL, FL, Ig-related/high grade (IgG) NHL, ALL, MM). Only within the group of Ig NHL of B-cell phenotype were similar serum levels to B-CLL measured. Serum concentrations of sCD23 correlated with disease activity as evaluated by RAI stage, lymphocyte doubling time and distinction between active and indolent forms of B-CLL, but not with absolute lymphocyte counts. The response induced by chemotherapy was reflected by a decrease of sCD23 serum levels. The FAC5 analysis revealed that 75% of MNC in B-CLL were CD23 positive. The CD23 antigen was located independently of stage on the malignant CD19/CD5 positive population. While in lymphomas the CD23 antigen was restricted to the CD19/CD5 positive population, it was found only on CD19 positive cells in healthy donors. Since sCD23 serum levels were only weakly correlated to the absolute numbers of CD23+ MNC in peripheral blood analysis related to the number of CD23 molecules per cell surface and the percentage of MNC nor to the product of these two parameters, sCD23 seems to reflect the tumor mass rather than the number of leukaemic cells. Supported by "Fonds zur Förderung der wissenschaftlichen Forschung" P 7060.
Non Hodgkin's lymphomas (NHL) are monoclonal, each with a unique and identifiable rearrangement in their immunoglobulin genes (with all cell clones having the same rearrangement in every malignant cell). Using Southern blot analysis it is possible to detect the presence of 1% of these malignant cells in a tissue sample thereby identifying occult minimal volume disease (MVD). We have prospectively evaluated Southern blot analysis in the detection of MVD as a prognostic marker in newly diagnosed patients with NHL. Approximately 0.1-1.0% of bone marrow (BM) and 50% of peripheral blood (PB) were obtained from 55 newly diagnosed NHL patients during routine pretreatment staging procedures between (1990-1991). DNA was extracted from the monoclonal fraction of BM (n=84) and PB (n=44) samples and digested using the restriction enzymes EcoR1, Hind3 and BamH1. Southern blot analysis was carried out and gene rearrangements were detected by hybridizing with both an immunoglobulin heavy chain (JH) probe and T-cell receptor constant region (TT) probe to detect clonality in BM and T-cell lymphomas respectively. The results of these studies were compared with routine histopathology. There was insufficient DNA for analysis in 2292 samples. While malignant tumour cells were identified pathologically in 14% (270) of specimens, in contrast, gene rearrangements were detected in 43% (3070). Patients with and without detectable MVD had similar pre-treatment prognostic characteristics. There was no difference in response rate or survival among patients with or without detectable MVD in their BM. However patients (13/35) with detectable rearrangements in their PB had a significantly better complete response rate of 48% versus 70%, and had an inferior disease free survival at 2 years, 20% versus 40%, when compared to patients (20/23) without evidence of MVD in their PB. These results suggest that Southern blotting in NHL is superior to standard histopathology in detecting MVD in PB and BM samples. The detection of MVD in BM, as suggested by these results may have prognostic significance in NHL. In this study the detection of MVD in the bone marrow does not appear to be of prognostic significance. Further studies are indicated to clarify the value of gene rearrangement analysis as a prognostic marker, and its role in the early detection of residual or recurrent disease in patients with NHL, thereby allowing the identification of those patients who would benefit from more intensive therapy including bone marrow transplantation.

Supported by Cancer Research Advance Board, Irish Cancer Society.

The analysis of prognostic factors in a series of 143 patients with low-grade lymphomas from our institution variables associated with a shorter survival were age, performance status, and the stage of lymphoma, involved lymph node areas involved, extranodal involvement, bone marrow infiltration, ESR, WBC count, leukemic expression, and serum LDH. Of note, some of these variables (namely, age, PS, stage, number of extranodal sites, and serum LDH) are those employed to build the IPI for large-cell lymphomas. Therefore, in order to determine whether the IPI is also useful in low grade lymphomas, we applied this index to our series. Main results are shown in the table:

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Patients (%)</th>
<th>CR (%)</th>
<th>10 years Survival (%)</th>
</tr>
</thead>
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<td>LOW</td>
<td>30.0</td>
<td>60</td>
<td>73.6</td>
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<tr>
<td>LOW INTERMEDIATE</td>
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<td>45.2</td>
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</tr>
<tr>
<td>HIGH INTERMEDIATE</td>
<td>23</td>
<td>53.5</td>
<td></td>
</tr>
<tr>
<td>HIGH</td>
<td>1.2</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>

The prognostic value of IPI was further investigated by including it in a multi-variable analysis along with the other variables previously identified. In such analysis, IPI failed to correlate (P>0.001) and it would be a worse predictor (P=0.038) of independent prognostic variables for survival. When response to treatment was included in the model, IPI retained its significance (P<0.001). Furthermore, IPI was the only parameter related to survival (P<0.001) in patients achieving a CR.

In conclusion, this study IPI has been found to be an important prognostic tool in low-grade lymphomas. If confirmed in other series, IPI could be used to predict prognosis not only in large-cell but also in low-grade lymphomas.

Waldenstrom's macroglobulinemia is a generalized chronic lymphoproliferative disorder characterized by the production of a monoclonal IgM well-differentiated plasmacytoid lymphocytes. Most patients respond to first-line chemotherapy at varying prefixed time points. Although Plakazide and 2-CDA are now reported to be active in this disease so, in order to evaluate their role, it would be desirable to identify patient categories at different prognostic risk level (age and extent survival seems to be the only available parameters). Therefore, we have prospectively evaluated a cohort of patients collected in four italian institutions (Bergamo, Vicenza, Genova, Parma) between 1976 and 1992 the diagnosis of Waldenstrom's macroglobulinemia was confirmed in 114 pts, 76 males and 38 females, median age 67 (range, 35-87) by the presence of an unequivocal infiltration > 30% of lymphocytes lymphoplasmocytes in bone marrow biopsy and a monoclonal IgM > 0.5 g/dl on electrophoresis.

Twenty percent of pts had hepatitis, 19% splenomegaly & 18% lymphadenomegaly. Eighty pts received chemotherapy (73% mono and 27% polychemotherapy) while 36 pts remained untreated. Median survival was 108 months (range, 4-147 months). Thirty three pts (29%) died of disease or intercurrent medical illness. Twelve clinicopathologic parameters at presentation were examined in univariate analysis: 
- age < 65 vs 65; sex M vs F; IGM < 2.5 g/dl vs > 2.5 g/dl; kappa vs lambda; albumin < 3.5 g/dl vs > 3.5 g/dl; absence vs presence of cryoglobulins; 10% vs 100% T blasts; > 10/5% vs < 10/5% T cells; > 15% vs < 15% lymphopenia, hepatomegaly, lymphadenomegaly. In univariate analysis 5 criteria were found to predict survival: 

1. age < 65 years (logrank 1.1, p <0.05) 
2. IGM > 2.5 g/dl (logrank= 11.6, p <0.001) 
3. Pb < 10 x 109/L (logrank= 11.4, p <0.001) 
4. white blood cells < 1.5 x 109/L (logrank= 13.5, p <0.001) 
5. plastele < 100 x 109/L (logrank= 13.5, p <0.001).

Using Cox proportional hazard model only 3 risk factors among the 5 were independently of prognostic significance: 1) Age >65 years (p <0.03) 2) platelets < 100 x 109/L (p <0.05) 3) IgM > 2.5 g/dl (p <0.005).

Thus, in this unsselected series 22% of pts were considered at low risk (i.e. without any of the three afore-mentioned risk factors) and had a 10 years actuarial survival of 83%, while in the high risk group (with all 3 risk factors) patients would benefit from more intensive therapy including bone marrow transplantation.
**P 61**

**A NEW PROGNOSTIC INDEX INCLUDING S-CA125 AND LDH LEVELS IN PATIENTS WITH NON HODGKIN'S LYMPHOMAS.** L. Benboubker, C. Vallet, C. Linossier, M. Delean, C. Pecleriol, F. Festrerol, J. Lamagnere, Ph. Colombet, CHRU (Brest), University of Brest, France.

CA125 serum level (s-CA125) was measured by radioimmunossay (IDS international®) at diagnosis in 45 patients with non-Hodgkin's lymphoma (NHL). They were 25 men and 20 women with a mean age of 59.2 years (range 24-80 years). Sixteen patients had a low grade histology whereas 23 had a high grade subtype. Ann Arbor stage was: IV in 23 patients, I in 3 patients, II in 6 cases, and I in 1 cases. B-Symptoms were present at diagnosis in 5 patients and a bulky tumoral mass was found in 11 patients. All patients were homogeneously treated according to the Paris Ouest France protocols.

s-CA125 was abnormal (> 30 U/ml) in 16 patients (35.6 %). s-CA125 level was closely related to the stage of the disease. In stage I-II, only 8 patients (11 %) had elevated s-CA125 level (mean value: 21.7 ± 15.6 U/ml) whereas 14 patients (50 %) had an abnormal dosage in stage III-IV patients (mean value: 325 ± 512 U/ml) (p = 0.0037). The LDH level was also related to the stage of disease. In stage III-II, only 5 % of patients had elevated level (mean value was: 240.6 ± 65.2 U/L) whereas 20 % had an abnormal dosage in stage III-IV patients (mean value of: 522.4 ± 359.6 U/L) (p = 0.0014). None of patients with stage I-II had both elevated s-CA125 and LDH levels increase, opposite to 40 % of patients with stage III-IV disease (p = 0.0005). We found no difference in s-CA125 levels between patients with either high grade or low grade NHL (p = 0.9).

All patients were assessable for response to induction therapy. Table 1 reports the early response after 3-4 courses of induction chemotherapy according to the LOH and CA125 levels at diagnosis. Complete remission (CR) rate was found significantly higher in patients with normal s-CA125 or LDH levels than in others. When taking both markers together, patients with normal LDH and s-CA125 levels responded better to therapy than patients with normal s-CA125 and elevated LDH levels and far more better than patients with both elevated markers. We conclude that the combination of s-CA125 and LDH is a good prognostic index in patients with NHL.

**Table 1:**

<table>
<thead>
<tr>
<th>Group</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>PD (%)</th>
<th>ND (%)</th>
<th>N (%)</th>
<th>CR rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-CA125 &lt; 30</td>
<td>35.7%</td>
<td>13.8%</td>
<td>1.8%</td>
<td>39.7%</td>
<td>100</td>
<td>42.5%</td>
</tr>
<tr>
<td>s-CA125 &gt; 30</td>
<td>22.5%</td>
<td>6.3%</td>
<td>0%</td>
<td>72.7%</td>
<td>100</td>
<td>24.5%</td>
</tr>
<tr>
<td>LDH &lt; 300</td>
<td>36.3%</td>
<td>12.5%</td>
<td>0%</td>
<td>50.8%</td>
<td>100</td>
<td>41.5%</td>
</tr>
<tr>
<td>LDH &gt; 300</td>
<td>20.8%</td>
<td>10.4%</td>
<td>1.8%</td>
<td>67.0%</td>
<td>100</td>
<td>27.2%</td>
</tr>
</tbody>
</table>

**P 62**

**UNUSUAL PROGNOSTIC FACTORS IN ALL NONHODGKIN'S PATIENTS WITH ADVANCED NON HODGKIN'S LYMPHOMAS.**


Division of Oncology, Hôpital Cantaloupe II, University of Maple, Division of Haematology and Division of Epidemiology, National Cancer Institute of Thailand.

We have reviewed the prognostic characterization of 62 consecutive patients [I] of advanced non-Hodgkin lymphomas who were observed at our institutions from 1977 to 1993. The patient population was divided into four groups according to size of disease: group I: pts diagnosed between 1977-1980 (n: 14), group II: pts diagnosed between 1981-1985 (n: 26), group III pts diagnosed between 1986-1990 (n: 35), group IV pts diagnosed between 1991-1993 (n: 10). All pts were treated with up-to-date chemotherapeutic programs. We evaluated the following prognostic characteristics, which are thought to represent the most significant prognostic factors in aggressive non-Hodgkin's lymphomas: age, stage, symptoms, bulk disease, LDH levels, bNHL status, bone marrow involvement, number of extranodal sites. Results of our analysis are shown below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Age &lt; 40</th>
<th>40-59</th>
<th>60-79</th>
<th>80+</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>22 (14)</td>
<td>12 (8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Group II</td>
<td>20 (12)</td>
<td>13 (8)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.0023</td>
</tr>
<tr>
<td>Group III</td>
<td>21 (14)</td>
<td>19 (12)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0.0114</td>
</tr>
<tr>
<td>Group IV</td>
<td>19 (12)</td>
<td>18 (11)</td>
<td>3 (3)</td>
<td>0 (0)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**P 63**

**DISSEMINATED GROWTH OF HODGKIN DERIVED CELLINES IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE.**

Ursula Kapp, Andreas Dix, Elizabeth Schell-Frederick, Michael Hummel, Susanne Mücke, Jönn Bulleriek, Claudia Gottstein, Andreas Engert, Volker Diehl and Jürgen Wolf

Local tumor growth has been reported after subcutaneous and intrasplenic injection of Hodgkin derived cell lines into different immunodeficient mouse strains. Since new immunotherapeutic strategies will be employed in patients with disseminated disease, an animal model with disseminated growth of tumor cells would be useful for preclinical testing. Therefore, the Hodgkin derived cell lines L640, L640sc, L428 and KM-H2 were injected intravenously into SCID mice. In contrast to L428 and KM-H2, widespread neoplasia occurred after a period of 4-6 weeks following injection of L640 and the subline L640cy. The lymph nodes were found to be the preferred site of tumor growth. The CD30 surface antigen on Hodgkin cells and the karyotype of the cells were preserved in the animal host. Thus, the SCID mouse model mimics to a large extent the dissemination pattern of Hodgkin's disease in man and may provide a useful tool for evaluation of the efficiency of conventional and newly developed therapies.

To evaluate the role of adhesion molecule expression in the dissemination of Hodgkin-derived cell lines, CD44 and members of the immunoglobulin, integrin, selectin and Fc receptor families were quantified by flow cytometry. CD30 expression was also measured. Although CD44 expression has been correlated with dissemination in non-Hodgkin lymphoma, this was not the case in the Hodgkin SCID mouse model. CD44 was not expressed on the disseminating cell lines L640 and L640cy.

**P 64**

**THE B7/B8 ANTIGEN IS EXPRESSED BY REED-STERNBERG CELLS OF HODGKIN'S DISEASE: FURTHER EVIDENCE FOR AN ACCESSORY CELL FUNCTION OF REED-STERNBERG CELLS.** J. Delabie, P. Vandenberge, J.L. Ceuppens, C. De Wolf-Feiters, Department of Pathology and Internal Medicine (Div. of Clinical Immunology), Catholic University of Louvain, B-3000 Leuven, Belgium

The B7/B8 molecule recently received much attention, because it has been found to be the natural ligand for CD28, expressed by T cells. CD28 provides upon binding of B7/B8 a signal that synergizes with the T cell antigen receptor to induce T-cells to proliferate and secrete cytokines and that prevents anergy induction of T cells. Since Reed-Sternberg cells are known to be strong stimulators in mixed lymphocyte reactions, we evaluated the expression of B7/B8 in lymph nodes affected by Hodgkin's disease. In addition, non-Hodgkin's lymphomas, including T cell rich B cell lymphomas, were evaluated for B7/B8 expression. B7/B8 was found to be strongly expressed by the Reed-Sternberg cells in all cases of Hodgkin's disease studied, irrespective of the subtype of Hodgkin's disease. In contrast, B7/B8 is not expressed by the neoplastic cells in the majority of non-Hodgkin's lymphomas including T-cell rich B-cell lymphomas. Evidence for a functional role of B7/B8 on Reed-Sternberg cells was obtained by our findings that the primary allogeneic mixed lymphocyte reaction using the B7/B8 expressing Hodgkin's disease cell lines L428 and KM-H2 as stimulators, could be partially blocked by adding anti-B7/B8 antibody.

Our data provide further evidence for an accessory cell function of Reed-Sternberg cells and strongly indicates that the accumulation of reactive T cells observed in Hodgkin's disease involves different mechanisms than those acting in non-Hodgkin's lymphomas.

The proliferative activity and the origin of multinucleated Sternberg-Reed cells in Hodgkin's disease have been studied using cell cultures, thymidine incorporation and immunohistochemistry techniques. The presence of both proliferating cell nuclear antigen (PCNA) and the Ki-67 antigen associated with cell proliferation have been reported in Hodgkin's disease. However, PC10 (an antibody to PCNA) may not reflect the true proliferating fraction as results may vary with antibody concentration and the use of Ki-67 has been limited to frozen material. The p34cdc2 kinase gene in Hodgkin's disease is probably cell cycle control gene. The p34cdc2 kinase functions at cell cycle control points and is necessary for mitosis. It also operates in G1 and is involved in the commitment of cells into the proliferative cycle. Using a monoclonal antibody against the protein p34 (courtesy T.Hunt), cases of different histological subtypes of Hodgkin's disease have been studied along with normal non-Hodgkin's disease controls. As expected, positive immunostaining in the controls with anti-p34 was reciprocal to that found using an anti-pcbl-2 antibody. In the ten cases of Hodgkin's disease, positive p34 staining was seen in the majority of Sternberg-Reed cells and mononuclear variants (>90%), along with a proportion of small lymphocytes, mainly T cells. Staining was predominantly cytoplasmic and occasionally additional nuclear signals were seen. In addition, double-stained sections with the anti-p34 antibody and CM-1 for p53 were examined. In these cases, a positive signal for both proteins was observed in the same cells. Whether the presence of p34 in Sternberg-Reed cells reflects mitosis or nuclear division without cell division (endomitosis) and hence explain the multinucleated appearance of these cells, remains to be determined.

P 66 CLINICAL FEATURES PREDICTING BONE-MARROW INVOLVEMENT IN HODGKIN'S DISEASE: ATTEMPT TO AVOID UNNECESSARY INVESTIGATIONS IN FAVOURABLE PRESENTATIONS. E. H. Spang, F. Bonichon, R. S. Donnellan and B. Hoch. Fondation Bergonié, 180, rue de Saint-Germain 33270 Bordeaux Cedex (France).

Bone marrow biopsy (BMB) is widely performed in untreated Hodgkin's disease (HD) staging in order to choose the most appropriate treatment according to clinical classification. It is commonly admitted that bone marrow is involved in 5 to 15% of HD, but it is also admitted that bone-marrow involvement (BMI) is in relation with clinical extension and disease aggressiveness. In order to assess this relation, we reviewed clinical and routine biological features of 261 unscreened newly diagnosed HD before randomized trials. After clinical examination there were 157 patients (pts) with no systemic symptoms according to Ann Arbor classification criteria (A = group A) and 104 pts with fever and/or night sweats and/or weight loss more than 10% (B - group B) respectively 61 and 38% of pts. Bone-marrow was involved in one case of group A (0.6%) and in 14 cases of group B (13.5%). In the second step, the analysis of radiological features (chest X-ray, CT scan) before BMB showed 3 cases of liver and/or lung involvement in group B. The only case of BMI in group A had liver involvement and generalized lymphadenopathy. In group B, regardless of BMI, there were 9 cases of liver and/or lung involvement. After BMI all of these 9 pts had BMI and 5 had BMI without other visceral involvement. Statistical analyses (CHIS) conclude that the most predictive factor is: 1) BMI-symptoms (p value < 0.0001); 2) liver involvement (p value < 0.0001) and more than 4 involved lymph node areas (p value < 0.0001). There is also a correlation with clinical spleen enlargement and leukopenia or thrombocytopenia, but we don't find correlation with age, histological type (2 vs 3) and erythrocyte sedimentation rate. These results suggest that BMI should be used for all cases of HD-symptoms but it is unnecessary and useless in clinical staging for HD patients, it is unlikely that in clinical staging no cases of BMI will be missed. We recommend not performing BMB for clinical staging in HD patients with HD-symptoms. If clinical staging is positive for clinical or radiological BMI, BMB should be performed to assess the extent of the disease. In conclusion, this study shows that BMB is not necessary for clinical staging of HD and even if we recommend not performing BMB for clinical staging of HD, if clinical staging is positive for BMI, BMB should be performed to assess the extent of the disease. In conclusion, this study shows that BMB is not necessary for clinical staging of HD and even if we recommend not performing BMB for clinical staging of HD, if clinical staging is positive for BMI, BMB should be performed to assess the extent of the disease.

P 67 INTERLEUKIN-8, S-TNF RECEPTOR AND P53 PROTEIN LEVELS ARE ELEVATED IN SERA OF HODGKIN'S DISEASE PATIENTS. L. Trümper, G. Dahl, A. Gause, W. Jung, V. Diehl, M. Pfreundschuh. Department of Internal Medicine I, University of Saarland, 6601 Homburg/Saar, and 1st Medical Clinic, University of Cologne, Germany.

Hodgkin's disease patients frequently exhibit severe systemic symptoms even with a low tumour load. These symptoms are thought to be mediated by cytokines released by inflammatory bystander cells or the tumour cells themselves. In addition, surface molecules shed into the serum by the tumour cells may serve as tumour markers and may have prognostic significance as has been shown for the soluble form of the CD 30 molecule. We examined circulating levels of Interleukin-8 (IL-8), Interleukin-7 (IL-7), the soluble form of the p60 TNF receptor (sTNFR) and circulating levels of the p53 growth suppressor protein in the sera of 40 (80 for IL-8) consecutive patients with Hodgkin's disease after chemotherapy. Interleukin-8 levels were elevated in 20/80 sera; the mean serum level was 1190 pg/ml (range: 82-9468 pg/ml; normal controls: < 30 pg/ml). Elevated levels corresponded to advanced stages (III and IV acc. to Rye), the presence of B-symptoms (16/20 patients) and elevated white blood cell count (11/20 patients) when compared with the remaining 60 patients. Since IL-8 is stimulated by IL-1 and TNF and activates neutrophils, IL-8 in Hodgkin's sera may be responsible for the activation of granulocytes frequently seen in these patients. In contrast, serum levels for IL-7, a B-cell stimulatory cytokine, were not significantly elevated in the sera of 740 patients only. sTNFR levels were elevated in 12/34 sera above 500 ng/ml, without correlation to stage or sCD 30 levels (elevated in 5/34 sera). Therefore, this parameter does not seem to have prognostic significance in Hodgkin's disease. Serum levels of p53, as assessed by a sandwich ELISA employing two different antibodies to different p53 protein conformations, were elevated in 7/34 sera. The presence of antibodies to p53 may play a significant role in other tumours assessed by immunofluorescence.


From November 1988 to December 1992, 967 clinical stage (CS) I-VI Hodgkin's disease (HD) adult pts were enrolled in 8 European centres. Pts were prospectively enrolled in two ongoing phase III trials (EORTC protocols # 20881 and 20884). Usual staging procedures were performed including laparoscopic staging. HD was reviewed by a panel of pathologists who assessed the diagnostic of HD. The Rye classification was used and, among the nodular sclerosing (NS) subtype, cases were classified according to the BNL2 grades I (G1) or II (G2). Complete remission (CR) was assessed after initial treatment in supradiaphragmatic CS I-II pts, after 6 courses of chemotherapy in CS III-IV pts. CR was assessed using the Fisher exact test, one-way or two-way analysis of variance, as appropriate. Factors predicting for CR were assessed through a logistic regression model which included age, gender, stage, systemic symptoms, number of nodal and extra nodal localizations, mediastinal bulk, and biological parameters (erythrocyte sedimentation rate (ESR), haemoglobin (Hb), leucocytes, neutrophils, platelets, erythrocyte corpuscular volume (EVC) and HCT) as dependent variables. Overall, there were 33% CS I, 50% CS II, 16% CS III and 11% CS IV pts. The sex ratio was 1.0 and 1.7 in CS I-II and CS III-IV pts, respectively (p<0.01). Mean age at diagnosis was 33.6 and 36.4 years (p<0.01), B symptoms were present in 23% and 59% (p<0.001) of the sex respective. After review (558 CS II-I and 179 CS III-IV cases) by the histology panel, 94% of the cases were considered true HD. 32% were non-Hodgkin's lymphomas. Of the 722 true HD cases, 3% were lymphomatous predominant (LP), 79% were NS, and 18% were mixed cellularity (MC) subtype. MC subtype was 7% while 2% were G2, whereas 5% were borderline cases. NS was associated with female gender (p<0.001) and CS I-II (p<0.001). B symptoms were more often present in NS and MC than in LP (p<0.01). subtype. Among NS cases, G2 correlated with stage (p<0.01), NS and MC correlated with low Hb and high ESR (p<0.05). An NS or MC absence of CR at end of treatment was associated with low Hb (p<0.01) and low ECV (p<0.01). Among NS subtype, G2 correlated with low Hb and high ESR (p<0.01), high platelets (p<0.01), and low ECV (p<0.01). An NS or MC absence of CR at end of treatment was associated with low Hb (p<0.01) and low ECV (p<0.01) as well as with NS (p<0.004) subtype. The same biologic factors, but not G2, significantly correlated with a absence of CR when the analysis was restricted to NS cases.
ABSTRACTS - Fifth International Conference on Malignant Lymphoma, Lugano

P 69 BETA-2MICROGLOBULIN (β2-MG) : A GOOD PROGNOSTIC FACTOR FOR RESPONSE AND SURVIVAL IN YOUNG ADULTS WITH Hodgkin's DISEASE (HD).

β2-MG appears a good indicator of responsiveness and survival in non-Hodgkin's lymphoma (NHL) [Legros M et al, Proc ASCO (1987)]. We studied level from 84 previously untreated patients (55 males, 29 females) less than 50 years old affected by HD. Serum β2-MG level was measured by radioimmunoassay (normal range: 1 to 2.4 mg/l). Thirty-seven were stage II- I at 47 stage IV according to Ann Arbor classification. Forty-four had histological Lennert's lymphoma, 25 bulky disease, 54 stage B. Nodular sclerosis (n = 33) and mixed cellularity (n = 32) were predominant in lomas-sclerosis classification. Patients were treated with chemotherapy alone or combined with radiotherapy. Chemotherapy regimens consisted of MOPP alone or alternating MOPP/ABVD.

The response and survival rates [Kaplan-Meier's method] are summarized as follows below:

<table>
<thead>
<tr>
<th>CR after 3 courses</th>
<th>CR at the completion of treatment</th>
<th>Relapse after CR</th>
<th>Primary refractory</th>
<th>Overall survival</th>
<th>DFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2-MG ≤ 2.4</td>
<td>(n = 65)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2-MG &gt; 2.4</td>
<td>(n = 19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease-free survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For all patients the median follow-up was 65 months (5-179).

A multivariate analysis (Cox model) was performed and included: age, sex, presence or absence of systemic symptoms, clinical stage I/II, IVM, tumor burden, biological stage, histology and initial serum β2-MG rate (≤ 2.4 mg/l) or > 2.4 mg/l.

Early response (after 3 courses) Significant β2-MG variables

- Females 40%
- Nodular sclerosis 40%
- Initial β2-MG > 2.4 mg/l 70%

The prognostic value of β2-MG was also found for stage IV and stage IVW. Moreover we noted a significant correlation between β2-MG rate and systemic symptoms (p < 0.01), bulky tumor (p < 0.01) and clinical stage (p < 0.02). This correlation was found with other biological parameters.

In conclusion, initial serum β2-MG appears in this study a good indicator of chemosensitivity and survival for young adults with HD.

P 70 VIM (VINBLASTINE, BLEOMYCIN, METHOTREXATE) CHEMOTHERAPY WITH INVOLVED FIELD RADIOTHERAPY IN THE MANAGEMENT OF 'EARLY' HODGKIN'S DISEASE.

The VIM regimen (Vinblastine, Bleomycin, Methotrexate) was reported by Horning et al. (1988, J Clin Oncol. [18]:1632-1631) to prevent relapse after involved field irradiation in laparotomy staged patients with PSIA and IIA Hodgkin's disease.

To assess the VIM in unselected clinically staged patients the BNLI initiated a prospective, multicentre pilot study. Two cycles of VIM were then used to prevent involved field irradiation (40 Gy in 20 fractions).

Four more cycles of chemotherapy were then given to complete the planned treatment.

30 eligible patients were enrolled in a year. Response to chemotherapy was assessed at 6 weeks (after two cycles of VBM). 26 patients had assessable disease and all showed an objective response to therapy: 9 showed a complete clinical response (not confirmed by CT scan) and 17 showed an objective partial response. At the completion of all therapy 29/30 patients had achieved complete remission and two have stable residual masses. Follow-up ranges from 18-30 months off therapy and one patient has relapsed at 19 months.

Cough, dyspnoea and abnormal pulmonary function tests occurred in 50% of subjects and led to the discontinuation of Bleomycin therapy. This toxicity interfered with normal activity, but was reversible. 22/30 patients received mediastinal irradiation. In those whose mediastinum was irradiated lung toxicity occurred in 12/22 (55%); without mediastinal irradiation lung toxicity was seen in 6/18 (33%). These differences were not significant (p = 0.06).

Three episodes of neutropenic sepsis occurred, one of which was fatal.

In our hands the VIM regimen with intervalated irradiation is effective but produced unacceptable pulmonary toxicity.


Treatment of patients (pt) with HD and large mediastinal masses (MM) traditionally includes extensive chemotherapy (CT) with or without radiation therapy (RT) regardless of stage. From 1970 to 1990, 137 newly diagnosed pt with MM greater than 10 cm received therapy at our institution. We excluded 19 patients from review because of protocol violation or incomplete records. Characteristics of the 118 evaluable pt included: female 50%, Nodular Sclerosis subtype-92%, median age-26 years (range 14 to 64); stages I-II 78%, B symptoms-56%, and hilar involvement-33%. Pt with stages I-II received one of four treatment regimens: (1) 6 to 8 cycles of MOPP or similar CT plus XT; (2) 2 cycles of MOPP or 3 of ABVD plus XT; (3) 6 of CVPP/ABCD (CIA) (cyphosplasmide, vincristine, procarbazine, prednisone/doxorubicin, bleomycin, dacarbazine, lomustine) plus XT; or (4) 3 of NOVP (mitoxantrone, vincristine, vinblastine, procarbazine) plus XT. CT doses included 30 Gy to areas of nodal involvement prior to therapy. By protocol design, no patient with stage IV disease received NOVP plus XT; the incidence of B symptoms was also different for those receiving MOPP (53%), 2 MOPP (48%), CIA (25%), and NOVP (30%). Complete remission (CR), freedom from progression (FFP), and freedom from tumor mortality (FTM) rates are shown:

<table>
<thead>
<tr>
<th>Treatment Stage</th>
<th>No of CR</th>
<th>3 yr FFP %</th>
<th>3-4 yr FFP %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 MOPP plus XT</td>
<td>68</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>6 CIA plus XT</td>
<td>56</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>2 MOPP plus XT</td>
<td>28</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td>2 NOVP plus XT</td>
<td>40</td>
<td>85</td>
<td>86</td>
</tr>
</tbody>
</table>

FFP results for those receiving NOVP with and without B symptoms were 75% and 94%, respectively. None of the above comparisons are statistically different. From this analysis, we propose that it is necessary to administer 6 cycles of CT to patients with MM greater than 10 cm with early staged HD, since it appears that less CT provides FFP and FTM results that are as good as those achieved with 6 cycles in combined modality programs.

P 72 ANALYSIS OF TREATMENT OUTCOME AFTER EBVP CHEMOTHERAPY AND INVOLVED FIELD RADIATION ACCORDING TO PROGNOSTIC FACTORS IN HODGKIN'S DISEASE STAGES I AND II. H. Egibali, P. Richaud, P. Soubyrean, F. Benichon and B. Henn. Fondation Bergonzi, 180, rue de Saint-Genes 33076 Bordeaux Cedex (France).

The main problem of Hodgkin's Disease (HD) treatment in past years and especially now, is the necessity of cure and avoidance of early and late complications such as aplasia, sterility or acute leukemia. In this purpose, ABVD-derived EBVP (epubicline, bleomycin, vindesine, prednisone) is used in order to reduce side-effects resulting from dacarbazine and in the same time late toxicity of alkylating drugs (myelodyplastic syndrome, leukemia, sterility) in MOPP or CVPP. The early results of this regimen were as good as ABVD and MOPP; all three used in a sandwich-framed protocol including induction chemotherapy (CT), involved-field radiotherapy (IF) and consolidation CT. Recently in the EORTC phase III trial H7, it was suggested that EBVP may be inadequate for unfavourable cases (ASCO Proceedings 1993). In order to check this assumption, we carried out a retrospective analysis of all patients (pts) with clinical stage I, I treated by EBVP + IF radiotherapy. One hundred and ten of pts of 14 to 75 years (median 33) were reviewed and divided into two groups favourable (F) and unfavourable (U) according to EORTC prognostic factors which are: 1) age > 50 years; 2) number of involved lymph node > 3; systemic symptoms B V A; 3) ESR < 50 mm in 1 hour or < 30 if B; 4) bulky mediastinum < 0.35 vs > 0.35, histology 1-2 vs 3. Thus, 52 pts of U group and 58 pts of F group were compared. All were clinically staged without laparotomy but with CT scan, lymphangiogram, bone-marrow biopsy and whole biological work-up. The treatment consisted on 3 courses of EBVP before and after IF irradiation. The total CT dosage was the same in two groups with an average of 397 mg/m² for epubicline, 68 mg/m² for bleomycin and 40 mg/m² for vindesine. All of them were irradiated only on IA fields. Complete remission was achieved in 53 cases (91%) for F and 50 cases (96%) for U group after EBVP or after radiotherapy plus chemotherapy. One patient labeled in group F, 8 pts relapsed in F and 9 in U group (p-value 0.4) and 8 died of HD (p = 0.2). The 5-year p-value 0.058. According to Kaplan-Meier method and Log rank test with and a median follow-up of 7 years, disease-free survival is respectively 90% for F and 84% for U. These results are consistent with previous suggestions: 1) despite a priori worse prognosis, the U group has the same relapse-free survival; 2) relapses are sooner in U group, and late in F group. Thus early relapses are not significant if compared; 3) more than 1/5 of all pts are cured by a non leukemogenic, non myelotoxic and well tolerated regimen. They shouldn't be forgotten; 4) in all HD trials a long follow-up is absolutely necessary before concluding.