FREQUENT BCL-6 GENE MUTATIONS IN POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS (PT-LPDs).
PT-LPDs represent a clinically and morphologically heterogeneous group of Epstein-Barr virus (EBV) associated lymphoid proliferations of varying clonal composition which arise in organ transplant recipients in the setting of immunosuppression. Some of these lesions regress following a reduction in immunosuppressive therapy, while some progress despite aggressive therapy. Neither morphologic, immunophenotypic or immunogenotypic criteria have been useful in predicting clinical outcome. We have previously noted that structural alterations in oncogenes and/or tumor suppressor genes identified in the monoclonal PT-LPDs (classified as immunoblastic lymphoma or multiple myeloma) correlate with a poor clinical outcome. However, the presence of these alterations has not been a consistently useful predictor of lesion regression following reduction of immunosuppression. We extended previous studies by examining 53 PT-LPD lesions obtained from 38 patients for the presence of mutations in the BCL-6 proto-oncogene. This gene encodes a putative zinc-finger transcription factor which is the only molecular marker that is altered in a significant proportion of large B cell lymphomas, being rearranged (40%) and/or mutated in the first intron region (7%). We determined the presence of mutations in the BCL-6 gene using the single strand conformation polymorphism (SSCP) analysis. While no mutations were identified in the cases classified as plasmacytic hyperplasia, all cases of PT-LPD classified as malignant lymphoma/multiple myeloma contained BCL-6 gene mutations. Among the lesions classified morphologically as polymorphic PT-LPD, 66% contained BCL-6 gene mutations. Correlation with clinical history revealed that BCL-6 gene mutations were not identified in any of the lesions which regressed following a reduction in immunosuppression, while all of the cases which failed to regress had mutations in the BCL-6 gene. It remains to be determined whether mutations in the BCL-6 gene are causally related to the development and clinical behavior of PT-LPDs, or whether they simply represent a marker of genetic instability secondary to neoplastic transformation. Nevertheless, results strongly suggest that the search for BCL-6 mutations may be a useful test to determine whether a reduction in immunosuppression should be attempted.
MUTATIONS OF BCL-6 REGULATORY REGIONS IN AIDS-RELATED LYMPHOMA
G. Gaidano1, C. Pastore2, A. Migliazza3, G. Volpe2, A. Gliochini4, G. Saglio2, A. Carboni3, R. Dalila-Pavera1,6
1Dipartimento di Scienze Mediche e 2Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, Novara and Torino, Italy; 3Ospedale
Oncologico, Department of Pathology, Columbia University, New York, NY, USA; 4Divisione di Anatomia Patologica, I.N.R.C.C.S.-C.R.O., Aviano, Italy.

Non-Hodgkin lymphomas (NHL) represent a major complication of AIDS. NHL occur consistently of B-cell origin and are classified as small non cleaved cell lymphoma (SNCLL), diffuse large cell lymphoma (DLCL), nodal large cell lymphoma (ALCL), and body cavity based lymphoma (BCBL). The molecular pathogenesis of BCL-6 is characterized by distinct genetic pathways involving rearrangements of proto-oncogenes (c-MYC, BCL-6), inactivation of tumor suppressor loci (p53, FOP) and viral infection (EBV, KSHV) (1). These pathways selectively associate with specific AIDS-NHL histotypes and peculiar clinical features. Recently, it has been shown that the spectrum of BCL-6 structural alterations in lymphoma of the immunocompetent host includes point mutations in addition to gross rearrangements (2, 3). These mutations consistently map within BCL-6 regulatory regions and appear to cluster with tumors arising from B-cells of germinal center (GC) or post-GC origin. On these grounds, BCL-6 mutations are thought to represent a marker for differentiating NHL of distinct histogenetic origin. The aim of this study was to define the frequency and the distribution of BCL-6 mutations in the various AIDS-NHL histotypes. Forty-seven AIDS-NHL, including 16 SNCLL, 14 DLCL, 3 ALCL, and 4 BCBL, were tested for BCL-6 mutations by PCR-SSCP followed by DNA direct sequencing. We analyzed 15 overlapping PCR fragments spanning 740 bp within BCL-6 intron 1 which are known to contain >95% of mutations detected in NHL of the immunocompetent host (3). Mutations of BCL-6 were detected in 14/26 AIDS-SNCLL (53.8%), 5/12 AIDS-DLCL (41.7%), 2/5 AIDS-ALCL (40%), and in 0/4 AIDS-BCBL (0%). The relevance of these data is threefold. First, our results indicate that BCL-6 mutations are a genetic marker of AIDS-NHL occurring both in cases carrying BCL-6 rearrangements (c. 20%

MOLEULAR ANALYSIS OF 70 AIDS-RELATED SYSTEMIC NON-HODGKIN LYMPHOMAS FROM THE EAST AND WEST COASTS OF THE UNITED STATES OF AMERICA (USA)

R.G. Nador, M.G. Horenstein, A. Chadburn, R. Paulose, E. Cesarian, J.W. Said and D.M. Knowles. The New York Hospital-Cornell Medical Center, New York, NY and Cedars-Sinai Medical Center, Los Angeles, CA, USA

Non-Hodgkin lymphomas (NHLs) occur frequently in HIV infected patients. Although the vast majority of AIDS-related NHLs had been found to harbor clonal immunoglobulin ( Ig) gene rearrangements, subsequent studies from the USA west coast reported a frequent lack of clonal rearrangements, suggesting the existence of a large number of polyclonal AIDS-related lymphomas. To address this controversy we collected a cohort of 70 systemic AIDS-related NHLs (30 east coast and 40 west coast) and examined whether significant molecular genetic differences exist in the NHLs from the two geographically distinct areas. 24 of 30 (80%) east coast and 32/40 (80%) west coast NHLs displayed one or two Ig heavy chain gene rearrangement bands indicating a monoclonal B-cell population. Among the remaining 14 germinate cases, 7 exhibited rearranged Ig light chain genes (56% east coast and 28% west coast). EBV terminal analysis revealed a monoclonal population in two additional west coast NHLs. Overall, clonal EBV infection was present in 40% of the cases, and both types of EBV were detected equally. The c-myc protooncogene was found to be structurally altered in 11/30 (36%) east coast and 10/40 (25%) west coast NHLs by gene rearrangements on Southern blot and/or by mutation of the first exon-intron boundary region on SSCP analysis. Only one N-ras mutation was found among the east coast cases. The molecular genetic lesions and clonal EBV infection patterns were similar on the east and west coasts of the USA. In conclusion, AIDS-related systemic NHLs lacking detectable clonal Ig and TCR 3 gene rearrangement and clonal EBV infection do exist but are rare, although they appear to be slightly more frequent on the west coast (4/10; 10%) than on the east coast (1/30; 3%). Whether these morphologically malignant NHLs are truly polyclonal or whether the rearrangements simply are not detectable with the methods employed remains to be determined.


BCL6 AND BCL2 REARRANGEMENT AND p53 OVER-EXPRESION AS PROGNOSTIC FACTORS IN FOLLICULAR LYMPHOMA, D.C. Louie, D. Hochman, A. Schugar, R. Jaslow, P. Roy, J. Oliver, K. Offit, and R.S.K. Chaganti, Departments of Pathology and Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Rearrangement of the BCL6 and BCL2 oncogenes and the TP53 tumor suppressor gene have been implicated as prognostic factors in diffuse large cell lymphoma. These parameters have not been extensively analyzed in follicular lymphoma (FL). We performed gene rearrangement, immunohistochemical, and chemical analysis of 79 cases of FL sequentially ascertained for cytogenetic analysis. BCL2 rearrangement was assessed by Southern blot hybridization using

mbr, mor, and 5′ BCL2 probes; or by karyotypic detection of a t(14;18)(q32;q21). Immunohistochemical analysis was performed using a monoclonal anti-p53 antibody on paraffin-embedded tissues.

The median age of the patients was 51.7 years (range 18-87). 59% of the patients presented with advanced stage disease, and 56% were treated with anthracycline-containing chemotherapy for their FL. Overexpression of p53 at the time of initial biopsy was seen in 10 cases, of which 6 demonstrated BCL2 rearrangement and 1 case showed BCL6 rearrangement. None of the cases with p53 overexpression had follicular small cleaved cell histology, compared to 25 of 69 cases without p53 overexpression (p < 0.001). There was no statistically significant difference in outcome between the group of 10 cases with p53 overexpression (median survival = 24 months) compared to those without p53 overexpression (median survival = 120 months) (p = 0.57). Eight of the 10 cases received anthracycline-containing chemotherapy regimens. The 2 patients who did not receive anthracycline rapidly succumbed to their disease. There was a trend for improved survival in the group of 21 patients without BCL2 rearrangement in their FL (p = 0.2). A group of 11 patients demonstrating BCL6 rearrangement had a projected 10 year survival of 86% compared to 35% for the BCL6 germline group (p = 0.02). Nine of the 11 cases also demonstrated BCL2 rearrangement. Only 1 of the 9 cases demonstrated p53 overexpression.

These data indicate that BCL6 rearrangement in FL is associated with prolonged survival. In addition, p53 overexpression in FL does not appear to be associated with adverse prognosis in patients treated with anthracycline-containing regimens.
8. High-dose chemotherapy

DETECTION OF TRISOMY 12 IN CD34 POSITIVE PROGENITOR CELLS OF PATIENTS WITH B-CLL BY FLUORESCENCE IN SITU HYBRIDIZATION

Divi, Hematology/Oncology in the Dept, of Internal Medicine, University Hospital, Göttingen, Germany

B-cell chronic lymphocytic leukemia (B-CLL) is a slow growing disease usually associated with the clonal expansion of mature B-lymphocytes. The most frequent chromosomal abnormality is trisomy 12. Recently more aggressive therapeutic approaches using myeloablative therapy and autologous stem cell support have been developed. Phase I/II studies have resulted in molecular remission and prolonged survival. One cause of relapse may be tumor cell contamination of the transplant. We asked whether hematopoietic progenitor cells in B-CLL are part of the malignant cell population. Peripheral blood of 17 patients with B-CLL was investigated with a DNA-probe to the centromeric region of chromosome 12 using fluorescence-in-situ hybridization. Trisomy 12 was detected in 3 out of 17 cases (18%). The number of trisomic cells ranged from 45% to 67% (mean 55%). In patients with trisomy 12 two subpopulations of hematopoietic progenitor cell, CD34+CD38- and CD34+CD38+ cells, were isolated by fluorescence-activated-cell-sorting, the sorting purity was 99%. Trisomy 12 was present in 34/46% of CD34+CD38- cells and in 34/46% of CD34+CD38+ cells of the three patients. We conclude that in patients with B-CLL trisomy 12 is frequent present in early hematopoietic progenitor cells. The data suggest that malignant transformation in this disease may occur at the level of CD34+CD38- stem cells. These results are of significance for future strategies using autologous stem cell transfusion.

SALVAGE TREATMENT FOR CHEMOTHERAPY RESISTANT DIFFUSE NON HODGKIN LYMPHOMA (NHL): REVIEW OF THE 215 PATIENTS OF THE PARMA STUDY AND RATIONALE FOR A SECOND PROSPECTIVE STUDY


A total of 215 patients with NHL in first or second relapses were treated between July 1987 and June 1994 in the Parma study. A logistic regression was performed including the parameters correlated to response: lactate dehydrogenase, CD4/CD8 ratio, duration of remission, histology, sex, age, size of tumor and Karnofsky score. Only duration of remission (cut off=6 months after the end of chemotherapy) and LDH level (cut off=normal level) were shown to be significant prognostic factors for response (p=0.0001 and 0.0269 respectively).

The term of relapse 'on' or 'off therapy' is frequently used to determined the time of relapse. This term may not be appropriate to distinguish patients according to prognosis. For a better comprehension, the term of early and late relapse are proposed. Here we try to find the most appropriate endpoint for this factor. The 188 patients in first relapse were reviewed every month using 3 possible definitions for early relapses:
- delay of relapse after complete remission: the most discriminant cut off was 4 months with 43 early relapses and 145 late relapses with respectively 25% and 43% of survival at 2 years (p<0.00001).
- delay of relapse after completion of chemotherapy: the most discriminant cut off was 5 months with 27 early relapses and 107 late relapses with respectively 19% and 48% of survival at 2 years (p=0.000001).
- delay of relapse from initial diagnosis: at 12 months, 75 patients were early relapses, 15 early relapses and 107 late relapses (survival at 2 years = 47%) (p<0.00001).

In conclusion, patients with first recurrence before 12 months of post diagnosis, phase II studies are mandatory. The PARMA project will study this group in which a double intensification (two peripheral blood stem cell transplants) will be tested.

THE CURATIVE POTENTIAL OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN CHRONIC LYMPHOCYTIC LEUKAEMIA

Dreger F, von Neuhoff N, Kaea R, Viehnmann M, Mundt T, Sattury M, Löffler H, Schmitz N. Second Department of Medicine, University of Kiel, and Dept of Haematology, AK St, Georg Hospital, Hamburg, Germany.

Chronic lymphocytic leukemia (CLL) cannot be cured by conventional therapy. We have started to evaluate the curative potential of high-dose radiochemotherapy followed by autologous stem cell transplantation (ASCT) in younger pts with early-stage CLL or lymphoplasmacytoid immunocytoma (IC). Results: 15 pts (median age 49 [20-61] years) with adverse prognostic factors (high leukocyte count and/or diffuse BM infiltration) entered the study after previous treatment with 1 (0-3) lines of conventional chemotherapy. Rai stages at referral were 0 (n=3), I/II (n=11), and III (n=1). All pts showed a clone-specific molecular marker as demonstrated by PCR amplification of CDR3 rearrangements. For stem cell mobilization and reduction of tumor load, 1-2 cycles of DEXA-BEAM chemotherapy were administered, resulting in complete histomorphological remission of BM infiltration in 7/15 pts and a strong reduction of circulating lymphoma cells (<1x10^5/L) in all cases. Stem cell mobilization/harvesting was successful in 12 pts. The grafts (BM, peripheral blood [PB]) were purified from lymphoma cells using immunomagnetic methods. So far, 8 pts were reinfused with purified ASC grafts following preperfusion with TNF-alpha. Engraftment was delayed in patients receiving BM (n=3) but prompt (ANC >0.5x10^9/L after 9-11 and platelets >20x10^9/L after 9-13 days) in pts restored with PB (n=5). Procedure-related deaths did not occur. With a median follow-up of 11 months (1-29), 8/12 pts survive without clinical or molecular evidence of disease recurrence. Conclusions: High-dose radiochemotherapy followed by reinfusion of purified ASC in pts with CLL/IC is safe and can result in durable complete molecular remission. Long-term follow-up is needed to assess if definite cures can be achieved by this strategy. Supported by Deutsche Krebshilfe (W/19/94/Schm1).

IMPACT OF HIGH DOSE SALVAGE THERAPY (BEAM) ON OVERALL SURVIVAL IN YOUNGER PATIENTS WITH ADVANCED LARGE CELL LYMPHOMAS ENTERED INTO BILPI TRIALS.


Chemotherapy with the CHOP regimen has been standard therapy for large cell lymphomas for over 20 years. Randomized trials have shown no overall advantage for newer generation regimens. The introduction of High Dose Therapy (HDT) with haemopoietic stem cell support has however resulted in improved salvage therapy as shown by the Parma Trial. In order to assess the overall impact of the availability of HDT as salvage we have compared the outcome of two cohorts of patients with stage III/IV large cell lymphoma, under the age of 60 years, treated with CHOP. The first cohort of 88 patients were entered between 1974 and 1982, when HDT was not available. The second cohort of 87 patients were entered between 1987 and 1992 when HDT was the recommended salvage therapy for chemosensitive disease. The CR rates in the two cohorts were similar at 47% and 53% respectively and the actuarial relapse rates at 5 years were also similar at 45% & 47%. The actuarial overall survival rates at 5 years were 40% and 44% indicating little improvement. Analysis of cohort 2 reveals that 62 patients failed CHOP. Nine of these patients died before receiving second line combination therapy and 8 were only offered 'palliative therapy'. Seven patients (6 Pts) were given radiotherapy to remaining disease and 3 remain in CR at 47, 49 and 61 months from diagnosis. Thirty eight patients received second line combination chemotherapy, of whom 14 were chemosensitive. Two of these patients received BEAM and one remains in remission at 51 months. Of the 24 remaining patients with chemosensitive disease only 6 received HDT. All are alive in CR at 27-50 months. Although the results of HDT were excellent they are clearly a highly selected group. If all the possible candidates for HDT had received it, and 50% remained alive at 5 years (extrapolation from literature), there would be an additional 2 survivors. This study shows that whereas HDT can achieve excellent results in selected individuals, it has made little impact on the overall prognosis of advanced large cell lymphoma.

8. High-dose chemotherapy
### AUTOLGOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR HODGKIN'S DISEASE (HD): CONTEMPORARY MANAGEMENT AND OUTCOMES OF RECURRENT AND REFRACTORY CANCER

| Age | C3S-I | IX | SJITI | 3MOPP | COND | ASCT | PRD 30yr | 92% | 9% | 18% | 16% | 47%±64% | 64%±26% | PRD | 30yr | 61% | 3% | 19% | 16% | 51%±25% | 68%±32% | PRD | R1 | 32yr | 54% | 5% | 48% | 19% | 64%±21% | 56%±41% | PRD | R2 | 32yr | 52% | 26% | 34% | 19% | 60%±29% | 75%±25% | PRD |
|-----|-------|----|------|-------|------|-----|-------|-------|-----|-----|-----|-----|----------|----------|-----|-------|-------|-----|------|-----|----------|----------|-----|-------|-------|-----|----------|----------|-----|
| CR clinical stage, 5-yr progression-free survival (PFS) (n=309) was 70% in SC, 86% in MP, and 82% in ASCT. Cond. conditioning regimen with BEAC, MOPP, or ASCT performed with bone marrow and peripheral blood stem cells (PBSC).

### 5-yr overall survival rates were 82%, 35%, 44% and 52% in P: PRD, R1 and R2 groups, respectively

SC and HD toxicity: SC* others

<table>
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<th>PSD (n=102)</th>
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<th>R1 (n=150)</th>
<th>R2 (n=69)</th>
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In Cox model, factors which influenced overall survival were indication for ASCT (PSD vs others, p<0.001), disease status at transplant (Complete remission (CR) vs no CR, p<0.001) and bone marrow ITN (p=0.031). Relative to the first general population, the incidence of SC was significantly increased (observed/expected = 18.6, p<0.001). The 3-yr cumulative incidence rate of SC was 4.9%. In Cox model, factors which influenced the SC risk were age 35 yr (p=0.003), psuedoatrophic lymphoma (p=0.046), and HV (p=0.067) and PHS (p=0.077). High-dose chemotherapy and ASCT given first-line or a relapse is associated with lower death rate. The PHS associated risk of SC, however, remains to be confirmed before ASCT is considered a safe therapy.
NEW KNOWLEDGE ABOUT T-CELL CYTOTOXICITY

J.C. Cerottini and J. Tachpoh, Ludwig Institute for Cancer Research, Lausanne Branch, and Institute of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland

In contrast to lysis mediated by antibody and complement, cytolytic T lymphocytes (CTL) mediated lysis involves target cell apoptosis rather than lysis. This means that intracellular events, in addition to membrane damage, take place in the target cell. Two independent lytic pathways have recently been identified. The first one is based on the local release of perforin granules of perforin, a membrane pore forming protein, together with a series of natural serine proteases called granzymes. The second one is based on the interaction between the CTL membrane-associated Fas ligand (FasL) and the target cell membrane-associated Fas (receptor). While the two pathways have been well documented in vivo, studies in mice in which either one of the proteins mentioned above is missing or non functional are presently being used to assess the relative role of each pathway in vivo or in vitro immunological processes that are thought to involve antigen-specific CTL-mediated lysis. The results of such studies will be discussed.

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GENERATION OF ANTI-FOLLICULAR LYMPHOMA SPECIFIC T CELL MEDIATED IMMUNITY, J. Schultz, A. Carduto, V. Boussois, G. Freeman, J. Gribben and L. Nadler, Dana Farber Cancer Institute, Boston, MA

The paucity of clinically significant anti-lymphoma specific T cell mediated immunity in patients is striking. To study this issue, we have previously demonstrated that follicular lymphoma (FL) cells are either highly inefficient or ineffective antigen presenting cells since they cannot present allogeneic. In an attempt to generate anti-FL specific autologous cytolytic T cells, FL cells were activated via the CD40 pathway by co-culturing them with CD40 ligand transfectants in the presence of IL-4. Such CD40 signaling upregulates adhesion, MHC, and costimulatory molecules including both B7-1 and B7-2 on FL cells. CD40 activated FL cells are now highly efficient allogeneic presenting cells. Although CD40 activated FL cells are efficient allogeneic presenting cells, induction of autologous anti-FL specific T cells requires the repair of two defective pathways. First, lymph node T cells isolated from most patients with advanced stage FL do not respond to CD40 activated FL cells since the zona staining signaling of the T cell receptor is both quantitatively and functionally down-regulated. In contrast, peripheral blood T cells isolated from these patients are not defective. The defect in these T cells is restricted to T cell receptor signaling since they respond normally to mitogens. Defective zona chain signaling in T cells isolated from lymph nodes can be repaired by culture in IL-2 since zona is upregulated and such culture conditions repairs defective signaling. IL-2 treated T cells isolated from FL lymph node were not cytotoxic for autologous FL cells. However, repetitive culture of lymph node T cells isolated from FL patients resulted in the generation of anti-FL specific T cells which were cytotoxic for the patients lymphoma but not for their normal cellular counterparts or FL cells from other patients. Addition of cytokines to these cultures enhanced proliferation or the generation of cytotoxicity. For example, interferon-gamma augmented proliferation whereas IL-12 enhanced cytotoxicity. These results demonstrate that CD40 activated FL cells are capable of functioning as tumor antigen presenting cells and therefore will form the basis for a clinical trial.

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Clinical Use of Immune Cytotoxic T Cells For The Treatment of EBV-Induced Lymphomas Complicating Autoimmune Marrow Transplantation

Richard J. O'Reilly, MD

EBV-associated lymphoproliferative disorders (EBV-LPD) are a well recognized complication of profound deficiencies of T-cell-mediated immunity. Recently, EBV-induced lymphomas have emerged as a significant complication of HLA-matched related and matched unrelated allogeneic marrow transplants, particularly when allografts are not extensively irradiated or the application of more intense T-cell targeted cytoreductive regimens to ensure engraftment or prevent GVHD. The EBV-LPD observed in marrow allograft recipients usually occurs as a malignant mononuclear cell infiltrate that destroys normal lymphocytes and B cell immunophenotypes involving both nodal and extranodal sites. These lymphomas are of T-cell origin need not be Hodgkinian or monoclonal, as assessed by analysis of DNA fingerprints and immunohistologic examination. The lymphomas are not clonal in origin and are unresponsive to traditional therapy, as assessed by absence of response to chemotherapy. In 1994, our group reported a series of 10 marrow allograft recipients who achieved complete and durable remissions of EBV+ nodal and extranodal lymphomas following infusion of small doses of peripheral blood mononuclear cells from their EBV-seropositive donors. Our results were confirmed by two groups who used either donor-derived PBSCs or T cells derived from EBV-seropositive donors as the effector of this adoptive therapy. The use of donor-derived T cells in vivo as a treatment for EBV-induced lymphomas is based on several considerations. First, analyses of EBV-specific T cells in normal individuals suggest that the frequency of EBV-specific T cells in normal individuals can be as low as 1-10 in 10^6. Second, the EBV-associated lymphomas developing in these patients express EBV antigens shared by EBV transformed B cells and EBV infected T cells as well. In animal models the adoptive transfer of EBV-specific T cells has resulted in the induction of EBV-specific responses and tumor regression in vivo or in vitro in response to in vitro or to in vivo rechallenge with EBV transformed cells. Third, several groups have shown that the frequency of EBV-specific T cells is increased in certain EBV-exposed individuals and is increased in frequency of EBV-specific T cells in patients with AIDS or patients with EBV infections and in patients with EBV-induced lymphoproliferative disorders. Finally, numerous studies have demonstrated the feasibility of the use the 1-10 EBV-specific CTL precursors can induce expansion of EBV-reactive T cells in vivo in the host with induction of durable and complete regressions of EBV lymphomas, clinically demonstrated as early as 1-21 days following infusion. More recently, studies with genetically modified populations of EBV-specific T cells generated in vitro have confirmed that up to 10% of the EBV-reactive T cells generated in vitro can be derived from EBV transformed cells in EBV+ cell populations. Furthermore, the EBV-specific T cells may persist in the host for periods as long as 18 months following their infusion. Studies of EBV-specific T cells derived from EBV infected populations of EBV-reactive T cells and their reactivity against EBV-reactive humoral lymphoma xenografted in SCID mice have led to early chemotherapeutic strategies directed at the T cells which can produce the durable remissions observed. These studies have also demonstrated the striking capacity of each cell to home in and induce regression of EBV lymphomas bearing appropriate viral epitopes. Based on these observations, new strategies for the prevention of EBV-reactive T cells, their current in xenografted hosts and their application to affected individuals other than EBV seropositive EBV-LPD recipients are now being developed. Specifically, allogeneic T cells are also being used to treat the patients with EBV-LPD in organ allograft recipients and patients with AIDS, as well as in the therapy of other EBV associated malignancies, particularly nasopharyngeal carcinoma of patients with Hodgkin's disease, in which tumor cells are demonstrated to express EBV-induced antigens which can be targeted by EBV-specific T cells.

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ACTIVE IDOTYPE VACCINATION FOLLOWING CHEMOTHERAPY INDUCES A SPECIFIC ANTI-ADCC IMMUNE RESPONSE TO PROLONG REMISSION IN PATIENTS WITH NON-HODGKIN'S PROLYMPHOMA.

F.J. Hsu, D.B. Cassady, D. Czerniak, L.W. Kiech, T.M. Liang, T. Tait, and R. Levy, Department of Oncology, Stanford University, Stanford, CA 94305-5306, USA

The idiotype (ID) of the surface immunoglobulin (Ig) of Non-Hodgkin's Lymphoma (NHL) is a tumor specific marker. Although nonimmunogenic on its own it can be made immunogenic by chemically coupling to foreign proteins such as a Keyhole Limpet Hemocyanin (KLH) and by mixing it with immunogenic adjuvants. Animals immunized with ID-KLH are protected against tumor challenge. Immunogenic NHL sera can be cured by combination with non-curative chemotherapy. A clinical study was initiated in 1989 evaluating active ID-KLH vaccination after chemotherapy to prevent relapse of NHL. To date more than 60 patients with NHL who have advanced stage follicular lymphoma have been enrolled and 20 patients have completed all treatments and are evaluable. The NHL sera from patients were followed for up to 4 years after therapy. We found that tumor specific ID-KLH antibodies were present in 98% of the patients with relapsed NHL. Nodal idotype was generated from idotype samples using hybridoma technology. A further treatment to ID-KLH in patients with NHL who had relapsed was ID-KLH administered to patients at 6 month intervals. Of 20 patients with advanced stage lymphoma, 17 showed a significant increase in the proportion of ID-positive cells compared to the baseline. 11 patients remained relapse free over 18 months. ID-KLH was effective in both relapsed NHL and follicular lymphoma. In conclusion, active ID-Idotype vaccination in NHL patients is a viable alternative to chemotherapy.
BCL-1/CYCLIN D1 IN MALIGNANT LYMPHOMA AND CLINICAL CORRELATIONS.
Kluin PM, de Boer CJ, Velders GA, Vaandrager JW, Schuuring E, van Krieken JHJM. Laboratory of Pathology, Leiden University Hospital, POBox 9600, 2300 RC Leiden, The Netherlands

Chromosomal breaks within BCL1 at chromosome 11q13 have been associated with specific subtypes of B-cell lymphoma. Breakpoints may be part of the (11;14) translocation by which the cyclin D1 gene is deregulated by immunoglobulin enhancer sequences at 14q32. Approximately 50-75% of mantle cell lymphomas, 5-15% of splenic lymphomas with villous lymphocytes and immunocytoma, but also rare cases of CLL and multiple myeloma/plasma cell leukemia may harbor this chromosomal breakpoint. So far it is unknown whether the breakpoints at 11q13 are similar for all malignancies. The BCL1 region originally has been described as a 120 kb large region centromeric of Cyclin D1 with clustering of breakpoints at the major translocation cluster (MTC). However, using a novel method, DNA fiber FISH, by which breakpoints in large genomic regions of 500 kb can be detected, we demonstrated extensive scattering of 19 different breakpoints over the entire 120 kb region, including a breakpoint 100 kb centromeric of the MTC. Other groups have described rare variant translocations involving the 3' end of Cyclin D1 and Ig light chain genes, reminiscent of the variant translocations in Burkitt's lymphoma. Thus almost all, if not all MCL have a breakpoint in the expanded (250 kb) region of BCL1. In agreement with these results almost all MCL have overexpression of Cyclin D1 as demonstrated by Northern blot, Western blot and immunohistochemical analysis. Additional abnormalities like deletions in the 3' untranslated region harboring RNA destabilizing sequences, may further contribute to the increased expression of the gene. We developed an immunohistochemical assay for detection of nuclear expression of Cyclin D1, using the DC56 monoclonal antibody. This assay can be used on formalin fixed and paraffin embedded tissue sections. Overexpression is restricted to MCL and rare other lymphomas, but a moderate level of expression is also consistently found in hairy cell leukemia (HCL). No translocations could be found in HCL, indicating that other mechanisms may lead to overexpression as well. MCL patients have a poor prognosis with a low response to current chemotherapy and a median survival of 2 to 3 yrs. Further characterization using age, clinical stage, but especially morphology, DNA ploidy, PS3 expression and the proliferative index as defined by KI-67, may further delineate subgroups of patients with different prognosis.

FROM ONCOGENES TO TUMOR CELLS IN MALIGNANT LYMPHOMA
Korsmeyer, S. J., Howard Hughes Medical Institute, Washington University School of Medicine, 660 S. Euclid, Box 8022, St. Louis, MO 63110 USA.

The Bcl-2 gene was identified at the chromosomal breakpoint of (14;18) bearing B cell lymphomas. Bcl-2 has proved to be unique among proto-oncogenes in blocking programmed cell death rather than promoting proliferation. In adults, Bcl-2 is topographically restricted to progenitor cells and long-lived cells but is much more widespread in the developing embryo. Transgenic mice that overexpress Bcl-2 in the B cell lineage demonstrate extended cell survival, and progress to high grade lymphomas. Bcl-2 deficient mice complete embryonic development and display relatively normal hematopoietic differentiation but undergo fulminating lymphoid apoptosis of thymus and spleen. Moreover, they demonstrate profound renal cell death and develop polycystic kidney disease and also hair hypopigmentation. A family of Bcl-2 related proteins regulate cell death and share highly conserved BH1 and BH2 domains. Bax counters Bcl-2 and promotes apoptosis. A Bax knockout mouse displayed hyperplasias and resistance to cell death in several lineages. However, Bax-deficient males display spermatogenesis with disordered seminiferous tubules and multinucleated giant cells accompanied by massive cell death of gametes. The loss of Bax results in hyperplasia or hypoplasia depending on cellular context. BH1 and BH2 domains of Bcl-2 were required for it to heterodimerize with Bax and repress apoptosis. A yeast two-hybrid assay defined a selectivity and hierarchy of further dimerizations. Bax also heterodimerizes with Bcl-xL, Mcl-1, and A1. Interactive cloning identified a new protein, Bad, which has homology clustered in BH1 and BH2 domains and heterodimerizes with Bcl-xL and Bcl-2. Bad reverses their protective effect restoring apoptosis. Thus, the susceptibility to a death stimulus in a given cell is dictated by a complex set point determined by the relative levels and interactions amongst these family members.
10. Clinical results in lymphoma I

THE INTERNATIONAL PROGNOSTIC FACTORS PROJECT ON ADVANCED HODGKIN’S DISEASE: A PROGNOSTIC INDEX, BUT NO VERY HIGH RISK GROUP

D. Hasenclever1 and V. Diehl1 for the International Prognostic Factors Project on Advanced Hodgkin’s Disease; 1IMISE, University of Leipzig, D-04103 Leipzig, Germany; Clinic I. T. N. Medical University, D-50924 Köln, Germany

An international cooperation was organized to define a prognostic index for advanced stage Hodgkin’s disease treated with modern state-of-the-art chemotherapy (+radiotherapy). Data were collected from 22 study groups from Europe and North America on 4937 patients considered as advanced stage and treated accordingly in a well-defined protocol. Individual patients’ data on course of disease and 20 covariate measurements at diagnosis were requested (age, gender, histology, stage, mediastinal mass, inguinal involvement, organ involvements, B symptoms, albumin, ESR, AP, HB/Hct, leukocytes, lymphocytes, platelets, LDH, serum creatinine). Analysis so far concentrated on tumour control as endpoint (censoring deaths not preceded by HD progression). The objective was to identify specifically those patients in which standard treatment fails to eliminate the disease and experimental approaches may be indicated. Low haemoglobin is the most informative single factor; chance of cure decreases continuously with the haemoglobin level at diagnosis. Most other factors investigated were confirmed as prognostic in univariate analyses with an effect in the direction expected from the literature, except: multiple organ involvement within stage IV, histology and creatinin. Marked leukocytosis (> 1610^9/L) was present in 15% of the patients and turned out to be relevant. Besides haemoglobin, all the observed univariate prognostic differences are moderate or small (in the order of 10% in tumour control rates at 5 years). Such small prognostic effects cannot be reliably detected (with sufficient statistical power) in a few hundred patients from single group data. This underlines the necessity of an intergroup effort in this setting. A Cox regression model comprising Hb, stage IV, symptoms, inguinal involvement, very large mediastinal mass, leukocytosis, lymphopenia and gender was constructed. Again, individual factors except Hb carry only moderate relative risks in the order of 1.3. Cross-validation (take-one-out) techniques were used to safeguard against overfitting and to calibrate the model for independent prediction. This eight-factor prognostic index quantitatively discriminates expected 5 year tumour control rates in the range of 50-80%. Using the model, only 5% of the patients have an expected chance of less than 50% to be progression free at 5 years. In conclusion, although some fine-tuning of the model is still under way, it is improbable that a substantial “very high risk group” within advanced stage Hodgkin's disease can be identified using routinely documented parameters when state-of-the-art treatment is given.

TREATMENT OF EARLY STAGE HODGKIN’S DISEASE: IMPROVED OUTCOME WITH BRIEF CHEMOTHERAPY AND RADIOTHERAPY WITHOUT STAGING LAPAROTOMY

R.J. Kiess, J.M. Connors, R. Farrow, R. Gassoigne, P. Hains, S. O'Reilly, T. Shekstier, N. Voss, K. Wilson, British Columbia Cancer Agency (BCCA), Vancouver and Victoria, B.C., Canada

From 589 to 12/95, all patients (pts) with low bulk (mediastinal ratio < 1/3) clinical stage (CS) IA (n=28) and 2A (n=85) Hodgkin’s Disease (HD) seen at the BCCA were managed without staging laparotomy (p=113). The median age was 31 (range 15-63), 63 were males, 34 had nodal extranodal histology (NS) and 103 had supradiaphragmatic disease. 98 pts were treated with 2 cycles of chemotherapy (CT) followed by extended field radiotherapy (RT). Chemotherapy was VLB (vincristine (V), doxorubicin (A), bleomycin (B), VAB) (76), VACOP-B (Vincristine V, doxorubicin (A), cyclophosphamide (C), prednisone (P)) (26) in 60 pts, COPP/ABV Hybrid (C, O, procarbazine, P, A, B, V) in 59 pts or ABVD in 6 pts. 91 of the 98 pts were treated according to protocol, 7 protocol violations, 6 pts received 4 to 8 cycles of CT without RT for various reasons (contraindication to RT n=3, physician choice (n=1), initial staging error (n=2) and 1 pt refused all treatment after excisional biopsy. Until 12/93 pts with CS1A and 2A non-mediatinal (neck and/or axilla) NS or lymphocyte predominant histology (LP) or CS1A NSH of only the mediastinum were treated with RT alone (n=15). Because the only 2 relapses observed in the entire 113 pts occurred in this group, after 12/93 this type of pt also received 2 cycles of CT followed by RT. All 113 pts achieved a complete remission with no severe toxicity. With a median follow-up of 31 m (range 1 to 83) there have been only 2 relapses, both in the 15 pts who received only RT. None of the 98 pts who received brief chemotherapy and RT have relapsed. 1 pt died in the 3rd week of CT and the other had a relapse on 3-4 weeks after CT. Conclusions: Brief chemotherapy added to extended field RT while omitting staging laparotomy in improved 7year relapse-free survival with minimal acute toxicity in early stage HD.

FOUR WEEKS OF NEO-ADJUVANT CHEMOTHERAPY SIGNIFICANTLY REDUCES THE PROGRESSION RATE IN PATIENTS TREATED WITH LIMITED FIELD RADIOTHERAPY FOR CLINICAL STAGE (CS) I/IIA HODGKIN’S DISEASE. RESULTS OF A RANDOMISED PILOT STUDY

J.A. Radford1, R.A. Can3, W.D.I. Ryder2, D.P. Dkin4, R.D. James2, P.M. Wilkinson4 and D. Crowther1 1CRC Dept. Medical Oncology, 2Dept. Radiology, 3Medical Statistics and 4Clinical Pharmacology, Christie Hospital NHS Trust, Manchester, M20 4BX, UK

Since 1989, 111 patients with previously untreated CS IA and IIA H.D. (no B symptoms or mediastinal bulk) have been randomised to receive either limited radiotherapy (RT) alone or 4 weeks of VAPESC-B neo-adjuvant chemotherapy (CT) followed by RT. CT consisted of doxorubicin 35mg/m2 i.v. weeks 1 and 3, cyclophosphamide 350mg/m2 i.v. week 1, etoposide 100mg/m2 p.o. days 1-5 week 3, vincristine 1.4mg/m2 i.v. weeks 2 and 4, bleomycin 10mg/m2 i.v. weeks 2 and 4, with prednisolone 50mg/m2 p.o. i.v. weeks 1-4 then tailed zero over 10 days and prophylactic ketoconazole/eco-simexole.

At the time of analysis 93 pts. (median age 38 years) who had completed treatment (RT, n = 47; CT + RT, n = 46) were available. Follow-up in the combined modality arm, 30 pts. had achieved CR, 4 pts. CR uncertain and 11 pts. PR. After subsequent RT 40 pts. were in CR, 5 pts. in CR uncertain and 1 pt. in PR. In the RT alone arm 38 pts. achieved CR, 4 pts. CR uncertain, 3 pts PR and 1 pt. failed to respond. Four pts. experienced significant toxicity with CT, 3 developed infections requiring i.v. antibiotics, 1 received treatment for a grade III mucositis and another had an exaggerated skin reaction to subsequent RT. All made a full recovery.

After a median follow up of 3.3 years, there have been 17 progressions (RT alone arm, 15; CT & RT arm, 2). 4 deaths due to HD (RT alone arm 3; CT & RT arm, 1), 1 death due to high grade NHL (CT & RT arm; diagnosis confirmed at relapse and presentation) and 1 second malignancy (RT alone arm). Actual survival-progression-free survival (PS) at 3 years is 73% for the RT alone arm and 91% for the combined treatment arm.

The results of this randomized pilot study suggest that a short course of weekly combined CT given before RT is sufficient to produce a significant improvement in PS compared with limited RT alone for patients with CS I/IIA H.D.

META-ANALYSIS OF RANDOMIZED TRIALS OF MORE EXTENSIVE RADIOTHERAPY (RT) AND OF ADJUVANT COMBINATION CHEMOTHERAPY (CT) IN EARLY STAGE HODGKIN’S DISEASE (HD). L. Specht and R. Gray, University of Copenhagen, Denmark, and Clinical Trial Service Unit, Oxford University, UK, for the International Hodgkin’s Disease Collaborative Group

Purpose: To determine if more extensive RT or adjuvant CT improve survival in early stage HD. Patients and methods: Collaboration was sought worldwide between coordinators of all known randomized trials in early stage HD comparing more vs. less extensive RT, or RT with or without adjuvant CT. Individual patient data, including age, stage, date of entry, treatment allocation, date of recurrence and date and cause of death, were provided for 1974 pts. in 8 trials of more extensive RT and for 1653 pts. in 12 trials of adjuvant CT, with tabular data on a further 226 pts. Meta-analyses used log-rank statistics. Results: Both more extensive RT and adjuvant CT produced highly significant improvements in relapse free survival. However, because of the success of salvage CT, overall survival was not improved by more extensive RT (reduction in odds of death ± 1 SD: 1.2% ± 8.5%). There was a trend towards improved overall survival with adjuvant CT (reduction in odds of death 9.3% ± 9.3%), which was more pronounced for deaths from Hodgkin’s disease (reduction 20% ± 12%), but neither was conventionally statistically significant. Conclusion: No significant improvement in overall survival or mortality of HD from more extensive RT or adjuvant CT in early stage HD could be demonstrated despite the large reductions in recurrences seen.
COMBINED MODALITY TREATMENT VS CHEMOTHERAPY ALONE IN HODGKIN'S DISEASE (HD): AN OVERVIEW ON RANDOMIZED TRIALS

Overview study group; 1IMISE, University of Leipzig. D-04103 Leipzig, Germany

The objective of this metaanalysis was to clarify the role of combined modality treatment (CMT) as compared to chemotherapy (CT) alone in intermediate and advanced stage HD-patients (ps). 30 randomized trials on CMT vs CT were identified by screening literature and databases. Data from 14 trials were accessible and analysed jointly. Data on gender, age, histology, stage, systemic symptoms, randomization arm, primary treatment outcome, relapse, date of last follow-up and death were almost complete. Information was partly available on mediastinal involvement (88%), mediastinal mass (50%), bulky disease (37%), date of relapse (83%), 2 trials consistently without date of relapse) and cause of death (4% of all deaths), 1752 pts were included for survival (SV) analysis, 1552 for analysis of freedom from HD-failure (FFH). Trials differ in design (5 confounded, i.e. long CT vs short CT+RT; 4 with randomization conditional on CR), duration (7 with 6 cycles) and type of chemotherapy planned in the CT-arm (4 Adriaycin containing) and extent of radiotherapy (RT) planned (7 with more involved field). Significant heterogeneity in the observed treatment effects was revealed by study groups (SV: p=0.05, HDF: p=0.005). Therefore, the trials were grouped according to duration of chemotherapy in the CT-arm (50%, >6 cycles). Trials with long CT correspond mainly to those with Adriaycin containing regimen and planned RT design. Short CT regimens were applied to advanced stages. A multivariate Cox regression analysis stratified by trials adjusting for stage, systemic symptoms, histology and invasion involvement was performed. Overall survival and relapse were improved by RT in short CT trials (p=0.0004), but this benefit is less pronounced in pts with stage IV, systemic symptoms or unfavourable histology (MC, LD). In trials with long CT, RT added little to overall survival or in pts with stage IIIA with major mediastinal mass. 9/10 pts with mediastinal involvement large mediastinal mass seem to experience a moderate benefit. The addition of RT in primary treatment had no impact on overall survival neither in long nor in short CT trials. In conclusion, the addition of RT after modern Adriaycin containing chemotherapy with more than 6 cycles showed little benefit in advanced stage HD. However, causes of death should be investigated in greater detail to reveal competing risks.

LONG-TERM RISK OF SECOND CANCERS IN YOUNG SURVIVORS OF HODGKIN'S DISEASE

F.E. van Leeuwen, W.J. Kloekman, A. Hogenbeek, A.W. van den Belt-van Buskirk, R. Sonneveld. The Netherlands Cancer Institute, Amsterdam, The Netherlands

Although many studies have assessed the overall risk of second cancers (SC) development in patients with Hodgkin’s disease (HD), there is no consensus focussed specifically on the long-term risk of this complication in patients who were adolescents or young adults at diagnosis of HD. We assessed SC risk in 641 5-10 year survivors of HD who were less than 30 years old at diagnosis of HD and who were admitted to the Netherlands Cancer Institute (Amsterdam) or the Dr. N. van Eijk-Heidema Cancer Institute (Rotterdam) between 1966 and 1986. The median follow-up duration was 15 years.

In all, 60 patients developed a SC (6.6 cases expected on the basis of cancer incidence in the general population; relative risk (RR): 9.1; 95% CI: 6.9-13.7). The mean 25-year actuarial risk of all SCs was 28%. Significantly increased RR were observed for digestive tract cancers (n=13; RR=5.4), lung cancer (n=1; RR=8.3), breast cancer (n=15; RR=8.5), urogenital cancer (n=5; RR=4.6), thyroid cancer (n=4; RR=28.4), NHL (n=4; RR=44.1) and leukemia (n=7; RR=35.1). The excess risk of solid tumors was related to RT; patients treated with a combination of RT and CT had similar risk of solid tumors as compared to risk in patients treated with RT alone (RR=3.3; 95% CI: 6.3-13.2). The excess RR varied from RR=8.6 (95% CI: 5.0-13.8), the excess risk of solid cancers (RR=16.8; 95% CI: 10.1-26.3) was highest among those who survived for 20 years or more, with strongly increased risks for cancers of the breast (RR=23.5), digestive tract (RR=24.8) and thyroid (RR=220). Patients irradiated before age 20 had a significantly higher excess risks of breast and digestive tract cancers than those irradiated after age 20. Among 115 survivors the relative risks of breast cancer in women irradiated before and after age 20 were 38.9 and 9.3, respectively. Also after breast cancer was excluded, women treated for HD experienced higher excess risks of SC than men (RRs: 13.6 versus 8.2; p < 0.05).

PATIENTS WITH STAGE III/IV HODGKIN’S DISEASE (HD) IN PARTIAL REMISSION (PR) AFTER MOPP/ABV CHEMOTHERAPY HAVE EXCELLENT PROGNOSIS AFTER ADDITIONAL INVOLVED FIELD RADIOTHERAPY: INTERIM RESULTS FROM THE ONGOING EORTC/ECIM Study 409084 on stage III/IV HD (462 patients accrued), patients in CR after MOPP/ABV are randomised between no further treatment and involved field RT (IF-RT). No results are available as yet from this randomised part of the trial. Patients with PR after 6 cycles of MOPP/ABV all receive additional IF-RT to all initially involved areas (dose: nodal 30 Gy, lung 18 Gy, liver 20 Gy, bone 24 Gy with boost if indicated). Here, we present the outcome of these PR-patients. A total of 118/130 patients are evaluable (12 too early). Clinical characteristics at entry: mean age 33 yrs, stage IIIf69%+31%, bulky disease (>10 cm or MT >20.35) 48%. In eight patients (7%) no IF-RT was given because of ABMT (n=2), expected intolerance (n=3), refusal (n=3). In 75 patients (68%) IF-RT was given according to protocol, in 20 (26%) most areas received IF-RT whereas some involved areas were given no RT, in three (3%) both nodal areas received a dose <24 Gy. No toxic deaths occurred. Twelve patients (11%) developed grade IIIi hematologic toxicity. After a median follow-up of 33 months, 84% of the 160 patients who actually received IF-RT, were in continuous CR. Only 16 patients (16%) developed relapse or progression. The calculated 4-year freedom-from-failure rate was 90% with a 4-year cause-specific survival rate of 92%. 10% of patients after MOPP/ABV-Induction and MF-RT of Hodgkin’s disease produce excellent failure-free and overall survival rates. There is no need for high-dose treatment with ABMT/PBSCT in this category of patients.

PRESENTATION FEATURES AND CLINICAL COURSE OF MANTLE CELL LYMPHOMAS - RESULTS A EUROPEAN SURVEY


Mantle cell lymphoma (MCL) represents a more recently recognized and accepted subentity of non-Hodgkin’s lymphomas with distinct histologic, immunophenotypic and molecular characteristics. Histomorphologically MCL have been classified among the low grade lymphomas although its clinical course and sensitivity to conventional chemotherapy appears more unfavorable. However, because of its relatively low frequency of cure, the information is based on small numbers of cases and prospective evaluations are limited to few studies only. Stimulated by the definition of clearly defined diagnostic criteria that emerged from a workshop of the European Task Force on Lymphomas at Amsterdam a retrospective collection of data was initiated to improve the basis of currently available knowledge. Presentation features and clinical data of 573 patients were collected from nine European institutions or multicenter studies. Diagnostic material was reviewed by expert pathologists and 392 cases were found to fulfill the Annency criteria. The median age of these patients was 63 years and male sex predominated with 72%. Eleven percent of cases were diagnosed at limited stages I and II while the majority of patients (75 %) were at stage IV. Eighty-four percent of patients had a performance status of 0 or 1 and 38 % presented with B symptoms. Sixty-nine percent of percent of cases had bony marrow involvement and 66 % revealed extranodal involvement of therapeutic response or data on survival were available in stages I and II. These data broaden the currently available knowledge about the features of presentation of MCL and may allow to define distinct factors of prognostic relevance.
11. Clinical results in lymphoma II

High response rate after 2-chlorodeoxyadenosine (2-CDA, cladribine) treatment in non-pretreated patients with follicular non-Hodgkin’s lymphomas (NHL) stage III-IV

D. C. Bitticher1, A. von Rohr1, T. Egger1, E. Zucco2, J. Radford2, K. Bürki4, B. Rufener4, B. F. Hsu4, C. Morris2, T. Perry3, 1Institute of Med. Oncology, Inselspital, University of Bern, 2Servizio Ospedaliero del Canton Ticino, Ospedale San Giovanni Bellinzona, 3Dept. of Med. Oncology, Christie Hospital, Manchester, UK, 4Institute of Pathology, University of Bern, ESTWAK Coordinating Center, Bern, Switzerland for the Swiss Group for Clinical Cancer Research.

2-CDA, a pyrimidine analogue, is active in low-grade lymphoproliferative disorders. Follicular NHL has been identified as a particularly sensitive subgroup. We designed a phase II multinstitutional trial to study toxicity and activity of 2-CDA in untreated follicular NHL. We also assessed the presence of cells carrying the t(14;18) translocation (Bcl-2/IGH rearrangement) in peripheral blood and bone marrow by PCR before, during and after treatment. Methods: Between May 1993 and October 1995, 37 patients were accrued: male/female: 15/22, median age 51 years (range: 20-78), stage III: 9; stage IV: 28. Confirmation of diagnosis was obtained by histopathological review. Patients received a total 2-CDA dose of 0.7mg/kg as a continuous sc or iv infusion over 7 days, every 28 days for a maximum of 3 cycles. Results: Thirty-seven patients are evaluable for toxicity and response. A total of 165 cycles were administered. In 21 patients analysis of the Bcl-2/IGH rearrangements are available before, during and after treatment. Overall confirmed response rate was 84% (95% CI, 68-94%) with 14% CR [n=5] and 70% PR [n=26]. Bcl-2/IGH rearrangement in peripheral blood or bone marrow was found in 11/21 of patients at study entry and in 5 patients, it became negative after three cycles. Change to negativity was not associated with response. In 10 patients, therapy was interrupted because of progressive disease (n=2), severe myelosuppression (grade 2-3), n=1 and other causes (pulmonary embolism, n=1, metabolic disorder, n=1 and patient’s decision, n=1). In 4 patients (11%), long lasting thrombocytopenia or neutropenia (grade 2-3) were associated with cumulative 2-CDA dose (≥2.0 mg/kg), and 4 patients (11%) suffered from infections (grade 2-3).

Conclusion: 2-CDA is highly active in untreated follicular NHL, but its ultimate role will depend on the remission duration and survival when compared to standard treatment. Although molecular remission is induced in a considerable proportion of patients with disappearance of the Bcl-2/IGH rearrangement, its possible prognostic significance warrants further study.

Superiority of a Brief Course of CHOP Plus Radiotherapy (RT) Over CHOP Alone in Localized Unfavorable Non-Hodgkin’s Lymphoma (NHL) is Seen in Lowest Risk Subgroups: A Southwest Oncology Group Study

TM Grogan, S. Carlin, E. Chase, R. Fisher, Tucson, AZ; Seattle, WA; Maywood, IL, USA

Localized NHL (stage I, non-bulky II) is frequently a systemic disease and requires initial treatment with doxorubicin-containing chemotherapy. To better define the best treatment, we randomized 401 eligible patients with localized intermediate and high-grade NHL to CHOP (3) followed by involved field RT or to CHOP (8) alone between 3/88 and 3/95 (SWOG 87/36). CHOP included 750mg/m² cyclophosphamide, 50mg/m² doxorubicin, 2.0 vincristine all given iv on day 1 of 21 day cycle, and 100 mg prednisone given po daily for 5 days. RT followed 3 cycles of CHOP and included all sites of initial disease (prior to biopsy or resection). A minimum of 4000 cGy was delivered to the tumor volume with an optional boost to a maximum of 5500 cGy for residual overt disease (daily dose 180-200 cGy). Overall survival (OS) including deaths from all causes, CHOP (3) plus RT (p<0.01). 4-year survival estimates are 75% for CHOP (8) and 87% for CHOP (3) plus RT. Progression-free survival (PFS) is not significantly different (p=0.09) and the difference between OS and PFS appears to reflect excess deaths in the CHOP (8) arm (primarily cardiac) occurring after completing treatment. Reversible toxicity occurring during therapy also favored CHOP (3) plus RT over CHOP (8) with 31% of patients having grade 4 toxicity vs. 40%, respectively (p<0.05). Subgroup analysis demonstrates OS and PFS varies with International Prognostic Index risk groups (p=0.0001 and p=0.0002, respectively). A significant difference in favor of CHOP (3) plus RT was seen only the low-risk subgroup where the 4-year survival for 176 CHOP alone treated patients was 88% and for 176 CHOP plus RT treated patients was 78% (p<0.01). We conclude that improved outcome (survival) for patients treated with CHOP (3) plus RT is seen in patients with the best pre-treatment prognostic factors.
RANDOMIZED COMPARISON OF MACOP-B WITH CHOP FOR INTERMEDIATE-GRAdE NON-HODGKIN’S LYMPHOMA: LONG-TERM FOLLOW-UP.

M. Wolf, I. Cooper, T. Robertson, R. Fox, J. Matthews, J. Stone for the Australian and New Zealand Lymphoma Group, Peter MacCallum Cancer Institute, St Andrew's Place, East Melbourne 3002, Australia.

Between October 1986 and June 1991, 304 patients with intermediate-grade non-Hodgkin’s lymphoma were randomized to either CHOP x 6 cycles or MACOP-B. In an analysis of 236 eligible patients followed for a median of 32 months, CHOP produced equivalent results to MACOP-B but with significantly fewer toxic complications (J Clin Oncol 12: 769-778, 1994). Patient follow-up has now been extended to a median of 5.8 years. The 5 year actuarial progression-free survival is 44% (s.e. 5%) for MACOP-B and 29% (s.e. 4%) for CHOP (p=0.015). Due to a number of late relapses, the 7 year actuarial progression-free survival is 36% (s.e. 5%) for MACOP-B and 25% (s.e. 5%) for CHOP (p=0.1). The 5 year overall survival is also significantly better for MACOP-B (54% vs. 45%) than for CHOP (40% s.e. 2%). P=0.03. However at 7 years this difference is not statistically significant (44% s.e. 6% vs 38% s.e. 5%), p=0.5. This long-term follow-up suggests that the more intensive MACOP-B chemotherapy has resulted in more patients being cured of their lymphoma in our study. Further follow-up is required to confirm these results.


High-dose therapy followed by autologous transplantation improves the outcome of sensitive-relapse patients (pts) with aggressive NHL and may also benefit partial responder pts. Whether this approach may be used as part of initial treatment is unclear and the question now is to determine the circumstances in which it should be employed. We previously reported (J.O.C. 12: 2543-51, 1994) a study of 646 poor risk pts in CR after induction treatment (ACVBP with Doxorubicin versus NCVBP with Mitoxantrone : 4 cycles) comparing by randomization two consolidative procedures. In this study, the CBV regimen (Cyclophosphamide : 6 g/m2, Carmustine : 300 mg/m2, VP16 1 g/m2) followed by ABMT was not superior to sequential chemotherapy (Ifosfamide plus Etoposide, Asparaginase, Cytarabine), although a trend in favor of CBV existed in the poorer-risk pts. Here we report the updated results of our series, which now includes 541 randomized-CR pts and focus on the comparison between the two consolidative arms when these pts are partitioned on the basis of International Index. From Oct 87 to Feb 93, 916 eligible pts were analyzed for eligibility, their main characteristics being : median age 40 y, large-cell subtypes (72%), performance status 0-2 (73%), stage III-IV (66%), number of extranodal sites ≥ 2 (24%), tumor mass ≥ 10 cm (55%), bone marrow involvement (24%), LDH ≥ 1.4 (61%), 68% of them achieved CR. The CR rate was 71% in the ACVBP (53%) and 63% in the NCVBP induction groups (390 pts) (P=0.01). Randomization of the autotransplant being stopped in Dec 91, 341 CR pts were assessible for the consolidation phase with a median follow-up of 53 mo.; the 5-year disease free survival (DFS) rate was 52% in the sequential chemotherapy arm and 59% in the ABMT arm (P=0.22). The 5-year survival rate did not differ either, at 67% and 68%, respectively (P=0.80). The International Index partitioned the 916 eligible pts into risk groups that significantly differed for DFS and survival. When applied to the pts randomized for consolidation, we found no statistically significant differences according to the consolidation arm in the low risk group (0 factor, 34 pts) and low-intermediate risk group (1 factor, 207 pts). On the other hand, the 5-year DFS rate was significantly higher in the ABMT arm (57% vs 36%, P=0.01) in the high-intermediate and high risk group (2-3 factors, 236 pts); the 5-year survival rate also differed (65% vs 52%, P>0.06). We now conclude that dose-intensive consolidation therapy should be proposed to pts at higher risk who achieve CR after induction treatment.

CHOP VERSUS MACOP-B IN HIGH GRADE MALIGNANT NON-HODGKIN’S LYMPHOMA: A PROSPECTIVE, RANDOMIZED STUDY BY THE NORDIC LYMPHOMA STUDY GROUP. M. Jerkeman1, E. Cavallin-Stahl1, H. Hågberg2 and B. Kvaløy3. For the Nordic Lymphoma Study Group: 1Department of Oncology, University Hospital, Lund, Sweden; 2Department of Oncology, University Hospital, Uppsala, Sweden; 3Department of Oncology, Radiumhospitalet, Oslo, Norway.

Patients and methods: Between 1989 and 1994, 403 patients with high grade malignant non-Hodgkin’s lymphoma according to the updated Kiel classification, Ann Arbor stage III-IV, aged between 18 and 67, were randomized to receive either eight courses of CHOP or twelve weeks of MACOP-B. Presently, data from 253 patients are available, upon which the following preliminary analysis is based. Analysis of the entire study population will be complete by early spring 1998. All patients’ records were reviewed by a panel from the Nordic Lymphoma Study Group, with special emphasis on response evaluation and the classification of residual masses. The majority of histopathological specimens were reviewed by a board of hematopathologists. Results: 23/253 patients were considered ineligible, mostly due to misdiagnosis. Among the remaining 230 patients, the median age was 54 years and 84% were stage IV. According to the International Index, 16% were high intermediate and 14% high risk patients. The two treatment groups were well balanced as regards age, stage, bulky disease, WHO performance status, S-LD, bone marrow involvement and International Index. The rate of complete remission (CR) was 42% in the CHOP group and 46% in the MACOP-B group. Among responding patients, a residual mass was present in 37% and 42%, respectively. At a median time of follow-up of 34 months, overall survival was similar: 60% in the CHOP group, 64% in the MACOP-B group. Median failure-free survival rate was 49% with CHOP and 52% with MACOP-B. Toxicity was acceptable. WHO grade III-IV leukopenia was present in 34% of the CHOP group and in 45% of the MACOP-B group. Grade II-IV mucositis occurred in 4% and 32%, respectively. Treatment-related mortality was low; 2.8% with CHOP and 3.3% with MACOP-B. Analysis of all 403 patients, including dose intensity and subgroup analysis of categories according to the International Index, will be presented at the conference.

ELDERLY LYMPHOMA PATIENTS HAVE A LONG SURVIVAL IF TREATED WITH A CURATIVE INTENT. A STUDY FROM THE G.E.L.A. ON 453 PATIENTS OLDER THAN 69 YEARS.


Optimal treatment for elderly patients (pts) with aggressive non-Hodgkin’s lymphoma is not known. The GELA has investigated in a randomized trial the addition of pirarubicin (THP-adriamycin, 50 mg/m2/CTVP) to the CVP regimen (cyclophosphamide 750 mg/m2, teniposide (VM-26, 75 mg/m2) and prednisone 40 mg/m2) in pts ≥ 70 every 3 weeks for 6 courses. 220 pts were randomized in CVP arm and 235 in CTVP arm. Median age was 75 years and 18% were older than 75 years. Most of the pts had clinically aggressive disease with 53% having 2 or 3 adverse prognostic parameters as defined by the International Index. More CTVP pts had high LDH level but otherwise the 2 groups were well balanced. 8% CTVP pts had a decrease in administered doses compared to 2% for CVP pts. CTVP was more frequently associated with neutropenia, thrombocytopenia, infections, mucositis, and alopecia. Toxic death rate was 12% and 15% for CVP and CTVP. Complete response was reached in 47% of CTVP pts and 33% of CVP pts (p=0.001). Median disease-free survival was 3.5 y for CTVB pts and 2.8 y for CVP pts (p=0.10). 5-year time to treatment failure (TTF) was 2.1% for CTVP vs 14% for CVP pts (p<0.05). Median survival was 13 m, not different for CTVB and CVP pts but more CTVB pts survived at 5 y (26% compared to 19%, p<0.05). Pts older than 80 y had the same survival as 69-74 y or 74-79 y pts. In a multivariate analysis, CTVB treatment was associated with a longer survival even after a lower rate of 3-5 year survival (p<0.05). We conclude that elderly pts have a good outcome if treated with a curative intent and that treatment is not associated with a high rate of adverse events. An anthracycline-containing regimen was associated with better outcome and, thus, such treatment is recommended as standard treatment for elderly pts without severe cardiac dysfunction.
PRIMARY LYMPHOMA OF THE STOMACH: 3-YEARS-RESULTS OF A PROSPECTIVE MULTICENTER STUDY

P. Koch, B. Groothaus-Pinke, W. Hiddemann, N. Willich, B. Reers, F. del Valle, H. Bodenstein, M. Pfundesruck, E. Muller, R. Parwaresch, M. Tiemann, for the German Multicenter Study Group on GI-NHL.

Department of Medicine, University of Muenster, D-48129 Muenster, Germany.

Since biology and appropriate management of gastrointestinal (GI) lymphoma is still an ongoing controversial debate, we initiated in October 1992 a prospective multicenter study to evaluate histological features, sites of involvement and therapeutic outcome after a standardised treatment. Primary surgery or conservative management depended on the physician's decision, followed by radio-therapy and chemotherapy. Standardised diagnostic workup included central histologic review and endoscopic and radiologic evaluation of the complete GI-tract.

Until 15th January 1996 312 pts. have been accrued, 250 being evaluable for clinic features. The distribution of GI-NHL is as follows: Stomach 73.2 %, small bowel 10.0 %, ileocecal region 6.4 %, multilocul GI-involvement 7.2 %. In gastric lymphoma low grade NHL accounted for 38.3 %. Of the remaining high grade NHL 35.5 % showed simultaneous low grade components thus being also of MALT origin.

Of 183 patients with gastric NHL 71.6 % were classified as stage Ia and IIa. Analysis of stage in relation to histology revealed a growing fraction high grade NHL with increasing stages.

After a median observation time of 20 months 152 pts. are evaluable for treatment outcome. CCR-rate in stomach lymphoma is significantly higher compared to those of small bowel, whereas involvement of multiple GI-organs has the worst prognosis.

So far only 12/112 pts. with gastric NHL presented with progressive disease or relapse. Over all stages there seems to be no difference in therapeutic outcome in surgically or conservatively treated patients. Even after R0-resection in limited stages patients appear to have no better outcome than those who did not undergo surgery.

There was no fatal bleeding or perforation during conservative treatment, whereas additive therapy in operated pts. was often delayed or incomplete because of reduced performance status.

The value of surgery in treatment of primary gastric lymphoma - as favoured by most authors - should be re-examined.
12. Clinical-pathological correlations

RARE FORMS OF PERIPHERAL T-CELL LYMPHOMA (PTL)

E. Jaffe, L. Kreams, M. Raffeld, Natl. Cancer Institute, Bethesda, MD 20892, USA

It is ironic to speak of rare forms of PTL, since all forms of PTL are rare, representing only 10-15% of non-Hodgkin's lymphomas. While one could challenge the need to delineate them, if these diseases have a distinct biology, natural history, epidemiology, and response to therapy, it is critical that they be recognized. Lumping together diverse disease entities only obscures results of clinical trials. Many extranodal PTL share some common features, and are related to NK- and NK-like cytotoxic T-cells.

Typically, PTL presents mainly in young adult males (15-35 yrs.), with marked heparosplenorenalomegaly, thrombocytopenia, and minimal PB involvement. BM involvement is usually present, but may be difficult to detect without immunocytochemistry. The course is aggressive (median survival < 2 yrs.)

The tumor is composed of monomorphic medium-sized cells, which preferentially infiltrate and expand sinuses. The cells have a Y- or P-cell phenotype and genotype. Immunocytochemistry is a specific cytogenetic feature.

Angiocentric T/NK cell lymphoma usually presents in the nasal area, with the clinical syndrome presenting as a nasal polyp. It is most common in Asia.

Strongly associated with Epstein Barr virus (EBV), the presence of EBV predisposes to a hemophagocytic syndrome (HPS). The cells do not show T-cell gene rearrangement (TCR) and are of NK cell origin. Local disease is responsive to radiation therapy, but there is a high relapse rate, and even with systemic therapy, late relapses with widespread disease are common. Secondary sites of involvement include GI tract, skin and subcutaneous tissue, testis, and other extranodal sites. The extensive necrosis seen may be secondary to angioinvasion as well as other factors.

Subcutaneous panniculitis-like PTL presents with multiple subcutaneous nodules, most common on extremities. There is a spectrum of cytologic grade, and early cases may be mistaken for benign panniculitis. HPS is a common complicating event, and is the immediate cause of death in most patients. The cells are EBV neg. Both Y- and P-Tcell forms have been described. Systemic spread is uncommon, but the median survival is < 3 yrs.

Intestinal PTL is histologically similar to other extranodal forms of PTL, and is frequently associated with sprue. The disease usually presents with an acute abdomen, often secondary to jejunal perforation. The lesions are ulcerative with extensive necrosis. Clonal TCR is present, and the cells express the homing receptor CD103. The clinical course is aggressive.

PLEOMORPHIC LARGE T CELL LYMPHOMAS HAVE A WORSE PROGNOSIS THAN DIFFUSE LARGE B CELL LYMPHOMAS

C. Gisselbrecht, Ph. Gaulard, E. Lepage, B. Collette, F. J. Briere, G. Herion, D. Casas, A. Bosio, L. Xerri, H. Thiéry, F. Berger, B. Bourhis, J. Diefeldt for the GELA, Hospital Saint Louis, Paris France

From 10/1987 to 3/1993, 1973 pts with diffuse mix and large cell Non-Hodgkin's Lymphomas (NHL) included in the LNHL87 protocol were eligible for review of both morphology and immunochemical typing. These included 276 pts with peripheral T cell lymphoma (15%), and 1595 (85%) pts with B cell lymphoma. According to the Ki67 classification, T-cell NHL were classified as follow : T-zone, lymphoepithelioid lymphoma, pleomorphic small cell, and angioimmunoblastic (32%), pleomorphic large T cell (50%), anaplastic T (17%). Mean age was 54 yr. old. T cell lymphomas when compared to B cell NHL had more disseminated stage (64% vs. 47%), B symptoms (60% vs. 40%), positive bone marrow (35% vs. 27%), skin involvement (20% vs. 4 %, increased LDH (45% vs. 32 %). B cell lymphomas had more bulky disease (40% vs. 25 %), GI involvement (29% vs. 15 %). Abnormal LDH level equal to or equivalent (51% and 49%). Five years overall (OS) and event free survival (EFS) were 52%, 45% and 42%, 33% for B cell and T cell lymphomas respectively (p < 0.001, < 0.0001). This difference was mainly observed when the 140 pleomorphic large T cell NHL were compared to the 1387 diffuse large B cell NHL. For large B and T cell NHL pts, OS and EFS were respectively, 52% vs. 35% (< 0.0001); 45% vs. 28% (p < 0.0001). Comparison between B and T NHL pts was made in the different stratum of the international prognostic index. In each stage, OS was inferior for T cell NHL but never reached a significant level. In the Cox’s model, age, LDH, performance status (PS), bone marrow involvement were highly independent significant factors (p < 0.0001) affecting OS while stage (p = 0.054), T immunophenotyping (p = 0.088) were not significant. However, when Cox’s was restricted to the pts with large B vs large T NHL age, LDH, PS, BM remained highly significant and T immunophenotyping was an independent parameter (p<0.003). Conclusion : Peripheral T cell lymphomas have a poor prognosis, mainly related to the presence of adverse prognostic factors at diagnosis. However, pleomorphic large T cell lymphoma patients have a worse prognosis independently of other parameters.

THE CLINICAL SIGNIFICANCE OF bcl-2 AND P53 PROTEIN EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA: A POPULATION-BASED STUDY

Kramer MHH, Hermans J, Parker J, Krol AGG, Kluin-Nelemans JC, Haak H, Kroesbergen KV, Kranken HVM van, de Jong D, Kluin PM, Dept of Pathology, Medisch Wetenschaps, Clin Oncology and Hematology, University Medical Center Leiden; Hematology and Pathology, Municipal Hospital Leyden, The Hague; Pathology, The Netherlands Cancer Institute, Amsterdam; Comprehensive Cancer Center West, Leiden. POBox 9900, 2300 RC Leiden, The Netherlands

Purpose: to assess the prognostic significance of bcl-2 and p53 expression in patients with de novo diffuse large B cell lymphomas as defined by the REAL classification. Methods: 372 patients were retrieved from a population-based registry of 1186 patients with non-Hodgkin’s lymphoma (NHL) and were enrolled between 1981 and 1988 with follow up until 1994. bcl-2 and p53 protein expression were studied by immunohistochemistry on paraffin sections and were scored quantitatively. Response to therapy and survival were analyzed in 165 patients who underwent full staging, received adequate treatment and for whom all prognostic data were available according to the International Prognostic Index (IPI).

Results: 45% of the tumors showed strong expression of bcl-2 (bcl-2 ++ ) with a score of 3 or 4. Sixty-seven % of patients with a bcl-2 negative/intermediate (bcl-2 +) lymphoma and 51% of patients with a bcl-2 + lymphoma had complete remission (CR). Complete remission rates were Disease-free survival (DFS) was better in patients with a bcl-2 + NHL than bcl-2 ++ NHL (p<0.01); at 5-years, bcl-2 + and bcl-2 ++ NHL patients showed a DFS of 74% and 41%, respectively (p=0.002). However, overall survival (OS) was not significantly influenced by bcl-2, probably due to the effect of rescue therapy after relapse. For DFS, bcl-2 was the strongest independent prognostic value after multivariate analysis (relative risk of 2.94) in comparison with p53 expression, age, LDH level, Karnofsky performance, stage and number of extranodal sites. P53 expression was found in 16% of NHL, but to a small extent in patients with extensive disease (31%). In the 165 patients, P53 expression did not influence CR, DFS and OS. Conclusions: bcl-2 protein is frequently expressed in large B lymphomas and is a strong independent prognostic factor for DFS. P53 expression is associated with a high tumor burden but is not an independent risk factor.

PROGNOSTIC SIGNIFICANCE OF BCL-2 EXPRESSION AND BCL-2 MBR REARRANGEMENT IN DIFFUSE LARGE CELL NHL: A WLAN STUDY

ME Hill, KA Macleman, D Cunningham, B Vaughan Hudson, M Buck, P Clarke, F Di Stefano, L Anderson, G Vaughan Hudson, D Mason and DC Linch. CRC Section of Medicine & Lymphoma Unit, Royal Maraden NHS Trust and Institute of Cancer Research, Sutton, and The British National Lymphoma Investigation, London, UK

Background The Bcl-2 protein is capable of preventing apoptosis and in vitro evidence suggests a role in drug resistance. Expression of Bcl-2 and rearrangement of the bcl-2 gene is detectable in some diffuse B-cell NHLs but the clinical significance of these findings is controversial. The purpose of this study was to determine definitively the influence of both Bcl-2 expression and bcl-2 MBR rearrangement in a large cohort of prospectively accrued patients treated in a standardised manner.

Patients and Methods All patients with Working Formulation (WF) F, G or H NHL, treated with CHOP chemotherapy in BLNHL studies between 1974 and 1992 were entered if paraffin blocks from the diagnostic specimens were available. Sections from the blocks were analyzed for evidence of MBR rearrangement using a PCR based method, and for Bcl-2 expression using immunohistochemistry. Failure to achieve CR, relapse, death from lymphoma and deaths from all causes were used as end points to measure CR rate, actuarial relapse rate, actuarial survival from NHL and actuarial overall survival.

Results 161 suitable patients were identified and tested for the bcl-2 MBR translocation, with 27 (17%) found to be positive. 153 of these patients were tested with immunohistochemistry and 84 (55%) showed evidence of bcl-2 expression. None of the pre-treatment characteristics were present in significantly different proportions in MBR positive and MBR negative patients or immunopositive patients compared to immuno-negative. However, for patients who achieved CR from their initial treatment, the relapse rate was significantly higher in those with Bcl-2 expression than in those without. In addition, multivariate analysis identified Bcl-2 expression significantly related to relapse at the 0.01 level of significance. The cause specific survival from NHL was significantly lower in patients with Bcl-2 expression than in those without. MBR status had no significant influence on any of the outcome measures.

Conclusions Expression of Bcl-2 is an important prognostic factor in WF F, G and H NHL of B-cell type, whilst bcl-2 MBR translocation appears to have no significant impact on clinical outcome.

12. Clinical-pathological correlations
HIGH GRADE B-CELL NON HODGKIN'S LYMPHOMAS: KIEL CLASSIFICATION IS A SIGNIFICANT PROGNOSTIC FACTOR
Dept. of Medicine, University of Essen, D-45122 Essen, Germany

The REAL classification of non-Hodgkin's lymphomas (NHL) defines one common category of diffuse large B-cell lymphoma which includes three major entities of high grade malignant B-type NHL distinguished by the Kiel classification, namely centroblastic, B-immunoblastic, and B-large cell anaplastic (Ki-1+) lymphomas. In order to clarify whether this simplification is clinically justified, the relevant subset of 217 patients from a prospective treatment trial (COP-BLAMIMVP-16 regimen ± radiotherapy, conducted in adult Ann Arbor stage II-IV patients) was analysed separately. In a final review all diagnoses were confirmed by histomorphometry (Giemsa staining) and immunochemistry. After a median follow-up of 3.5 years significant differences between centroblastic, B-large cell anaplastic (Ki-1+), and B-immunoblastic lymphoma were detected both for overall (p < .0001) and relapse-free (p = .0028) survival. These differences persisted in multivariate analyses including the risk factors of the International Index. Overall survival was significantly determined by the Kiel classification (p < .019), the LDH (p = .0187), and the performance status (p = .0001). Relapse-free survival was critically influenced by the Kiel classification (p < .019) and the age (p = .016). These results establish the Kiel classification as an independent prognostic factor appropriate for the identification of high risk patients otherwise not recognised within the REAL-category of diffuse large B cell lymphomas.

DIFFERENTIAL EXPRESSION OF APOPTOSIS IN MALIGNANT LYMPHOMA.
M. Hermann, H. Stein* and F. Schriefer, Virchow Hospital, Department of Hematology, Humboldt University, Berlin and *Institute for Pathology, Benjamin Franklin Hospital, Free University Berlin, Germany

Deregulation of programmed cell death (apoptosis) seems to be involved in the malignant transformation of lymphoid tissues. It is yet unknown, however, what leads to the dysfunction of apoptosis in non-Hodgkin's lymphomas (NHL). The present study investigated whether NHL and non-lymphoid tissues differ in the expression of apoptosis and in the phagocytosis of apoptotic cells by macrophages. In addition, tissue localisation of proteins, that influence apoptosis, e.g. the Bcl-2 family, c-Myc, Fas, fas-ligand (Fas-L), and Fas, was studied. Method: Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling in situ was combined with immunostaining using mAbs against Fas, c-Myc, Bcl-2 and against the macrophage antigen CD68. Tissue expression of Bax, Bcl-xL, Bcl, McI-1 and Fas-L was detected using rabbit antisera. Results: Normal tonsils and lymph nodes contained large numbers of apoptotic cells within germinal centers, which were positive for Bax, Bcl-xL, Bcl-2 and Fas-L. Compared with normal lymphoid tissues NHL (n=35) expressed significantly less apoptotic cells. The extent of apoptosis, however, did not correlate with the grade of malignancy of the NHL subtype. In addition, expression of Bcl-2, Fas or c-Myc was not related to the extent of apoptosis in the different NHL. Whereas in tonsils >85% of all apoptotic cells were contained in macrophages, some NHL entities differed significantly in their expression of phagocytosed apoptotic cells. In CB/CC NHL (n=8) >81% of all apoptotic cells were phagocytosed by macrophages. In contrast, in CB (n=9) NHL 55±13% and in CC (n=3) NHL only 34±10% of all apoptotic cells were engulfed by macrophages. Conclusions: Bax, Bcl-xL and Fas-L seem to have a role in ensuring the high degree of apoptosis in the germinal centers of normal lymphoid tissues. Bcl-2, Fas or c-Myc, do not appear to influence the grade of apoptosis in NHL. The amount of apoptotic cells appears not to be related to the grade of malignancy of the NHL. The differences in phagocytosis of apoptotic cells by macrophages in some subtypes of NHL could indicate a defect in macrophage function or alterations of cell surface receptors of lymphoma cells.

AMPLIFICATION OF GENOMIC DNA DEMONSTRATES THE PRESENCE OF THE (2;5)(p23;q35) IN ANAPLASTIC LARGE CELL LYMPHOMA, BUT NOT IN OTHER NON-HODGKIN'S LYMPHOMAS, HODGKIN'S DISEASE, OR LYMPHOMATOID PAPULOSIS
A H Sarris, R. Luber, V. Papadimitriouczopoulos, M. Wasaard, A. M. Dimopoulos, J. A. Mcclure, F. Casamitjana, M. Deplano, J. Latner, F. Comoll, R. Calandrella, R. Calandrella. Department of Hematopathology, Hematology, and Dermatology, The Univ. of Texas M.D. Anderson Cancer Center, Houston, Texas; and Department of Experimental Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee.

Anaplastic large cell lymphoma (ALCL), is a distinct clinicopathologic variant of intermediate grade Non-Hodgkin's lymphomas (NHL), that carries the (2;5)(p23;q35) chromosome translocation fusing the nckopathoimmun (NPM) gene on chromosome 5p35 to a novel protein kinase gene (Anaplastic Lymphoma Kinase, ALK) on chromosome 2p23. We determined the frequency of this translocation with a novel DNA polymerase chain reaction (PCR) technique using 0.5 mg of genomic DNA, 5' primers derived from the NPM gene and 3' primers derived from the ALK gene and validated the assay by detecting amplification using DNA from all three ALCL cell lines tested, or from all four primary ALCL tumors known to contain the t(2;5)(p23;q35) translocation. Amplification were detected in 100% of specimens diluted 10^4-fold and in 30% of those diluted 10^5-fold. We subsequently examined genomic DNA from 22 patients with ALCL, 39 with diffuse large cell, 2 with mantle cell, 20 with peripheral T cell, 13 with low grade NHL, 31 with Hodgkin's disease (HD), and 6 with lymphomatosus papulosis (LP). Fusion of the NPM and ALK genes was detected in 4 of 22 (18%) of ALCL with 95% confidence intervals (CI) of 5%-48%. Among ALCL it was confined to the 18 with nodal clinical presentation (22%, 95% CI 6%-48%) and to 40% of those younger than 40 years (95% CI 12%-74%). Amplion sizes differed in all cell lines and patients reflecting unique genomic DNA breakpoints that were confirmed by direct DNA sequencing, and served as controls against laboratory contamination. The rearrangement was not detected in other NHLs, HD or LP. We conclude that with genomic DNA-PCR the rearrangement of the NPM and ALK loci is restricted to patients with ALCL, and is not seen in HD or LP that carry t(2;5). A does not difficult to distinguish from ALCL. Further studies are needed to determine the prognostic significance of the NPM-ALK rearrangement, to determine whether its detection can aid in the differential diagnosis between ALCL, HD, and LP, and to establish the usefulness of the genomic DNA PCR in the monitoring of minimal residual disease in those patients whose tumors bear the t(2;5).

12. Clinical-pathological correlations

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POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD): CLINICOPATHOLOGIC CHARACTERIZATION AND RESPONSE TO IMMUNOMODULATORY THERAPY WITH INTERFERON-ALPHA.

D. Lietzowitz, J. Anastasi, F. Hagos, M.M. LeBeau, and O.I. Olopade. Department of Medicine, Section of Hematology/Oncology and Department of Pathology, University of Chicago Medical Center, 5841 S. Maryland Avenue, Chicago, IL 60637.

Post-transplant lymphoproliferative disease (PTLD) is recognized as a heterogeneous group of lymphoproliferations occurring in patients immunosuppressed after organ transplantation. The majority of these cases are of B cell origin and are Epstein-Barr virus (EBV)-associated. We have examined 38 cases of PTLD in adult and pediatric organ transplant recipients. The purpose of the present study is to characterize the clinicopathological features of PTLD examining the EBV status and viral gene expression pattern in the tumors and identifying cytogenetic abnormalities which may be associated with PTLD, and to examine the role of interferon therapy in this disorder. Our patient population consisted of 20 adult and 18 pediatric patients. Twenty of the patients had undergone liver transplant, 10 had renal transplants, 7 had cardiac transplants and 1 had a liver/renal double transplant. There were 27 males and 11 females who ranged in age from 8 months to 62 years. The median time to development of PTLD post-transplant was 4.5 months, and ranged from 1 to 50 months. The EBV status was examined in 22 cases, and was positive in 18/22. Two of the negative cases were B cell in origin and two were T cell lymphoproliferations. The pattern of viral antigen expression did not vary with other variables such as histology or karyotype. Cytogenetic analysis on 18 cases revealed six distinct groups: (1) 3 patients (17%) had Burkitt's translocations involving chromosome 8; (2) 3 patients (17%) were trisomy 9; (3) 2 patients (11%) had abnormalities at 3q27 involving the Bcl-6 gene; (4) 2 patients (11%) were trisomy 11; (5) 2 patients (11%) had rearrangements involving immunoglobulin loci, but not involving chromosome 8; and, (6) 7 patients (39%) had non-clonal abnormalities or normal karyotypes. Eighteen adult and pediatric patients with EBV-positive disease were treated with interferon-α2b and reduction in their immunosuppression. There were 14 (77%) complete responses (CR) and 1 (6%) partial response (PR) for an overall response rate (CR + PR) of 83%. Three patients were not evaluated for response due to early death. Complications of therapy included increased organ rejection and life threatening infection in 50% (9/18) of patients. The median overall survival was only 6 months.