

# **ABSTRACTS**

## **ORAL PRESENTATIONS**

## 1 PATHOGENESIS OF EBV IN LYMPHOPROLIFERATIVE DISORDERS IN IMMUNOCOMPROMISED HOST.

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In lymphocytes transformed *in vitro* or in acute lymphoproliferative disease, in humans, EBV expresses six nuclear proteins or EBNA's, two integral plasma membrane proteins or LMPs and two small RNAs or EBERS. Studies of the biologic and biochemical activities of the EBNA's or LMPs in rodent fibroblasts or non EBV infected BL lymphocytes has revealed that (i) EBNA 1 is essential for episome maintenance (B. Sugden et. al.) (ii) EBNA 2 and EBNA 3C are transactivators of specific cell or virus genes (iii) LMP1 is a dominant transforming oncogene which enables Rat 1 cells to become contact uninhibited, anchorage independent and tumorigenic in nude mice. (iv) LMP1 interacts with vimentin and forms a patch in the plasma membrane of lymphocytes, constitutively inducing much of the lymphocyte activation effects associated with EBV infection and lymphocyte growth transformation or with antigen activation. (v) LMP2 associates with LMP1 in the plasma membrane and also associates with the Src family tyrosine kinases *fyn* and *lyn* and with a 70 kda cell protein. LMP2 is a tyrosine and serine threonine phosphorylation substrate. Importantly, EBNA's or LMPs expressed in primary rodent fibroblasts could not complement *myc* or *Ras* in transforming these cells suggesting a requirement for specific interaction with other EBV genes or a limited host range for primary cell growth transformation. In order to investigate the specific and direct activity of the EBNA's, LMPs and EBERS in primary B lymphocyte growth transformation, specifically mutated EBV recombinant molecular genetic strategies have been developed. Thus, EBNA 1P, 2, 3A, 3C and LMP1 are critical or essential for primary B lymphocyte growth transformation; and, EBNA 3B, LMP2 and the EBERS are fully dispensable for all aspects of *in vitro* B lymphocyte infection and growth transformation. Strategies have been developed for obtaining mutant recombinants even in these essential transforming genes using non EBV infected BL cells as hosts for passage of non transforming EBV recombinants. Novel B lymphocyte genes which are up regulated by EBV infection have been identified. The most interesting are those which have motifs similar to known cytokine receptors or gene transactivators. These are being pursued to evaluate their role in normal and malignant B lymphocyte growth and differentiation.

Aside from acute lymphoproliferative disease which is seen with advanced immune compromise or in unusually susceptible humans, EBV is associated with Hodgkin's disease, Burkitt's Lymphoma and anaplastic Nasopharyngeal carcinoma. These latter malignancies occur long after EBV infection and presumably result from a multi step process of malignant evolution in which EBV can play an early etiologic role. A characteristic pattern of EBV genes are expressed in these malignancies. The role of EBV gene products in these late stage malignancies is uncertain; although, they can be diagnostically useful.

## 2 ROLE OF VIRUSES IN THE ETIOLOGY OF LYMPHOMAS. L. Weiss. Division of Pathology, City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010 USA

Several viruses have been proposed to be involved in the etiology of non-Hodgkin's lymphomas, including human T-cell leukemia virus-1 (HTLV-1), Epstein-Barr virus (EBV) and human herpes virus-6 (HHV-6). HTLV-1 is a human retrovirus that is endemic in several regions, including the southernmost islands of Japan. It remains latent for many years after infection, but a minority of patients will develop adult T-cell leukemia/lymphoma (ATLL). Proviral integration sites are mono- or oligoclonal, with no apparent integration sites that are common among different cases of ATLL. HTLV-1 produces several proteins, including *tax* and *rex*, that may interact with the viral genome or cellular transcriptional factors. Evidence of HTLV-1 infection, either complete or defective, has also been found in approximately 10% of patients with mycosis fungoides, particularly the CD30+ large cell variant. EBV and HHV-6 are herpes viruses with a prevalence rate of approximately 90% in developed nations. Initial infection with either virus produces a permanent infection of lymphoid cells. EBV has been associated with lymphomas arising in immunocompromised patients, Burkitt's lymphoma (particularly in endemic regions of Africa and South America), sinonasal T cell lymphomas (particularly in Asia and South America), and sporadically with other B and T cell lymphomas. HHV-6 has been associated with a premalignant disorder termed atypical polyclonal lymphoproliferation and with rare cases of B cell lymphoma. Despite the documented associations between EBV and HHV-6 and various types of malignant lymphomas, no direct evidence of a role for the viruses in the etiology of the lymphomas has been obtained. It is possible that these viruses serve as a constant promoter of cellular proliferation, increasing the pool of cells at risk for lymphomagenesis by other mechanisms, and also perhaps increasing the frequency of other genetic events.

3

Epstein-Barr Virus Associated Lymphoproliferations.

H. Stein.

Summary

In recent years, techniques, probes, and reagents became available to reliably visualise individual Epstein-Barr virus (EBV)-infected cells, to assess EBV gene expression, and to analyse the clonal composition of EBV genomes in human tissues. Application of these techniques to more than 1000 lymphoid tissue specimens revealed (1) characteristic cellular and compartmental distribution patterns of EBV-infected cells in normal lymph nodes, reflecting the interference of EBV with physiologic B cell differentiation pathways, (2) an association of EBV with various mono- and oligoclonal lymphoproliferations ranging from benign conditions to overtly malignant lymphomas, and (3) characteristic patterns of EBV gene expression among EBV-associated lymphoproliferations. In the context of the established immortalising and transforming properties of EBV, the findings support the concept of an etiologic role of EBV for cases of certain lymphomas such as Burkitt's lymphoma, anaplastic large cell lymphoma, plasmablastic lymphoma, Hodgkin's disease, and lymphomas arising in immunocompromised individuals. In contrast, lymphomas harbouring EBV in only proportions of the tumour cells (such as cases of peripheral T cell lymphoma and some B cell lymphoma types) argue against an etiologic role in the primary process of malignant transformation for the virus in these instances. Since in many of these cases a proportion of the EBV-infected tumour cells express the EBV oncoprotein LMP (latent membrane protein) the virus may influence, however, the proliferative properties as well as the morphological and molecular phenotype of the neoplastic cells.

4

#### EPSTEIN-BARR VIRUS (EBV) LATENT AND REPLICATIVE GENE EXPRESSION IN AIDS-RELATED NON HODGKIN'S LYMPHOMA (NHL) AND POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD)

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NHL and PTLD arising in the setting of immunodeficiency states such as HIV infection and organ transplantation are frequently associated with EBV. 3 forms of latent infection have been described in vitro. In type I latency (LMP-, EBNA2-) only EBNA1 is expressed. In latency II (LMP+, EBNA2-), EBNA1 and LMP are detected. In type III latency (LMP+, EBNA2+) the 6 EBNA1s (1, 2, 3A, 3B, LP, 3C) and the 3 LMPs (1, 2A/2B) are expressed. Furthermore EBV replicative cycle can be activated from latent infected cells. In order to investigate EBV cycle in vivo, the pattern of EBV latent and replicative gene expression was analyzed in respectively 18 and 9 EBV positive AIDS-related NHL and PTLD. EBV status was determined either by Southern blot with the BamHI W probe or with the EBER1 riboprobe. EBV latent and replicative proteins were detected by the immunoperoxidase and the alkaline phosphatase anti alkaline phosphatase techniques. The following monoclonal antibodies were used: Cs1-4 and PE2 directed against the latent proteins LMP1 and EBNA2, BZ1 directed against the immediate early replicative protein BZLF1, F3.23, H667 and 65 directed against the late proteins VCA, MA and BCRF1. Histologic subtypes of AIDS-related NHL included 10 large cell NHL (LCL) and 8 BL. PTLD consisted in 4 polymorphic polyclonal cases (PP), 3 polymorphic monoclonal cases (PM) and 2 monomorphic monoclonal cases (MM). In AIDS-related NHL, the 3 forms of latent infection were detected in LCL whereas BL more often expressed type I latency. A few proportion of BL showed type II latency but failed to express type III latency. In PTLD, polymorphic proliferations expressed the 3 types of latency whereas MM only showed type I latency. Replicative proteins were present in 5 LCL and 1 BL which all expressed the BZLF1 protein, indicating a switch from latency to replication. However late viral proteins were not detected in BL and VCA was detected in 2 LCL without expression of other late viral proteins. In PTLD, BZLF1 was detected in 5 polymorphic cases but only 2 cases expressed late viral proteins. MM failed to express EBV replicative proteins. Our results indicate that the 3 forms of latent infection are represented in vivo in AIDS-related NHL and PTLD. LCL and polymorphic PTLD frequently express type II or type III latency whereas BL and MM show more often type I latency. Noteworthy, latency II is observed in a few proportion of BL. EBV replicative cycle occur in a great proportion of cases. However the initiation of EBV replicative cycle may not always lead to viral production. Further studies should be undertaken to better understand the role of EBV replication in malignant cells in AIDS-related NHL and PTLD.

- 5** HODGKIN'S DISEASE (HD) AND NASOPHARYNGEAL CARCINOMA (NPC) SHARE IDENTICAL AND SIMILAR DELETIONS WITHIN THE LATENT MEMBRANE PROTEIN ONCOGENE (LMP). H.Knecht, E.Bachmann, R.Sahli<sup>1</sup>, P. Brousset<sup>2</sup>, D.Nadal<sup>3</sup>, F.Bachmann, B.F. Odermatt<sup>4</sup>. Division of Haematology and <sup>1</sup>Institute of Microbiology, CHUV, Lausanne; <sup>2</sup>Institute of Pathology, CHU Purpan, Toulouse; <sup>3</sup>Kinderspital and <sup>4</sup>Institute of Pathology, USZ, Zürich.

The LMP oncogene of the Epstein-Barr virus (EBV) is about 1300 base pairs long and specifically expressed in Sternberg-Reed cells of about 50% of HD cases and in tumour cells of about 60% of NPC cases.

The LMP sequences of two NPC (NPC 1510 and NPC CAO) have recently been published. Compared with the standard EBV sequence (EBV B95-8) both show deletions of 30 base pairs near the 3' end and small insertions within the internal repeat (IR) region. In 31 cases of HD expressing the LMP oncogene, we amplified the coding sequence by PCR. In three patients we localised small insertions also within the IR region and in two patients deletions in the same place as described for NPC. DNA sequencing revealed a 30 base pair deletion in case HD 22 (nearly identical to those observed in NPC 1510 and NPC CAO (table) and a 70 base pair deletion in case HD 61A encompassing the region of the 30 base pair deletion (table).

Base No: 168288

168250

EBV B95-8:	GGT CAT AGT CAT GAT TCC GGC CAT GGC GGC GGT GAT CCA
NPC 1510:	GGTCAT.....CCA
NPC CAO:	GG.....T GAT CCA
HD 22:	GG.....T GAT CAA
HD 61A:	.....

**Conclusion:** Deletions and insertions within the LMP oncogene occur in precise localisations (hot spots) shared by HD and NPC. It is likely that these mutations - known to render the LMP oncogene more aggressive in NPC- have the same capacity in HD.

- 6** MODERN TREATMENT OF MALIGNANT LYMPHOMAS: A MULTIDISCIPLINARY APPROACH? Gianni Bonadonna, Istituto Nazionale Tumori, Milan, Italy.

Following rigorous staging procedures that also included staging laparotomy, Henry Kaplan has amply demonstrated that the correct application of megavoltage irradiation is curative in a fraction of patients with Hodgkin's and non-Hodgkin's lymphomas. At the end of the 1970s it became equally evident that several drug combinations were able to cure a fraction of patients with advanced Hodgkin's disease and large cell non-Hodgkin's lymphomas. Although a number of clinical trials have indicated, particularly in Hodgkin's disease, that combined treatment modality resulted in a superior outcome compared to radiotherapy alone, most clinical efforts were focused on refining drug treatments and have somewhat neglected the curative potential of radiotherapy. One of the reasons is represented by the frequency of iatrogenic morbidity (e.g. second malignancies, cardiac and pulmonary dysfunctions) from combining chemotherapy with radiotherapy. Thus, today there is lack of consistent information which clearly demonstrates the optimal use of combined modality treatment. However, current data suggest that in many clinical situations the multidisciplinary approach is useful both to reduce the recurrence rate from chemotherapy and to spare important morbidity from extensive irradiation.

## 7 CD30 Ligand: Molecular Cloning and Pathobiological Role in CD30-Positive Malignant Lymphomas

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CD30, a cell surface antigen, has been identified on the neoplastic Hodgkin and Reed-Sternberg (H-RS) cells in Hodgkin's disease (HD), Ki-1 large cell anaplastic lymphoma (LAL) cells, and on activated lymphoid cells of either T or B cell origin. The CD30 molecule is a member of the TNF/NGF receptor superfamily. The corresponding CD30-ligand (CD30L) was identified on the membrane surface of a stimulated murine T cell clone (7B9) using a soluble, recombinant form of the extracellular portion of CD30. By direct expression cloning a 1.6 kB cDNA for the murine CD30L was isolated. A 1.7 kB human CD30L cDNA clone was isolated by cross hybridization of a stimulated peripheral blood T cell lambda library. The CD30L sequence revealed a 239(muCD30L)/234(hCD30L) amino acid type II membrane protein with approximately 75% homology. The C-terminal domain of the CD30L shows significant sequence homology to TNF- $\alpha$ , TNF- $\beta$ , CD27L and CD40L. CD30L is another member of a growing ligand superfamily for the TNF/NGF receptor superfamily. CD30L mRNA is strongly expressed in activated T cells, monocytes/macrophages and granulocytes, but not in cultured H-RS cells. We are investigating the expression pattern of CD30L in primary HD tissue sections and in Ki-1 LAL sections by using in situ hybridization. The CD30L induces proliferation of cultured H-RS cells and can induce apoptotic cell death in the CD30-positive LAL cell line Karpas 299. CD30L is a pleiotropic cytokine with a possible role as growth factor for the pathogenesis of Hodgkin's lymphoma. Further studies for the role of CD30L in the pathobiology of CD30-positive malignant lymphomas are under investigation.

## 8 PRIMARY CD30+ ANAPLASTIC LARGE CELL LYMPHOMA — A DISTINCT CLINICOPATHOLOGIC ENTITY —

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The morphology of anaplastic large cell lymphoma (ALCL) is associated with a clinical syndrome of peripheral lymphadenopathy (>80%) and frequent extranodal disease (>40%) in children and young adults (median age <40 yr). Skin lesions occur in more than 20% of patients; other extranodal sites are bone, soft tissue, GI tract, lung and pleura. Marrow involvement is infrequent (<10%). Distinguishing features from Hodgkin's disease (HD) are non-contiguous nodal disease (>50%), infrequent mediastinal mass (<20%), and common inguinal lymphadenopathy (>40%). Most patients present with stage III/IV disease. Stage is highly predictive of achieving complete remission, disease free survival, and overall survival. Localized skin lesions have an excellent prognosis and occasional spontaneous regressions are noted. Distinctive histopathologic features of ALCL are partial lymph node involvement, sinus infiltration, sparing of B-cell regions and tumor cell pleomorphism. Other features are high mitotic rate, necrosis, fibrosis, and plasma cell infiltrate. Morphologic variants resemble anaplastic carcinoma, syncytial variant of nodular sclerosing HD, true histiocytic lymphoma / interdigitating cell sarcoma, and mycosis fungoides. ALCL can be distinguished from morphologically similar disorders by immunophenotype (CD30+, CD45+, EMA+, BNH9+, CD15-, keratin-, lysozyme-). A recurrent cytogenetic translocation, t(2;5)(p23;q35) has been observed among morphologic variants and a CD30+ cell line which includes both small cerebriform and large Reed-Sternberg-like cells. 70% of ALCL are T-cell lineage, 15% B, 5% T/B, and 10% undefined. ALCL appears to be distinct from other peripheral T-cell lymphomas such as HTLV-1+ adult T-cell leukemia, angioimmunoblastic lymphadenopathy, angiocentric T-cell lymphoma and cutaneous T-cell lymphoma, occurring mainly in older patients. These combined clinical, pathologic, immunophenotypic and cytogenetic observations support the concept that ALCL is a distinct clinicopathologic entity.

**9 ANAPLASTIC LARGE CELL LYMPHOMA: MORPHOLOGY AND CLINICOPATHOLOGIC CORRELATIONS.**  
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The expression of CD30 antigen has been demonstrated in a variety of non-Hodgkin's lymphomas. These large cell lymphomas are commonly referred to as "anaplastic large cell lymphomas (ALCL), Ki-1 positive", if tumor cells have no "regular" morphologic counterpart.

Retrospective analysis of certain cellular features demonstrated that ALCL exhibit heterogeneous morphology: the larger group was composed of large pleomorphic cells intermingled with multinucleated giant cells; the second group showed a relatively monomorphic appearance with less pronounced nuclear pleomorphism of the large tumor cells and very rare multinucleated giant cells. A subgroup of the latter consisted of rather small neoplastic cells with some similarities to immunoblastic lymphoma of T-cell type. Clinically, both types differed in that the more monomorphic variants (large cell monomorphic and immunoblastic-like variants of ALCL) presented with advanced stage of disease (stage III and IV in >70% of cases), whereas the former group (large cell pleomorphic with giant cells) belonged to stage III and IV in a lesser extent (about 30%). Bone marrow infiltration was found predominantly in the more monomorphic variants.

Immunophenotypic heterogeneity was observed regarding B or T cell phenotype. However, ALCL did not differ with respect to the expression of CD 44, CD 44 variants as well as LFA-1 $\alpha$  and  $\beta$ . "Secondary" ALCL (arising from mycosis fungoides, Hodgkin's disease, peripheral T-cell lymphoma and even from B-cell lymphoma) were virtually indistinguishable by morphology alone from primary ALCL.

We conclude that ALCL represent a morphologically and immunophenotypically heterogeneous category of high grade lymphomas derived from dedifferentiated lymphoid cells.

**10 Ki-1 POSITIVE LARGE CELL LYMPHOMA: IS IT A CLINICAL ENTITY?** F. Cabanillas, J. Romaguera, J. Manning. U.T. M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

While searching for a unique immunophenotypic marker for Hodgkin's disease, Stein et al. discovered an antigen which marked Reed-Sternberg cells. Later on, they realized that it also stained a group of diffuse large cell lymphomas (DLCLs) that were morphologically characterized by their anaplastic morphology. Further investigation identified this "Ki-1" antigen as a marker of activated lymphocytes. We analyzed the phenotype of a group of DLCLs to determine if the Ki-1 antigen would identify a group of tumors that can be considered as a unique disorder. We compared 24 cases of Ki-1 positive to 52 Ki-1 negative large cell lymphomas (LCLs). There were four features that appeared different: 1) Ki-1 positive cases were younger than Ki-1 negative cases (median age=37 vs. 59,  $P<.01$ ); 2) skin involvement was more common in Ki-1 positive cases (29% vs. 2%,  $P<.01$ ); 3) bone marrow was negative in all 24 Ki-1 positive cases, while positive in only 4% of Ki-1 negative cases ( $P>.05$ ); 4) at five years, the failure free survival (FFS) was better for Ki-1 positive cases (75% vs. 60%,  $P>.05$ ).

Finally, we divided the 24 Ki-1 positive cases into anaplastic (N=17), and non-anaplastic (N=7), to determine if there were any differences in their clinical features or behavior. The anaplastic cases tended to be younger (median age=31 vs. 37,  $P>.05$ ), had fewer Ann Arbor stage IV presentations (29% vs. 43%,  $P>.05$ ), and the survival was better (94% vs. 57%,  $P=.05$ ).

We conclude that Ki-1 positive LCLs represent a unique group of tumors with a different age distribution, frequent skin involvement, and a low incidence of marrow involvement. The high cure rate we have observed for Ki-1 LCLs appears to be predominantly in the anaplastic variant. Thus, it appears important to separate the anaplastic from the non-anaplastic variant of Ki-1 positive LCLs.

- 11** BCL2 ANTISENSE OLIGONUCLEOTIDES SUPPRESS t(14;18) B-CELL LYMPHOMA GROWTH IN A SCID-HU MOUSE MODEL. FE Cotter, P Johnson\*, C. Pocock, P Hall\*, N Al-Mahdi, L Hawthorn, G Morgan. LRF Dept of Haematology and Oncology, Dept of Immunology, The Institute of Child Health, London, \*ICRF Dept of Med Oncology, St Barts Hosp, and Dept of Histopath., \*St Thomas Hospital, London.

The t(14;18) translocation is found in 80% of follicular lymphomas and 25% of high grade B-cell lymphomas. This results in deregulation of the BCL-2 gene and appears to play a role in oncogenesis. Increments between 5 and 50 x 10<sup>6</sup> cells from a cell line derived from a patient with B-cell lymphoma bearing the t(14;18) translocation were injected by IV, IP and SC routes into a total of 122 SCID mice. The cell line was tested and shown to be negative for the Epstein-Barr virus (EBV). The mice developed lymphoma bearing the t(14;18) translocation with as few as 5 x 10<sup>6</sup> cells within 28 days. This was determined by histological examination. The higher the cell inoculation the more rapidly the lymphoma developed. Engraftment of the tumour cells was determined by PCR for the t(14;18) breakpoint region on peripheral blood samples and could be detected prior to development of overt lymphoma. In addition the t(14;18) translocation within the lymphoma was demonstrated by interphase fluorescence in situ hybridisation with chromosome paints to the derivative 14 and 18 translocations derived by chromosome flow sorting. It was possible to passage the lymphoma from one generation to the next. Having established a lymphoma model the cells were treated in vitro with antisense oligonucleotides to the first open reading frame of the BCL-2 gene. Control treatments with sense and nonsense oligonucleotides were also performed. Cell viability studies were completed and 5 x 10<sup>6</sup> viable cells were inoculated into SCID mice. In vitro sustained down regulation of BCL2 was observed after three days of treatment with the antisense oligonucleotide. At 28 days the sense, nonsense and untreated cell SCID mice had developed lymphoma, however, the antisense treated group failed to develop lymphoma suggesting that in vitro down regulation of BCL2 leads to an in vivo anti lymphoma effect. SCID-hu modelling of B-cell lymphoma bearing the t(14;18) translocation has been demonstrated and the importance of BCL2 expression in the lymphoma process suggested. Reduction of the BCL2 protein suppresses the oncogenic potential of these lymphoma cells in vivo confirming that it plays an essential role in the development of malignancy.

- 12** INTERLEUKIN-4 (IL-4) AND INTERFERON- $\alpha$  (IFN) INHIBIT APOPTOTIC CELL DEATH AND PREVENT THE LOSS OF THE BCL-2 PROTEIN IN B-CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) CELLS IN VITRO. P Panayiotidis\*, K Ganeshaguru, SAB Jabbar, AV Hoffbrand Royal Free Hospital & School of Medicine, London, England. \*ESMO Fellow

Although CLL cells have a long half life in vivo, when cultured in vitro they die rapidly by apoptosis (programmed cell death). Apoptosis is at least partially regulated by the presence of the bcl-2 protein. A number of lymphokines are involved in the proliferation and differentiation of CLL cells. Among them IL-4 suppresses IL-2 or tumor necrosis factor induced increase in <sup>3</sup>H-thymidine uptake in CLL cells and IFN alpha may be used clinically in early stage CLL patients. We analysed the effects of IL-4 and IFN in apoptosis, DNA synthesis, cell morphology and bcl-2 protein expression on CLL cells in vitro. Purified CLL cells from 24 patients were analysed; 18 were in Binet clinical stage A, 2 in stage B and 4 in stage C. Apoptosis was determined by morphology, propidium iodide staining of cell nuclei and by the demonstration of a DNA ladder pattern of ~180 base pair multimers. In purified CLL cells from 20 patients, apoptosis was observed after 24-30h culture in medium (RPMI +10% fetal calf serum). When purified CLL cells were cultured in the presence of IL-4 or IFN, DNA fragmentation was inhibited and cell viability was enhanced. CLL cells were kept alive for up to 3 weeks in the presence of IL-4 (5ng/l) or IFN (10<sup>6</sup>U/ml), while in control cultures 95-100% of CLL cells were dead after 10 days culture. In CLL cells from 4 patients no apoptosis was observed (<5% of DNA was fragmented after 24-30h culture) and they survived up to 4 weeks in the presence of culture medium alone. Western blot analyses of cell lysates of 10 CLL patients revealed the presence of the bcl-2 protein in all. However, during in vitro culture in medium alone, levels of the bcl-2 protein were stable only in patients resistant to apoptosis and were reduced in others. The reduction of the 26kDa bcl-2 protein levels was inhibited when cells were cultured in the presence of IL-4 or IFN. FACS analysis of fixed and permeabilized cells stained with anti-bcl-2 antibody reveals that reduction of bcl-2 levels occurs in the apoptotic cells. Inhibition of apoptosis of cultured CLL cells by IL-4 or IFN was independent of induction of DNA synthesis or morphological transformation. Our results indicate the existence of humoral factors that protect CLL cells from apoptotic cell death and the loss of the bcl-2 protein. The long half life of CLL cells may be due to the presence of IL-4 and other cytokines in the in vivo milieu. It is possible that a similar process may explain the long life span of some low-grade lymphoma cells lacking the 14;18 (bcl-2) translocation. The beneficial clinical results observed in some CLL patients treated with IFN maybe due to interaction with production of humoral factors vital for CLL cell survival.

# ABSTRACTS - Fifth International Conference on Malignant Lymphoma, Lugano

## 13 PERSISTENCE OF t(14;18) BEARING CELLS IN PATIENTS WITH FOLLICULAR LYMPHOMA AFTER MYELOABLATIVE THERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT). P.W.M. Johnson, C.G.A. Price, T.A. Smith, J. Meerabux, A.Z.S. Rohatiner, B.D. Young, T.A. Lister. I.C.R.F. Department of Medical Oncology, St Bartholomew's Hospital, London, England.

The cells of up to 90% of follicular lymphomas carry the t(14;18) translocation which may be used as a marker for the presence of residual disease. Using the polymerase chain reaction (PCR) with nested primers, DNA from samples of bone marrow and peripheral blood was examined for the presence of the translocation after treatment with cyclophosphamide (CY) and total body irradiation (TBI) with ABMT. Direct sequence analysis was used to confirm the continued presence of the lymphoma-associated clone and to minimise false positive results.

Forty-one patients with recurrent follicular or transformed follicular lymphoma having a PCR-amplifiable t(14;18) were studied. All received CY 60mg/kg x 2 and TBI 1200cGy in 6 fractions over 3 days. The mononuclear cell fraction of harvested bone marrow was treated in vitro with three cycles of CD20 monoclonal antibody (Coulter Immunology, Hialeah, Fla) and baby rabbit complement (Pel-freez, Wi).

At a median follow up of 3 years the Kaplan-Meier estimate for overall survival was 70% with 42% in continuous remission. The harvested bone marrow of 29 patients was available for study before and after treatment in vitro. In only 4 cases was the marrow found to be rendered PCR-negative for the t(14;18) after CD20 antibody and complement, having initially been positive in all. Three of the patients who received PCR-negative marrow developed recurrent disease 4, 11 and 35 months after reinfusion, compared to 11 of 25 in whom it had remained PCR-positive.

One hundred and twenty-two follow up samples of peripheral blood (75) and bone marrow (47) were available for 27 patients. In all cases but one the original t(14;18) clone could be detected by PCR. A median of 2 samples per patient had the clone detectable 3 months to 7 years after ABMT. Thirteen patients remained in remission 1+ to 7+ years with the clone detectable and one died of secondary myeloid leukaemia at 4 years without evidence of recurrent lymphoma.

This form of treatment does not eliminate the t(14;18) clone, although prolonged remissions may occur despite its continued presence.

## 14 POLYMERASE CHAIN REACTION (PCR): A REFINED TOOL FOR ASSESSING RESPONSE IN FOLLICULAR LYMPHOMAS (FL). F. Cabanillas, M. S. Lee, P. McLaughlin, A. Majlis, F. B. Hagemeister, F. Swan, M. A. Rodriguez, J. Romaguera, A. Sarris, A. Younes. U.T. M.D. Anderson Cancer Center, Houston, Texas 77030

The constant and late relapse rate of stage IV FL is very likely associated with minimal residual disease that persists after treatment. The PCR test, when applied to detect bcl-2 rearranged DNA, provides an extremely powerful means of detecting minimal residual disease in follicular lymphoma. We tested the blood of patients with FL before and after therapy with three objectives: 1) To correlate the clinical remission status with the molecular response status. 2) To examine the correlation of PCR result with clinical outcome. 3) To compare the molecular CR rate of a new intensive therapy regimen based on 3 alternating combinations ("ATT" regimen) consisting of Adriamycin/ Cytosin, AraC/Platinum and Mitoxantrone/Procarbazine based combos given sequentially x 12 against standard CHOP or COP type therapy.

Of 31 cases whose PCR was positive before therapy and who were tested serially, 25(81%) achieved a clinical CR. Of these 25 clinical CR's, only 14(56%) were CR's at the molecular level. Another three molecular CR's were seen in patients judged clinically to be PR's. So far none of the 17 molecular CR's have relapsed while 3/25 clinical CR's have relapsed. Of the 13 molecular failures, 5(38%) have relapsed. The table below summarizes the molecular CR rate according to treatment given. Summarized here are all cases with a positive PCR test within 12 months of initiating treatment, including those who did not have a baseline PCR pre-treatment sample. Minimum followup is six months and median is 35 months. Median number of PCR tests per case is three.

Protocol	N	# Molecular CR's	# Molec. CR's Relapsed	# Molec. Failures	# Molec. Failures Relapsed
CHOP-B	20	4(20%)	0	16(80%)	5(31%)
ATT	23	19(83%)	0	4(17%)	2(50%)
Total	43	23	0	20	7(35%)

Conclusions: 1) The clinical assessment of response correlates poorly with the molecular assessment. 2) Molecular response correlates better with outcome than clinical response. 3) ATT produces a high fraction of molecular CR's and standard CHOP-Bleo type therapy usually fails to induce molecular CR's. 4) PCR is a more sensitive and objective method for detecting minimal residual disease and represents a refined tool for assessing the results of therapy of FL.

## 15 REGULATION OF CHEMORESISTANCE BY BCL-2. John C. Reed, Toshiyuki Miyashita, Shinichi Kitada, Lawrence Hovey III, and Shinichi Takayama. La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The bcl-2 gene becomes activated by the t(14;18) chromosomal translocations that occur in the majority of non-Hodgkin's lymphomas, leading to over-production of the 26-kD Bcl-2 protein. Equivalently high levels of bcl-2 expression can also be found in about 70% of B-CLL specimens, despite the absence of detectable structural alterations in the bcl-2 gene. Previous studies showed that over-production of the Bcl-2 protein contributes to neoplastic cell expansion primarily by promoting cell survival through interference with "programmed cell death" ("apoptosis"), a finding consistent with the biology and clinical manifestations of the low-grade non-Hodgkin's lymphomas (NHLs) and B-CLLs that frequently contain high levels of Bcl-2 protein. Because many chemotherapeutic drugs are capable of activating pathways leading to apoptotic cell death, we used gene transfer methods to achieve elevations in the levels of Bcl-2 protein in various lymphoid cell lines and then tested their relative resistance to killing by several drugs (both cell cycle-dependent and -independent) commonly used in the treatment of NHLs and B-CLL, including: Dexamethasone, Methotrexate, Ara-C, VP16, Cisplatin, Vincristine, 4-HC, 2-CdA, Adriamycin, and Daunomycin. Cells that had been stably infected with a control retrovirus and that had low levels of Bcl-2 were induced to undergo apoptosis by all of these drugs as defined by morphological changes, cell shrinkage, and DNA degradation into oligonucleosomal-length fragments. In contrast, cells that had been stably infected with a recombinant bcl-2 retrovirus and that contained 10-20-fold elevated levels of Bcl-2 protein were relatively more resistant to killing all drugs tested. Though all of these chemotherapeutic drugs were still capable of inducing cell cycle arrest in cells containing high amounts of Bcl-2 protein, for some drugs surviving cells with high Bcl-2 were able to re-initiate cell proliferation upon removal of drugs from cultures. Thus, by extending cell survival in the presence of cytotoxic drugs, over-production of the Bcl-2 protein appears to provide cells with an opportunity to repair drug-induced DNA damage and to resume their proliferative activity, as might occur begin cycles of chemotherapy in clinical scenarios.

To further test the relevance of Bcl-2 to chemoresistance, antisense approaches were used to reduce levels of Bcl-2 protein in t(14;18)-containing NHL cell lines. NHL cell lines were either treated with 18-mer antisense bcl-2 and control oligonucleotides delivered via cationic liposomes, or were stably transfected with an high copy number, episomal expression plasmid that used a metallothionein promoter for inducible production of antisense bcl-2 mRNAs. In both cases, antisense-mediated reductions in Bcl-2 protein levels markedly increased the sensitivity of these t(14;18)-containing NHL cell lines to killing by cytotoxic drugs such as Dexamethasone, Methotrexate, Ara-C, and Adriamycin. Taken together, these findings strongly argue that the relative level of Bcl-2 protein is an important determinant of the sensitivity of lymphoid cells to killing by chemotherapeutic drugs, and suggest that methods to reduce Bcl-2 protein levels or impair Bcl-2 function could markedly improve the efficacy of conventional antineoplastic drugs in the treatment of NHL, CLL, and perhaps other malignancies.

## 16 BCL-2 PROTEIN EXPRESSION IN AGGRESSIVE NON-HODGKIN'S LYMPHOMA (NHL). A NEW ADVERSE PROGNOSTIC FACTOR? A GELA STUDY. O.Hermine, C.Haioun, E.Lepage, MF d'Agay, J.Brière, H.Tilly, G.Fillet, B.Couffier, C.Gisselbrecht, J.Diebold, F.Reyes, Ph.Gaulard, Département de Pathologie, Hôpital Henri Mondor, 94010, Créteil, France.

Bcl-2 gene encodes for a protein of 25 kd, which extends cell survival by blocking programmed cell death. Recent in vitro studies have shown that bcl-2 may block chemotherapy-induced cell death in human leukemia cell lines. Among NHL, bcl-2 has been shown to be expressed and deregulated in most follicular lymphomas. However, little is known about the expression of bcl-2 protein in intermediate and high grade NHL, and its clinical and prognostic significance has not been investigated. This prompted us to study the expression of bcl-2 in 371 patients with high or intermediate grade NHL included in the LNH87 protocol. They were selected at random among patients uniformly treated with ACVBP in the induction phase (LNH84 protocol, JCO 1989, 7: 1018). The main characteristics of the patients were: performance status (PS)≥2 (19.5%), Ann Arbor stage III - IV (63%), bone marrow (BM) involvement (24%), number of extranodal sites≥2 (27%), LDH level>1N (53%). The lymphomas were classified according to the Working Formulation(WF) and the Kiel classification. On immunohistology, they included B (76%), T (13%), and T-and B- negative (11%) NHL. Expression of bcl-2 was analyzed in routine deparaffinized sections of tumoral tissues by the alkaline -phosphatase: anti-alkaline-phosphatase (APAAP) technique using the monoclonal antibody bcl2/124.

71 cases were excluded because no staining was present, either on tumoral cell (TC) or on normal reactive small lymphocytes (NRSL). 128 cases (43%) disclosed homogeneous positivity (bcl-2+) in virtually all tumor cells. In 72 cases (24%), bcl-2 expression was heterogeneous (bcl-2-/+) with a variable number of TC being positive, others being negative. 100 cases (33%) were negative (bcl-2-) (TC negative, and NRSL positive). According to the main histologic subtypes of the WF, the expression of bcl-2 was as follows (bcl-2+; bcl-2-/+; bcl-2-): follicular large cell (13;7;5), diffuse small cleaved (11;0;0), diffuse mixed (11;9;12), and diffuse large cell (69;43;52), immunoblastic (6;3;11), anaplastic (5;7;10), Burkitt (2;0;4) and lymphoblastic (4;0;2). In univariate analysis, a high level of bcl-2 expression (bcl-2+ subgroup) was significantly more frequent in NHL with: (1) Ann Arbor stage III - IV vs I - II (p=0.0006); (2) B- vs T-cell phenotype (p=0.007); (3) nodal vs extranodal presentation (p=0.02); (4) BM involvement (p=0.03). In the bcl-2+ subgroup, 66% of the patients achieved complete remission after induction compared to 77% in the bcl-2- and bcl-2-/+ subgroups (p=0.05). Disease free survival (DFS) and survival for the entire group were 70% at two years. Two-year DFS in the bcl-2+ subgroup significantly differed from that observed in the bcl-2- and bcl-2-/+ subgroups (57 vs 75%) (p=0.006). In multivariate analysis, a high level of bcl-2 expression (bcl-2+) was the only factor which influenced DFS negatively. However, at the time of analysis, a high level of bcl-2 expression did not influence survival in contrast to the wellknown adverse prognostic factors, ie age, PS, and LDH level.

In conclusion, these data show that high level of bcl-2 expression on tumor cells is predictive of poor disease-free survival, which may be in agreement with the role of bcl-2 in chemotherapy-induced apoptosis. They also suggest that bcl-2 staining, which can be performed in routine paraffin-embedded material, might be included in routine diagnosis of aggressive NHL.

## 17 Why are B-cell Lymphomas rare in Asia? A personal view

Koji Nanba, M.D., Ph.D.

(Department of Health Science, Faculty of Integrated Arts & Sciences, Hiroshima University)

This is a difficult question to answer, though scientifically provocative. My experience is primarily based on Japanese who are anthropologically classified as Mongoloid which includes most of Asians and native Americans.

Major histologic differences of malignant lymphoma (ML) between Japanese and Caucasians, in terms of relative frequencies, were made clear in the Seventies using the Rappaport and Rye classifications: Hodgkin's disease (HD), especially nodular sclerositis (NS), was very rare compared to U.S.A. Among the non-Hodgkin's lymphoma (NHL), follicular lymphoma (FL) was also very rare. On the other hand, diffuse lymphomas of mixed and histiocytic (now called "large cell") types were very frequent.

In the Eighties, immunologic phenotyping of ML became increasingly popular and the International Working Formulation (IWF) was proposed in 1980, which made comparative patho-epidemiological study of the lymphoma easier. Cooperative efforts by The Lymphoma Study Group (LSG) in Japan elucidated a unique distribution of T vs B-cell MLs in the archipelago. Initial study revealed relative excess of T-cell MLs including Adult T-cell leukemia (ATL) for which HTLV-1 was later attributed as the etiologic virus. Recent multi-institutional studies demonstrated the overall incidence of T-cell lymphoma excepting ATL was 25%.

Incidence study using the IWF and the cases of a Tumor Tissue Registry demonstrated the annual incidence rates of ML were 5.1 (per 100,000 population) in Japan and 12.1 for American Caucasians. The difference was mostly explained by the low incidences of HD and FL in nodal MLs among Japanese. Unexpectedly, extra nodal MLs did not reveal significant difference not only in histology but in the incidence rate.

In 1985, it became clear that t(14;18) often found in Western FL was rarely observed among Japanese. The rearrangement of an oncogene bcl-2 located at 18q (which is involved 14;18 translocation) was also significantly lower in Japan.

Environmental influence was evaluated by reviewing MLs of Japanese American in Hawaii. The results indicated that as for nodal lymphomas the ratio of B-cell ML inclusive of FL was increased to an intermediate of the two countries, but that of extranodal ML did not reveal a difference. Moreover, incidence of HD was extremely low (4%) as it was in Japan.

Development of extranodal B-cell MLs among the posttransplant patients is very high in U.S.A. and Europe. The risk was 35 times greater than expected in renal transplants. Only five cases of NHL (all B-cell) developed in Japan among almost 8,000 renal grafts performed by the end of 1990.

Relative excess of T-cell ML in Japan is partly explained by residual HTLV-1 (which was likely a ubiquitous virus in the beginning, and disappeared in the area where infant was fed with animal milk). This virus is by and large seems to be connected with Paleomongoloid traits in Japan. Not the rarity of B-cell ML but the low incidence of MLs in Asia should be the core of the question. "Nature or nurture" problem might not be easily solved. Recent analysis of mitochondrial DNA revealed the relative homogeneity of Caucasians, compared to other ethnic groups. Analysis of HLA-DR genes which is known to be associated with autoimmune disease might provide a clue to the enigma.

## 18 EPIDEMIOLOGY OF NON-HODGKIN'S LYMPHOMA. D. Weisenburger, Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, Nebraska USA

Between 1973 and 1989, the incidence of non-Hodgkin's lymphoma (NHL) increased by nearly 60% in the United States (US), one of the largest increases of any cancer. In contrast, the incidence rates of Hodgkin's disease and leukemia were stable during this period. In 1992, over 40,000 persons in the US were diagnosed with NHL and approximately 20,000 died of the disease. Although much attention has been devoted to NHL arising in persons with acquired immunodeficiency syndrome (AIDS), most of this increase in NHL cannot be attributed to AIDS. However, by the mid-1990's, AIDS will be a major cause of NHL, accounting for 10 to 15% of all new cases. The annual incidence rate of NHL per 100,000 persons in the US has risen from 5.9 in 1950 to 9.3 in 1975 and to 13.7 in 1989. The increase has occurred in both males and females, and in whites more than blacks, with age groups over and under 65 years both showing increases of over 50% since 1973. The incidence rate in 1989 was 56% higher in white males (17.8) than white females (11.4). The largest increase in incidence has occurred in the elderly, and rates have been increasing more rapidly in rural areas than urban areas. Histologically, the largest increases have occurred in the diffuse large cell and immunoblastic categories, and there has been a disproportionate increase in extranodal lymphomas. Similar findings have also been reported in other developed countries. Epidemiologic studies have suggested that environmental factors may play an important role in the etiology of NHL. Occupational studies have found that persons with certain types of jobs have an increased risk of NHL, including farmers, pesticide applicators, grain millers, meat workers, wood and forestry workers, chemists, painters, mechanics, machinists, printers, and workers in the petroleum, rubber, plastics and synthetics industries. Thus, chemical exposures of various types may be etiologic for NHL. Chemicals that have been linked to the development of NHL include a variety of pesticides (2,4-D, organophosphates, chlorophenols), solvents and organic chemicals (benzene, butadiene, carbon tetrachloride, carbon disulfide), wood preservatives (creosote, pentachlorophenol), drugs (alkylating agents, immunosuppressives, hydantoin), dusts (wood, cotton), and some components in hair dyes. In a recent study in Nebraska, we found that male farmers who frequently used the herbicide 2,4-D had an over 3-fold increased risk of NHL. Similarly, frequent organophosphate insecticide use, adjusted for 2,4-D use, was also associated with a 3-fold increased risk of NHL. For women, the use of hair coloring products, particularly permanent and dark coloring products, was associated with a 1.5- to 2.5-fold increased risk of NHL. These findings indicate that additional epidemiologic studies are needed to elucidate and quantitate etiologic factors for the current epidemic of NHL.

**19** THE INTERRELATIONSHIP BETWEEN HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMAS. E.S. Jaffe, A. Zarate-Osorno, and L.J. Medeiros. National Cancer Institute, NIH, Bethesda, MD 20892, USA

While Hodgkin's disease (HD) and the non-Hodgkin's lymphoma (NHL) have long been regarded as distinct disease entities, recent observations suggest a closer association. The analysis of cases in which these diagnoses are made in the same anatomic site (composite lymphomas), or in separate sites (simultaneous or sequential HD and NHL), indicates that this phenomenon occurs more frequently than would be expected by chance alone. The most common form of composite lymphoma is coexistent nodular lymphocyte-predominant Hodgkin's disease (NLPHD) and a large cell lymphoma (LCL) of B-cell phenotype. This finding is consistent with a B-cell origin for the abnormal cells in NLPHD, suggesting that the LCL represents a form of histologic progression, with the existence of a clonal relationship between the two components.

The association of other forms of HD (nodular sclerosis or mixed cellularity) and NHL is less common but still significant. As with NLPHD and LCL, one may observe composite lymphomas, or the diagnosis of HD may precede or follow the diagnosis of a NHL. The vast majority of the NHL associated with HD are of B-cell origin, most commonly follicular lymphomas. An association between HD and B-cell CLL is also observed. In selected cases, Reed-Sternberg cells are seen in a background of otherwise typical CLL, and some of these patients have progressed to disseminated HD. These findings suggest that, at least in some cases, HD may be clonally related to an underlying B-cell malignancy, and that the Reed-Sternberg cell may be an altered B lymphocyte.

A process that may have a different pathogenesis is the late occurrence of aggressive B-cell lymphomas in patients successfully treated for HD. This phenomenon most likely relates to an underlying and persistent immunodeficiency in these patients, and does not necessarily suggest a clonal relationship between the two tumors.

**20** IS HODGKIN'S DISEASE AN INFECTIOUS DISEASE ?  
V. Diehl and J. Wolf. Department of Internal Medicine I, University of Cologne, 5000 Cologne 41, FRG.

Hodgkin's disease (HD) is mainly considered a malignant disorder of the lymphatic system. This concept has been strengthened by the impressive cure rates of anticancer treatment like radiation and polychemotherapy. Nevertheless, in view of the peculiarities of HD and the recently described association with Epstein-Barr virus (EBV-) infection it is tempting to question the concept of HD being a true neoplasia from beginning of the disease.

Commonly accepted criteria defining a malignant cell clone, i.e. monoclonality and aneuploidy, are controversially discussed for HD/RS cells, at least in early stages of the disease. Our own studies show outgrowth of B-cells with cytogenetic aberrations after transplantation of HD lymph nodes into SCID mice. Thus, aneuploidy obviously is not restricted to the putative malignant HD/RS cells. Moreover, in early stages HD strongly resembles an immune response. This is evidenced by clinical signs of inflammation, the scarcity of HD/RS cells, the majority of mitotically active cells being reactive lymphocytes and their characterization as a subset of CD4 positive T-lymphocytes. The progredient 'malignant' course of HD might indicate the inability of the organism to eliminate a cell expressing the target antigen for this non self limiting immune response.

EBV latent proteins are likely to be involved in this process. Keeping in mind the lesson from other EBV-linked malignancies like Burkitt's lymphoma and lymphoproliferative disease in immunocompromised individuals, EBV latent proteins can act in a somehow contradictory fashion. They might have a transforming, i.e. oncogenic, potential, they may, however, also have a protective effect, i.e. representing target antigens for the host's immuneresponse. EBNA 1, the only latent gene expressed in all EBV-associated malignancies, might therefore exert a transforming potential also in HD. LMP might be one of the putative target antigens involved in the atypical immuneresponse characteristic of HD.

In conclusion, early stages of HD can be understood as the unsuccessful attempt of the organism to eliminate a cell expressing a putative target antigen, which might have been introduced into the cell by viral infection. With progression of disease the immune system loses its ability to control this cell. It remains to be established how specific characteristics of the host cell expressing the putative target antigen and of the antigen itself on one side as well as defects in the host's immune system on the other act together in this scenario.

**21 High plasma levels of tumor necrosis factor alpha ( $\alpha$ -TNF) are correlated with adverse prognostic factors and associated with a poor outcome in lymphoma patients.** G. Salles, M. Barbier, J. Bienvenu, Y. Bastion, C. Dumontet, L. Couion, Y. Barbier, and B. Coiffier. Centre Hospitalier Lyon-Sud, 69310 Pierre-Bénite, France.

Plasma values of  $\alpha$ -TNF were determined by immunoradiometry (IRMA, Medgenix) in newly diagnosed lymphoma patients (pts) between 04/91 and 12/91. These 69 pts included 23 pts with low grade lymphoma, 37 with high grade lymphoma, 4 with Hodgkin's disease, and 5 unclassified. Among those pts, 62% presented stage III/IV disease, 63% B symptoms, 68% a PS  $\geq 2$ , 42% BM involvement, 33% bulk disease and 18%  $\geq 2$  extra-nodal sites involved. High LDH level was found in 46%, high  $\beta 2$ -microglobulin level in 42%, low serum albumin level in 35% and low hemoglobin level in 60%. Plasma TNF values were found normal ( $\leq 9$  ng/l) in 9 (13%) pts, moderately elevated ( $> 9$  and  $< 50$ ) in 36 pts (52%) and markedly increased ( $\geq 50$ ) in 22 (35%). High level of  $\alpha$ -TNF correlated with advanced stage and  $\beta 2$ -microglobulin  $\geq 3$  mg/l ( $p < .0001$ ), with abnormal LDH ( $p < .0005$ ), and to a lesser extent ( $p < .005$ ) with age  $> 60$  and anemia (Hb  $\leq 12.5$ ) but were not significantly associated with B symptoms, poor PS, bulk tumor, low serum albumin, and histologic or immunologic subtypes.  $\alpha$ -TNF level was not significantly associated with C-reactive protein (elevated in 45/66 patients), IL-6 (17/55) or IL-1 (6/28) levels suggesting that increased  $\alpha$ -TNF was not necessarily the part of a lymphoma associated acute-phase reaction. Pts were treated according to histology and presence of prognostic factors in phase II or III prospective trials (GELA trials for aggressive or follicular lymphoma, CNVP for 9 elderly pts, and fludarabine for some low grade pts). Among evaluable pts, 45/57 (79%) achieved complete response (CR) after therapy and 27/69 (39%) experienced progressive disease. High  $\alpha$ -TNF level was associated with progression ( $p < .05$ ) but not CR or death. In summary, this study suggest that  $\alpha$ -TNF is well correlated to adverse prognostic factors in lymphoma pts and predict a poor outcome.  $\alpha$ -TNF has been reported to promote the growth promoting of certain malignant B-cells. The mechanism of  $\alpha$ -TNF production in lymphoma and its clinical relevance deserve further attention.

**22 T LYMPHOCYTE FUNCTION AND ONTOGENY IN GENE-TARGETTED MUTANT MICE.** T.W. Mak, The Amgen Institute, The Ontario Cancer Institute, 500 Sherbourne Street Toronto, Ontario M4X 1K9, Canada

T lymphocytes recognize their antigen peptides and Major Histocompatibility Complex products with the use of their T cell antigen receptors (TcR). In addition to the  $\alpha$  and  $\beta$  chains of TcR, the interaction between T cells and their target cells or antigen presenting cells is also assisted by a series of other cell surface polypeptides. Most notable of these are CD4 and CD8, which are selectively expressed on mature helper/inducer and killer/suppressor T cells, respectively. Upon engagement of their ligands, a series signals are being transduced intracytoplasmically via some of these molecules and their associated proteins. Perhaps the most important enzyme in this signal transduction process is the lymphocytes specific tyrosine kinase *lck*. Another important component is the cell surface tyrosine phosphatase CD45. This molecule is alternatively spliced and the different isoforms are expressed on the various hemopoietic and lymphopoietic cells. Signalling thru the TcR-CD4/CD8-*lck*-CD45 complex is thought to be insufficient to activate T lymphocytes. A co-stimulatory signal is believed to be essential. Many investigators have suggested that CD28, a ligand for B7/BB1 is an essential co-stimulatory signal. In an attempt to gain better understanding on the roles of these molecules in T lymphocyte functions and ontogeny, we generated a series of mutant mice with disruptions in these genes. These mutant mice are being analysed in order that we can evaluate the importances of these genes in T cell development.

## 23 THE TREATMENT OF HODGKIN'S DISEASE. Saul A. Rosenberg, Division of Oncology, Stanford University, Stanford, CA, USA.

New therapies of Hodgkin's disease are being directed toward reducing the acute toxicity and long-term morbidity of the highly curative therapies now available. New management programs for patients with favorable, highly curable disease reduce the total dose of alkylating agents and other leukemogenic drugs; minimize the long-term effects on cardiac and pulmonary function; and reduce the volume and dose of irradiation so that the incidence of secondary cancers can be reduced. The use of exploratory laparotomy and splenectomy is being limited whenever possible. These new management programs must not reduce the overall cure and survival rates for patients with good prognoses.

For patients with poor prognoses, new dose intensive regimens are being developed with or without adjuvant irradiation. These programs should also be planned so that long-term secondary malignancies and acute leukemia rates should be kept at a minimum. A secondary goal is to reduce the overall incidence of sterility for young patients with high curative potential.

The proper utilization of autologous bone marrow and/or stem cell support to achieve very high dose intensity of combined modalities should be applied appropriately and whenever possible, compared to secondary or salvage treatment programs with less intensive therapies.

A summary of the long-term results and treatment complications of patients treated at Stanford will be presented to illustrate these problems and approaches to their solution.

## 24 LOW GRADE LYMPHOMA 1993: STATE OF THE ART S.J. Horning, MD., Stanford University, Stanford, CA, USA.

Randomized trials initiated more than twenty years (yr) ago at Stanford tested single alkylating agent, combination chemotherapy and combined modality treatment of advanced low grade lymphoma. Mature follow-up of these studies demonstrate an actuarial freedom from relapse and survival of 25% and 51% at 10 yr and 14% and 27% at 20 yr. Complete response rates in current studies appear to be considerably lower than these early trials, possibly due to selecting patients (pts) with a greater tumor burden for immediate therapy and the use of more rigorous criteria in restaging. Selected patients have been managed with no initial therapy at Stanford for over 20 years. The actuarial median survival of 314 deferred therapy pts is statistically superior to that of 550 pts treated immediately after diagnosis at Stanford (11.8 yr vs. 10.4 yr;  $p < 0.01$ ). These results reflect the variable biology of low grade lymphoma which can be appreciated at diagnosis. Measures of tumor burden and host factors such as age and gender, recognized as prognostic factors, must be considered in interpreting current trials, especially when attempting historical comparisons. Concurrent induction or adjuvant therapy with alpha interferon has been reported to favorably impact response and/or remission duration in several studies, although a survival advantage has not been demonstrated. Several centers, including Stanford, are testing high dose chemoradiotherapy and autologous stem cell transplant in first remission. Preliminary results of these studies will be presented as well as comments regarding their interpretation. Histologic transformation (HT) from a low grade to an intermediate or high grade lymphoma is a major cause of morbidity and mortality. While assessment of the timing and incidence of HT has a large inherent error, the actuarial risk of HT in pts managed at Stanford continues to increase over 20+ yr. Prognostic factors upon HT include extent of disease, bulk of disease and exposure to chemotherapy. A median survival after HT of 95 months was surprisingly good in a subgroup of 29 pts achieving a complete response to therapy. Management of low grade lymphoma at relapse is also an important issue, given the overwhelming likelihood of this event. Response to initial therapy is the most important prognostic factor in this situation; duration of remission and age have also been proposed. Studies from several transplant centers testing the use of high dose therapy and autologous stem cell transplant in this setting will be reviewed. A major difficulty encountered in the clinical investigation of the low grade lymphomas is the length of follow-up required to interpret the results of an intervention. A major question at this time is whether the persistence or emergence of t(14;18) lymphoma cells as determined by DNA amplification techniques is a reliable surrogate marker. The discovery of the *bcl-2* proto-oncogene and its inhibitory effects on programmed cell death suggests that strategies which do not rely on proliferation but are effective in resting cells are needed. A number of these is in clinical trials, including radiolabeled monoclonal antibodies, immunotoxins and tumor-specific vaccines. Cure or even improved survival of the advanced stage low grade lymphomas remains an elusive goal in 1993. Future therapies must be directed by an understanding of the biology of the low grade lymphomas and the recognition of considerable heterogeneity among individual pts.

**25** TREATMENT OF AGGRESSIVE NON-HODGKIN'S LYMPHOMAS, Richard I. Fisher, Loyola University Medical Center, Maywood, IL. 60153

Therapy for aggressive non-Hodgkin's lymphomas (identified as intermediate and high-grade lymphomas under the Working Formulation Classification) has undergone significant evolution in the last 25 years. Early combination chemotherapy studies with CHOP in the Southwest Oncology Group (SWOG) produced complete response rates (CR) of 50-55% with 30-35% long term survivors. Single institution third generation regimens such as ProMACE-CytaBOM, m-BACOD, and MACOP-B resulted in 68-86% CR with 58-69% survival. Late relapses have been observed in each of these studies. SWOG subsequently conducted a series of Phase II confirmatory trials using the last three regimens. CR varied from 49-65% and survival varied from 50-61%. Since the results from these recent studies are closer to those obtained with the first generation regimens in a national cooperative group setting, ultimate conclusions concerning the efficacy of these new regimens awaited the results of prospective randomized trials.

Between May, 1986 and June, 1991, 1138 previously untreated patients with bulky Stage II, Stage III, or Stage IV, intermediate or high grade, non-Hodgkin's lymphoma were randomized to receive treatment with either standard therapy, CHOP, or one of the third generation chemotherapy regimens, m-BACOD, ProMACE-CytaBOM, or MACOP-B. Each treatment regimen was administered exactly as initially described. Treatment arms are well balanced for patient characteristics. Median follow-up for all patients is 31 months; There is no significant difference in the overall response or complete response rates between treatment arms. At 4 years the percent of patients alive without disease is as follows: CHOP, 36.4%; m-BACOD, 34.4%; ProMACE-CytaBOM, 45.1%; MACOP-B, 38.8% (p = 0.14). No difference in overall survival is seen between the treatment arms (p = 0.67). Fatal toxicities have been observed in 1% with CHOP, 5% with m-BACOD, 4% with ProMACE-CytaBOM, and 6% with MACOP-B.

Based on these results, new treatment approaches must be developed. We have chosen to focus on two new strategies: preventing the development of the multidrug resistance phenotype and using colony stimulating factors to significantly dose escalate the current treatment regimens.

**26** RECENT ADVANCES IN MANAGEMENT OF PEDIATRIC LYMPHOMAS. S.B. Murphy, Children's Memorial Hospital, Northwestern University School of Medicine, Chicago, Illinois, USA.

Successive improvements in outcome for all stages and histologic types of pediatric Hodgkin's Disease (HD) and non-Hodgkin's lymphoma (NHL) have been achieved over the last decade through conduct of controlled clinical trials in many centers and by multi-institutional cooperative groups worldwide. Current cure rates are 80-90% of all cases of pediatric HD and NHL. These outstanding results of modern trials reflect the application of a stage- and phenotype/histology-specific, risk-adapted approach to treatment and the development of more intensive and effective therapies for the advanced stage cases. Results will be illustrated from the experience of the Pediatric Oncology Group (POG) where, for Stage III and IV small non-cleaved B-cell NHL and B-cell (SIG+) ALL, we have achieved a more than three-fold improvement in outcome compared to previous POG results: 76% 2-year event-free survival (EFS) for the best arm (Total B) for Stage III (N=52), 75% for Stage IV (N=34), and 60% for B-ALL (N=47). Current research efforts are being directed at reducing adverse late consequences of therapy for the increasing proportions of long-term-survivors, as exemplified by sequential randomized POG trials which have shown that nine weeks of chemotherapy without radiotherapy will cure most children with localized (Stage I and II) NHL. Advanced stage lymphoblastic lymphomas and large cell histologic subtypes require distinctly differing therapeutic strategies which yield cures in 60-75% of the cases. The goals of future research are to further reduce the morbidities of intensive multidrug protocols by improved schedules, incorporating hematopoietic growth factors, and to better understand the molecular pathogenesis.

- 27** LATE NON-MALIGNANT COMPLICATIONS OF THE TREATMENT OF LYMPHOMAS : EMPHASIS ON LONG-TERM CARDIAC TOXICITY. J.M. COSSET,<sup>1,2,4</sup> M. HENRY-AMAR,<sup>1,3,4,5</sup> E.M. NOORDIJK,<sup>4</sup> R.HOPPE<sup>5</sup>  
1/ Institut Gustave Roussy - 94800 Villejuif, France.  
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4/ EORTC lymphoma group.  
5/ International Database for Hodgkin's disease (IDHD).

Most of what is known in 1993 about the late non-malignant complications after treatment of lymphomas actually comes from long-term follow up of Hodgkin's disease (HD) cohorts.

The picture of these late complications changed dramatically in the last decade, both because irradiation techniques have been improved and because more and more is known about the toxicity of the association of antineoplastic drugs with radiotherapy.

**Late pulmonary toxicity** directly related to irradiation has been reduced by the use of linear accelerators, the use of more limited irradiated fields, reduction in radiation dose and adequate fractionation. Chemotherapeutic agents, such as Bleomycin, may add to the effects of radiation and currently remain a potential problem; however, life-threatening complications seem to be rare.

Overt clinical hypothyroidism is exceedingly rare. In contrast, **late compensated hypothyroidism** (isolated elevated TSH) is observed in 20-40 % of the patients treated by mantle field irradiation. This asymptomatic hormonal unbalance is simply and efficiently treated by thyroid hormone replacement.

**Late digestive injuries**, mainly due to radiotherapy, were significantly reduced by the use of appropriate fractionation (often associated with a dose decrease).

**Post-irradiation myelitis** has been, in almost all cases in the past, the result of some technical irradiation error. A recent survey among radiation oncologists indicated that the "acceptable rate" of myelitis in Hodgkin's disease was ... 0 %.

Patients are more and more spared iatrogenic sterility by adequate shielding of the testis, oophorexy, and the reduced use - or even complete avoidance - of drugs toxic to the reproductive stem cells.

**Late viral infections** remain frequent in the first two or three years after treatment. The introduction of oral or intravenous acyclovir, however, considerably reduced the severity of these infections.

When it comes to long-term cardiac toxicity, the last decade saw a significant decrease in the incidence of post-irradiation pericarditis, due to several technical improvements, mainly based on the use of subcarinal blocks to reduce the dose delivered to the heart, and on the use of suitable fractionation.

However, more recently a "new" cardiac complication has emerged from the long-term studies of large cohorts of patients, and seems to be, together with second cancers, one of the most plausible candidate responsible for the "overmortality" observed in HD series, up to 20 years after treatment completion. This life-threatening complication is **myocardial infarction (MI)**.

A retrospective study at the Institut Gustave Roussy showed that among 499 adult patients given a mantle field radiotherapy (RT), 13 presented MI while no case was observed in 138 HD adult patients who were not given mediastinal RT ( $p < 0.05$ ).

In an EORTC cohort of 1650 clinical Stage I-II adult patients, the 20-year cumulative cardiac mortality incidence was 6.7 %. This rate was more than 10 times higher than expected in the normal population ( $p < 0.001$ ).

In the recently reported Stanford series of 2232 patients, the risk of dying from heart disease was not significantly elevated when the patients did not receive mediastinal irradiation, or when they received a dose inferior or equal to 30 Gy. In contrast, this risk was 3.5 times higher than in a control group for the patients who were given more than 30 Gy in the mediastinum.

In a recent reanalysis of updated data obtained for 13703 patients of the International Database of Hodgkin's disease (IDHD), 155 deaths due to acute MI were recorded. Factors related to the risk of dying from MI in this series will be presented.

In a **prospective** study carried out at the Institut Gustave Roussy, 40 patients agreed to perform Thallium 201 tomoscintigraphy 3 to 16 years after mediastinal irradiation. Only 8 cases (20 %) were considered as unequivocally normal; 14 (35 %) showed a typical ischemic aspect, while 18 tests (45 %) were considered as ambiguous. These results are to be compared with the 56 % rate of ischemic and dubious aspects observed in the only other published study in which tomographic Thallium scintigraphy was utilized.

In conclusion, in 1993 myocardial infarction is probably one of the major life-threatening late non-malignant complication occurring after lymphoma therapy. Additional data on the role played by specific radiotherapeutic and chemotherapeutic parameters in the occurrence of late coronary toxicity are urgently needed.

**28** CURRENT TREATMENT FOR MULTIPLE MYELOMA. B. Durie. Director Myeloma Research and Treatment Center. Cedars-Sinai Medical Center/UCLA School of Medicine, Los Angeles, California 90048, U.S.A.

The selection of appropriate treatment in multiple myeloma has become more complex in recent years because of the introduction of various high dose schedules with transplantation and the use of several new cytokines or cytokine antagonists/antibodies. As a primary approach to treatment initial complete remission can be achieved in over 50% of patients utilizing either autologous and allogeneic transplantation approaches. Allogeneic transplant, even using a matched sibling donor, has the disadvantage of significant initial morbidity and mortality which is as high as 25-30%. However, this has to be balanced against the approximately 70% relapse free survival at 5 years which can be achieved in good risk patients who achieve CR with allogeneic BMT (e.g. EMBT and Seattle data). Although the initial morbidity with autologous transplantation is much lower (< 5%) the subsequent relapse rates are much higher. Use of alpha-interferon maintenance has produced the most encouraging results in terms of remission duration and survival post BMT. The problem has become whether or not to recommend transplantation approaches versus standard chemotherapy in the absence of long term data on survival. Based on currently available data the long term survival benefit with alpha-interferon maintenance after standard chemotherapy is remarkably similar to that achievable thus far with BMT. Large randomized studies are under way to help clarify these issues and delineate the role of other types of intervention. There is particular interest in the use of multi-drug resistance reversal agents, erythropoietin to improve anemia, diphosphonates to improve bone disease, and antagonists or antibodies to IL-6, IL-1-beta and TNF to reduce myeloma activity and bone disease. Studies are also under way to improve peripheral stem cell recovery utilizing G or GM-CSF with or without cylophosphamide or some purging method. Many innovations in myeloma therapy can be anticipated within the next few years.

**29** CYTOKINE EFFICIENCY IN THE TREATMENT OF HIGH-GRADE MALIGNANT NON-HODGKIN LYMPHOMAS (hNHL): RESULTS OF A RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED STUDY WITH INTENSIFIED COP-BLAM ± rhGM-CSF. M. Engelhardt\*, H. Gerhartz, G. Brittinger\*, T. Binder, A. Engert, R. Fuchs, B. Geiseler, D. Gerhartz, A.R. Hanauske, J.-H. Hartlapp, D. Huhn, W.-D. Ludwig, P. Meusers\*, P. Müller, H.-W. Pees, Ch. Peschl, H. Radtke, C. Schadeck-Gressel, J. Schick, G. Schlimok, W. Siegert, W. Spann, E. Terhardt, E. Thiel, W. Wilmanns, R. Brandmaier, A.C. Stern, T.C. Jones, K. Lennert for a German NHL Study Group. \*Div. of Hematology, Dept. of Medicine, University of Essen, Germany

The potential of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) in supporting aggressive induction chemotherapy (CTX) in hNHL was evaluated. In a phase III trial 172 patients (pts) (age 18-73 years) with advanced hNHL (stage II 38%, III 29%, IV 33%) were risk-stratified according to LDH and lymph node size, treated with an intensified COP-BLAM regimen and randomized to receive as study medication (SM) rhGM-CSF (400 µg) (87 pts) or placebo (85 pts) sc. day 8-14 of each CTX cycle. An intent-to-treat analysis was performed on all 172 and an efficacy analysis on 125 pts (> 70% of SM) plus 12 pts with early progressive and/or fatal disease. Among all observed side effects only cutaneous reactions (mostly injection site related) occurred more frequently in the rhGM-CSF than control group. In the efficacy population a significant influence of rhGM-CSF became evident in reducing the frequency of all (p=.01) and of severe (p=.02) infections, the length of neutropenia during CTX (p=.01), the mean days of hospitalisation for infections (p=.02), of fever (p=.04), and of iv. antibiotic requirements (p=.03). Complete response rates in the rhGM-CSF vs control group were 55% vs 58% (n.s.) for all pts, 84% vs 95% (n.s.) in the low and 69% vs 48% (p=.04) in the high risk group. Adherence to CTX doses was similar in both SM groups with less cycle interval prolongations in the rhGM-CSF arm (p=.06). After a median observation time of 17 months, freedom from treatment failure did not differ significantly between treatment arms, and overall survival was identical. Rates of early relapse appear to be lower as compared to the standard-dose COP-BLAM/IMVP-16 regimen investigated earlier by the same study group. Thus, GM-CSF is well tolerated, abates toxic side effects of CTX, and may help to maintain dose intensity and to improve response in high risk hNHL.

