

ABSTRACTS

ORAL PRESENTATIONS

ABSTRACTS - Third International Conference on Malignant Lymphoma, Lugano

1 IMMUNOGLOBULIN PRODUCTION IN TRANSGENIC MICE. G. Koehler, Max-Planck-Institut fuer Immunbiologie, 7800 Freiburg, Federal Republic of Germany

Rearranged μ and κ genes encoding a mouse immunoglobulin (Ig) M molecule with anti-trinitrophenyl (TNP) specificity have been introduced into the germ line of Swiss albino mice (ref. 1). Both chains are found on the membrane of B-cells and secreted in the serum, where they give rise to a 300 to 800-fold increase in TNP titers. Heavy (H) and light (L) chain variable region genes undergo DNA rearrangement steps to become mature genes. Three spatially separated DNA-segments (V_H , D_H and J_H) for the H chain gene and two (V_L and J) for the L chain gene have to be joined together. B cells produce H and L chains only from one of the two homologous chromosomes, a phenomenon termed allelic exclusion. In 75% of B cells incomplete or no rearrangement is observed in the silent alleles implying a tight control of the rearrangement process. Taking results from several laboratories (1-4) the following picture of control of the Ig rearrangement process emerges:

- 1.) The heavy chain variable region gene (V_H) rearranges first.
 - 2.) Heavy chain (μ) protein inhibits further V_H rearrangement and activates light chain variable gene (V_L) rearrangement.
 - 3.) Light chain (κ) protein in association with the μ -chain inhibits further V_L rearrangements.
- We have analysed the humoral immune response of our (μ, κ) transgenic mouse line. We found an impaired response to type II antigens such as Dextran, Levan and Phosphorylcholine. Almost normal responses against protein antigens were observed with however a delayed onset of the IgG response. These results demonstrate that the possibility to use Ig-transgenic animals for disease protection purposes has its limits.

References

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2. K.A. Ritchie, R.L. Brinster and U. Storb (1984) Nature **312**: 517
3. D. Weaver et al. (1985) Cell **42**: 117
4. M. Reth et al. (1985) Nature **317**: 353

2 Ki-1 LYMPHOMA: EXPERIMENTAL AND CLINICAL FINDINGS. H.Stein, J.Gerdes, G.Tippelmann, D.Dienemann, R.Schwartzing, N.O'Connor*, S.Pileri**, G.Pallesen*** and G Delsol+, Inst.Path. FUB Berlin,FRG; *J.Radcliffe Hosp. Oxford,UK; **Inst.Path. Univ.Bologna, Italy; ***Inst.Path. Univ.Aarhus, Denmark; +Dept.Path. Purpan Hosp., Toulouse, France.

A large number (more than 400) lymph node tumors have been immunohistologically examined with antibodies directed at the Ki-1 antigen which is expressed on Hodgkin and Sternberg-Reed cells and activated T- and B-cells but not on resting B- and T-cells, germinal center cells and macrophages. The results obtained led to the description of a Ki-1 positive lymphoma group that is characterized by a preferential perifollicular involvement of the lymph node, intrasinusoidal dissemination and a morphology ranging from immunoblastic tumors (rare) to neoplasms of large bizarre cells with abundant cytoplasm. The initial diagnosis given to these lymphomas varied widely and included anaplastic carcinoma and malignant histiocytosis. Although these tumors appeared to represent a morphologically clearly recognizable lymphoma category originating from lymphoid cells at certain activation state phenotypical and genotypical studies revealed that these lymphomas are heterogenous: the majority proved to be of T cell type, some of B cell type and some of null cell type. Retrospective studies of clinical data showed two peaks in the age distribution, the larger one in the second/third decade and the lower one in the sixth/seventh decade. The clinical course and prognosis proved to be more unfavorable than that of high grade malignant NHL described by the Kiel-classification. Comparison of the treatment protocols revealed that the polychemotherapy regime developed for childhood ALL is also effective in Ki-1 lymphomas if the patients are younger than 25.

- 3** RECEPTORS OF B CELL ACTIVATION AND DIFFERENTIATION ARE VARIABLY EXPRESSED ON EBV NEGATIVE AND POSITIVE CELL LINES. A.C. Favrot¹, I. Philip¹, V. Combaret¹, M. Cooper², T. Philip¹, G. Lenoir³
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The expression of receptors for proliferation and differentiation factors was analyzed by indirect immunofluorescence on 29 Burkitt lymphoma (BL) cell lines previously classified in three groups on the basis of their reactivity with 8 monoclonal antibodies, including anti-CALLA, BL₁₃ and Tü₁. BL₁₃ and HB₅ antibodies recognize different epitopes of the EBV/CR₂ receptors. The determinant recognized by BL₁₃ has been previously shown to be expressed only on cell lines of the first two groups, supposed to derive from the germinal center and to be negative on a third group of lines of putative BM origin and established from sporadic cases of BL. On the opposite, and as expected from its reactivity on normal B cells either in the BM or the lymph node, HB₅ antibody reacts with all BL lines except one. The receptor for transferrin is expressed on the 29 lines. Two new monoclonal antibodies, Bac-1 and H₁B₅ could recognize respectively receptors for BCGF1 and BCGF2. Bac-1 reacts with 15 of 17 BL lines belonging to the first two groups and 7 of 12 BL lines of the third group; 14 of 15 EBV + lines express Bac-1. No BL line expresses H₁B₅. The IL2 receptor is weakly expressed on 5 EBV + cell lines and one EBV(-) line. The six are BCGF1 positive. The almost constant expression of BCGF1 receptor on EBV + cell lines is the only strict relation between the expression of receptors for growth factors and their characteristics (ie. EBV association, translocation, ethnic origin and clinical presentation). The maturation stage or the origin of BL cell lines in relation with the expression of growth factor receptors and the functional significance of these receptors will be discussed.

- 4** B-CELL NEOPLASIA RECAPITULATES THE NORMAL HUMORAL IMMUNE RESPONSE. D.D. Weisenburger, D.S. Harrington, J.O. Armitage, Departments of Pathology and Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA

Surface marker studies of 240 cases of B-cell lymphoma indicate that the various types of B-cell neoplasia represent successive stages at which neoplastic differentiation is arrested. Pre-B acute lymphoblastic leukemia arises from the rapidly-dividing pool of normal bone marrow lymphoblasts (cytoplasmic μ , CALLA +). Chronic lymphocytic leukemia/small lymphocytic lymphoma usually arises from an immature, bone marrow-derived, virgin B-cell that mediates the primary immune response (sIgM+D, Leu 1 +). Intermediate lymphocytic (mantle-zone) lymphoma arises from a slightly more differentiated cell that resides in primary follicles and the mantle zones of secondary follicles (sIgM+D, Leu 1 +). Thus, intermediate lymphocytes appear to be the precursor cells of normal germinal centers. The follicular center cell lymphomas are derived from reactive germinal centers, where clonal expansion and Ig class switching occur (IgM+D \rightarrow IgG/A) and from which memory B-cells arise. The sequence of differentiation within germinal centers is thought to be: small non-cleaved \rightarrow large non-cleaved \rightarrow large cleaved \rightarrow small cleaved cell (IgG/A, CALLA +). Plasmacytoid small lymphocytic lymphoma and myeloma may be derived from either mature IgM-producing B-cells of the primary immune response or IgG/A-producing B-cells of the secondary response (cytoplasmic Ig +). Immunoblastic lymphomas may be derived from large non-cleaved cells of the prolonged germinal center reaction, mature small lymphocytes of the primary response, or memory B-cells (cytoplasmic Ig +). Partial blocks in differentiation result in the development of "mixed-cell" lymphomas. Transformation of an indolent B-cell neoplasm to a more aggressive counterpart may involve differentiation, dedifferentiation, or a block at an active stage in the cell cycle. Thus, relationships of the various types of B-cell neoplasia may be understood in the context of the normal humoral immune response. These findings should lead to a more meaningful system of classification of B-cell neoplasia.

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5 MULTICLONAL LYMPHOMAS. J. Sklar, Department of Pathology, Stanford University, Stanford, California 94305.

The configurations of DNA within rearranged immunoglobulin genes of neoplastic B lymphocytes represent a clonal marker for cell populations within lymphoma tissues. Consistent with this fact, most B cell tumors show one or two rearranged bands in Southern blot analyses for a given immunoglobulin gene depending on whether one or both alleles of that gene have rearranged. Recently we have discovered a number of B lineage tumors in which more than two rearranged bands were detected for a single immunoglobulin gene, suggesting the possibility of more than one clonal population of lymphocytes within the tumor. This finding is particularly prevalent among cases of common acute lymphoblastic leukemia, in which about 25 percent show three or more rearranged heavy chain immunoglobulin bands. Often different rearranged bands were found to derive from separate populations of cells when such populations were physically separated one from another either *in vitro* or *in vivo*. This situation was found among lymphoproliferative disorders in immunosuppressed patients and in selected cases of low grade B cell lymphomas. Several mechanisms may, in theory, account for the appearance of multiclonality within B cell tumors. One possibility is that the appearance of multiclonality is an artifact due to the instability of rearranged bands as clonal markers. A second possibility is that the clonal populations within presumed multiclonal tumors arise from independent transformation of separate lymphocytic progenitor cells. A third possibility is that a single stem cell is transformed and gives rise to progeny subclones containing various different rearrangements of immunoglobulin gene DNA. We have found evidence indicating that apparent multiclonality of B lineage tumors may arise by each of these three mechanisms in different cases. Data supporting this conclusion will be presented and discussed.

6 PERIPHERAL BLOOD (PB) GENE REARRANGEMENT ANALYSES IN MALIGNANT LYMPHOMA. SJ Horning, N Galili, M Cleary, J Sklar, SA Rosenberg. Departments of Medicine/Oncology and Pathology, Stanford University Medical Center, Stanford CA 94305 USA.

The diagnostic sensitivity and clinical usefulness of gene rearrangement analyses are being studied in PB from a large group of patients (pts) with malignant lymphoma. DNA probes for the immunoglobulin heavy chain J region, constant portions of kappa and lambda light chains and beta chain of the T cell receptor (TCR) are used in Southern blot analyses to detect tumors of B- and T-cell lineage and to demonstrate monoclonality.

PB has been studied in 36 pts with low grade (LG) lymphoma in remission. Immunoglobulin gene rearrangements (R+) were found in 5 (14%), one of whom has since recurred. After treatment of intermediate/high grade (I-HG) lymphoma, R+ were found in 9 of 67 (13%) pts [in remission for <24 months (28 pts) or >24 months (39 pts)], one of whom has since recurred. Recurrence in 4/89 pts with no PB rearrangement (R-) were also seen.

	Recurrence of Lymphoma	
	R+ (14 pts)	R- (89 pts)
LG (36 pts)	1/5	1/31
I-HG (67 pts)	1/9	3/58

In pts with indolent LG lymphoma receiving no therapy (NT) [25 pts followed for 2-48 mos, 4 pts followed >7 years], R+ in PB were found in 11/29 (38%), including 6/7 of small lymphocytic (SL), 3/15 follicular small cleaved (FSC) and 2/7 follicular mixed (FM). At diagnosis (dx), 8 of 13 LG lymphoma pts had PB R+.

	Sensitivity of R+ in LG Lymphoma	
	New Dx (13 pts)	Indolent NT (29 pts)
SL (10 pts)	3/3	6/7
FSC (21 pts)	4/6	2/15
FM (11 pts)	1/4	2/7

R+ were found in 3 of 15 newly diagnosed I-HG, one of whom has relapsed. R+ in PB were identical to those in lymph node and bone marrow aspirates and biopsies. R+ were not seen in 5 LG pts after spontaneous regression. R+ of the TCR beta chain were not seen in 7 T-cell lymphoma pts.

To date, the frequency of PB R+ correlate with clinical disease activity and histologic subtype. The prognostic significance of PB R+ and the specificity of the analyses will require larger pt numbers and followup. Additions to and an update of this data will be presented.

- 7** REFRACTORINESS TO CHEMOTHERAPY AND POOR SURVIVAL RELATED TO ABNORMALITIES (ABNL) OF CHROMOSOMES 17 AND 7 IN LYMPHOMA. F. Cabanillas, S. Pathak, G. Grant, F.B. Hagemeister, P. McLaughlin, F. Swan, M.A. Rodriguez, J. Trujillo, A. Cork, J. Butler, R. Katz, S. Bourne, E.J. Freireich, University of Texas M.D. Anderson Hospital, 1515 Holcombe, Houston, Texas 77030 USA.

We have previously shown in a group of 60 previously treated and untreated pts that the presence of monosomy 17 or 7 or a deletion in the short arm of these chromosomes ("-17 or -7") is associated with refractoriness to chemotherapy. A difference in survival (surv) was not yet evident in that report. In the present study, we have examined the response rate (RR) and surv of 95 previously untreated pts and related it to cytogenetic findings in the three Working Formulation histologic grades:

Histol. Grade	Cytog. Abnl.	N	%		P	%	
			CR+PR	Fail		2-Yr Surv	P
Low	-17,-7	7	57	43	.02	67	.008
	Others	29	97	3		93	
Int.	-17,-7	14	63	36	.004	27	.006
	Others	23	100	0		58	
High	-17,-7	8	63	37	.2	58	.09
	Others	14	86	14		38	

Both the RR and surv are adversely affected by the presence of a -17 or -7 abnl in both low and intermediate grade types, but only the RR appears to be affected in high grade types. The absence of a surv difference in high grades is due in part to several AIDS pts who died of infection. In addition, the 2-year relapse-free surv of responders with -17,-7 was shorter (48%) than for others (62%). We also analyzed the 2-year surv rates for the combination of LDH and karyotype in the intermediate histologic grades.

LDH	N	Cytogenetic Abnormalities		P
		-17,-7	Others	
<350	19	28%	90%	.003
≥350	18	0%	38%	.003

Thus, the karyotype as a variable behaves independently from LDH and cell type. In combination with LDH, it is capable of defining at least three major prognostic groups. The presence of t(14;18) was associated with a more favorable surv within both the -17,-7 group as well as the others. These data suggest that one or more genes in the short arm of 17 and 7 are related to response to therapy and prognosis.

- 8** CHROMOSOMAL ABERRATIONS IN CHRONIC B-LYMPHOCYTIC LEUKAEMIA - CONSISTENCY DURING PROGRESSION OF DISEASE. Gunnar Juliusson, Gösta Gahrton. Division of Clinical Oncology and Haematology; Department of Medicine, Huddinge Hospital, S-141 86 Huddinge; and Department of Medical Cell Genetics, Medical Nobel Institute, Karolinska Institute, S-104 01 Stockholm, Sweden

Karyotypes with extra chromosome 12 and multiple clonal aberrations indicate progressive disease in chronic B-lymphocytic leukaemia (CLL). We here present results from serial cytogenetic analyses of leukaemic cells in 35 patients with CLL, in which malignant cells were obtainable at least 18 months from the first cytogenetic study. Isolated cells from peripheral blood, bone marrow, lymph nodes, and pleural effusions were activated with lipopolysaccharide, Epstein-Barr virus, tetradecanoyl-phorbol-acetate, and cytochalasin B, and cultured for 4 days in Eagle's medium supplemented with human AB serum. The karyotype was analysed in Q-banded metaphases.

Fifteen patients retained the same abnormal clone through all analyses, with a median interval between the samplings of 2.6 years (range 1.5 - 6.5 years). Two thirds of these patients had received treatment during the interval, and 80% had had a progressive disease. Six patients showed an abnormal clone at one sampling only (median interval 3.8 years, range 1.7 - 6.6 years; 50% treated, 83% with progressive disease). In twelve patients no clone was found in any sample (median interval 2.9, range 1.6 - 6.6 years; 25% treated, 33% with progressive disease). Only two patients showed a significant change of the karyotype. One patient had a 46,XY,+12,-17 karyotype in 6 metaphases and 46,XY,-17,+t(12;17) in 3 metaphases at diagnosis, whereas six years later (following cytostatic treatment and splenectomy) 100% of the metaphases had the 46,XY,-17,+t(12;17) abnormality. Another patient had 3 metaphases with a 45,XY,-19 karyotype, one metaphase with 47,XY,+12, and 19 normal metaphases at diagnosis. At the study 2.6 years later during stable untreated disease 40% of the metaphases showed an extra chromosome 12, whereas no metaphases with monosomy 19 was found.

Thus, karyotypic evolution rarely occurs in CLL, despite progressive disease and cytostatic treatment. This is in contrast to the case in chronic myelocytic leukaemia, in which progression into a blastic crisis is accompanied and predicted by recurrent additional chromosome abnormalities. Abnormal karyotypes in CLL are frequently found at diagnosis, and the incidence is not related to the blood lymphocyte count. The role of the karyotype as an individual phenotypic marker with prognostic implication in early CLL disease is thus emphasized.

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- 9** MIXED-LINEAGE LEUKEMIAS AND PHENOTYPIC SHIFTS OCCURRING IN RELAPSED CASES OF ACUTE T LYMPHOBLASTIC LYMPHOMAS. D. Delia, F. Morandi, R. Giardini, E. Ballerini, E. Fontanella, G. Cattoretti, A. Orazi, F. Rilke, G. Della Porta. Istituto Nazionale Tumori, Milano, Italy.

The finding that leukemias can occasionally exhibit a "lineage infidelity" (eg. lymphoid plus myeloid characteristics) led us to investigate this aspect in lymphomas. Lymph-node biopsies and bone marrow aspirates from 19 patients were phenotyped at the onset of the disease; 8 of them were typed in the relapse phase as well. We used monoclonal antibodies directed against CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD33, MHC class II, TdT. All cases had an immature T cell phenotype and although no "lineage infidelity" was detected at any stage of the disease, the following findings were observed:

A) Phenotypic "de-differentiation": 5 relapse cases though retaining the original T cell phenotype, showed the loss of at least one of the CD5, CD4, CD8, CD2 antigens (but not of CD5, CD7, CD10). Acquisition of these antigens was never seen.

B) Mixed-lineage relapse: the second relapse of a T-lymphoblastic lymphoma case (Eros-, HLA-DR-, SIg-, TdT+) was characterized by blasts which, by FACS sorting, could be dissected into a lymphoid clone (CD5+, CD10+, CD11b-, CD13-, CD33-, HLA-DR-, TdT+) and into a myeloid clone (CD5-, CD7-, CD10-, CD11b+, CD13+, CD33+, HLA-DR+, TdT+, Perox+, Auer+) and CFUc negative.

C) Myeloid relapse: two cases, one with a cortical thymic phenotype (CD1+, CD2+, CD4+, CD5+, CD7+, CD8-, CD10+, CD11b-, HLA-DR-, TdT+) and one with a supposedly early thymic phenotype (CD1-, CD2-, CD4-, CD5+, CD7+, CD8-, CD10-, CD11b+, HLA-DR+, TdT+/-) relapsed into a myeloid leukemia (CD1-, CD2-, CD7+ or -, HLA-, DR+, MY7+, MY9+, Perox +, CAE+). The latter case gave no CFUc. Both myeloid-relapse cases had the TCR- β in the germ-line configuration. These data point out that the progression of the disease often leads to the appearance of lineage-restricted clones with a less mature phenotype (and never the opposite); relapse into a myeloid lineage can also occur, though more rarely. These findings may be restricted to lymphoid malignancies of T cell origin since neither non T-non B nor B cell lymphomas (Burkitt's included) we have studied presented such clinical evolution. The phenotypic follow-up of this patients may be important to implement chemotherapeutic protocols which are more adequate to the biological evolution of the disease.

- 10** COMBINATION THERAPY WITH CYTOKINES AND MONOCLONAL ANTIBODIES. B. Issell and J. Winkelhake, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, California 94608, USA

The immune response comprises an orchestration of cellular, antibody and other soluble mediator (cytokine) interactions. The production of specific cytokines and monoclonal antibodies in large quantities has now allowed these physiologic interactions to be explored in a pharmacologic way. The administration of monoclonal antibodies against cancer associated antigens has resulted in clear antitumor regression. A mechanism of action has been presumed to be through activation of monocytes and other effector cells by antibody binding to Fc receptors present on these cells in the antibody dependent cellular cytotoxicity (ADCC) process. The potential enhancement of this effect by the addition of cytokines such as interleukin-2 (IL-2) and the interferons, which may enhance effector cell function and antigen expression, has been demonstrated *in vitro* and in animal studies. Human studies are in progress and the experience reported to date will be reviewed.

There is also a rationale for the administration of cytokines in combination based on differential effector cell antigen recognition and cytolytic functions. The potential additive effects of combining IL-2 with the interferons or tumor necrosis factor (TNF) have been confirmed *in vitro* and in various animal tumor models, and are now being tested in patients with various tumor types. For any combination therapy, proportional dosage, scheduling of each component, and sequence of administration are variables which may be critical to a successful outcome. Our experience with IL-2 combined with TNF, which demonstrates some remarkable therapeutic synergy and sequence dependency, will be reviewed.

THE CLINICAL PHARMACOLOGY OF A RECOMBINANT HUMAN IL-2 ANALOG IN PATIENTS WITH CANCER

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Phase II clinical trials in patients with refractory malignancies have been in progress since January, 1985. Since that time over 600 patients have been entered into Phase II trials designed to determine the pharmacology, immunologic effects, toxicity, and antitumor response following treatment with this novel IL-2 analog. The half-life of IL-2 following IV administration is biphasic, with a serum distribution phase of five minutes and a half-life of approximately seventy minutes. IL-2 induces a transient and prompt margination of circulating lymphocytes, followed by a progressive and dose related lymphocytosis. Patients show prompt and marked increase in NK activity *in vivo*. A subset of patients show LAK induction *in vivo*. This *in vivo* induction of LAK activity is highly correlated with clinical tumor regression. LAK activity is present in IL-2 activated T lymphocytes, B lymphocytes, and null lymphocytes. Toxicity of IL-2 is dependent upon dose and schedule. Moderate doses given to outpatients is well tolerated, associated with tumor regression, and safe, with no irreversible toxicities or treatment-related deaths. The relative contribution of adoptive cellular therapy with *ex vivo* IL-2 stimulated lymphocytes in addition to IL-2 as a single agent will be discussed. Consistent anti-tumor activity in patients with melanoma, renal cell carcinoma, colon carcinoma, lymphoma, and ovarian carcinoma has been observed, and tumor regressions in patients with non small cell and small cell lung carcinoma, mesothelioma, bladder carcinoma, thyroid carcinoma, chronic leukemia, and Kaposi's sarcoma have been seen. No neutralizing IL-2 antibodies have been seen in patients receiving intravenous IL-2 therapy. Trials are underway exploring the combinations of IL-2 with conventional cytotoxic chemotherapy, interferons, tumor necrosis factor, monoclonal antibodies, tumor vaccines, and as an adjuvant following surgery in patients with stage II melanoma.

12 DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST HODGKIN - DERIVED CELL LINES. M. Pfreundschuh, E. Mommertz, G.F.R. Krüger, P. Möller, A.C. Fel-ler, R. Hassa, V. Diehl. Med. Univ. Klinik I, D-5000 Köln, F.R.G

Monoclonal antibodies were developed by immunizing mice with the Hodgkin - derived cell line L428. Of 10 000 hybridomas screened, about one third reacted with the Hodgkin cell line used for immunization. By immunohistological screening two clones could be identified which produced the monoclonal antibodies HSR-1 and HSR-2. HSR-1 and HSR-2 react with all Hodgkin and Sternberg-Reed cells *in vitro* and *in vivo*. Both antibodies react only with a small subpopulation of cells in a normal lymph node. The antigen detected by these antibodies is not expressed on the surface of unstimulated cells of the peripheral blood or bone marrow. However, after stimulation with mitogens or infection by viruses the antigen is expressed on activated T-lymphocytes, B-lymphocytes, but also on monocytes and macrophages. Thus the antigen detected by these antibodies represents a non-lineage specific activation antigen. The antigen seems to be identical with the one detected by other antibodies of the cluster CD30. Blocking experiments with the antibodies HSR-1, HSR-2 and Ki-1 showed that each detects different epitopes of the same antigen. Whereas none of the antibodies is cytotoxic by itself, a combination of two or three of these monoclonal antibodies is strongly cytotoxic. Therefore, a cocktail of these Hodgkin-associated antibodies holds promise for *in-vivo*-imaging, purging of bone marrow for autologous bone marrow transplantation, and immunotherapy in patients with Hodgkin's disease.

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- 13** LOW FIELD STRENGTH MAGNETIC RESONANCE IMAGING OF THE LIVER IN PATIENTS WITH MALIGNANT LYMPHOMA. M.A. Richards, J.A.W. Webb, R.H. Reznick, S.E. Jewell, P.F.M. Wrigley, T.A. Lister. ICRF Department of Medical Oncology and Department of Radiology, St. Bartholomew's Hospital, London EC1A 7BE.

Magnetic resonance imaging with spin lattice relaxation time (T_1) measurement of the liver was performed on 89 patients with lymphoma (48 HD, 41 NHL). Liver T_1 measurements from patients were compared with those from 61 volunteers. The influence of clinical status (previously untreated, remission, relapse) on liver T_1 was assessed. For 48 previously untreated patients liver T_1 was compared with the results of clinical staging investigations. Comparison with the results of open liver biopsy was made in 27 patients.

All 7 patients with biopsy proven hepatic lymphoma had elevated liver T_1 . Nineteen patients had normal liver biopsies, 15 of whom had normal liver T_1 . T_1 was marginally elevated in 2 patients and markedly elevated in the other 2 patients with normal liver histology. The remaining patient had biopsy evidence of chronic active hepatitis and had slight elevation of liver T_1 . Thus the sensitivity of T_1 for the detection of hepatic lymphoma was excellent but the specificity was less good.

In untreated patients with HD liver T_1 correlated closely with clinical stage. ESR, alkaline phosphatase and B symptoms ($r > 0.7$ in all cases). For untreated patients with NHL no such correlations were detected ($r < 0.24$ in all cases).

MRI was performed before and after chemotherapy in 15 patients. Liver T_1 returned to normal after treatment in all 9 patients who achieved clinical remission, but was abnormal in 6 patients who achieved only a partial remission or who failed to respond. The implications of these results for the detection of hepatic lymphoma and for monitoring response to treatment by MRI will be discussed.

- 14** MALIGNANT LYMPHOMAS ASSOCIATED WITH ASBESTOS EXPOSURE. R. Jacobson, D. Memoli, E. Kagan, Depts of Med. and Path. Georgetown Univ. Med. Center, 3800 Reservoir Road N.W. Washington D.C. 20007, U.S.A.

Asbestos has been incriminated as a putative lymphoid carcinogen. An increase in lymphoproliferative and plasma cell neoplasms has been noted in epidemiologic studies of individuals exposed to asbestos (Gerber *Am. J. Clin. Path.* 53:204, 1970; Robinson *et al* in *Dust and Disease*, edit. Lemen and Dement, *Pathotox Pub. Ill.* p.131, 1979 and Ross *et al* *Lancet* 20:1118, 1982). We previously reported (*Am. J. Clin. Path.* 8:14, 1983) a series of patients with asbestos related B cell neoplasms of the bone marrow (chronic lymphocytic leukemia and multiple myeloma). We now present the clinicopathologic findings in 12 male patients with non-Hodgkin's lymphoma who were occupationally exposed to asbestos. The patient's mean age was 58 yrs (range 27-68 yr) and the mean duration of asbestos exposure was 22.7 yrs (range 3-37 yrs). Stigmata of asbestos related pulmonary disease were present in all 12 patients and included interstitial fibrosis, localized pleural plaques and diffuse pleural thickening. None of the men had coexistent malignant mesothelioma or bronchogenic carcinoma. Tumor latency periods ranged from 14-41 yrs (mean 32 yrs). Diffuse large cell lymphoma (DLCL) was present in 7 patients, in 6 it involved the lungs or intrathoracic lymph nodes and in 1 the stomach only. Two patients had follicular small cleaved cell lymphoma, 2 had diffuse small lymphocytic lymphoma (1 with plasmacytoid features) and 1 had diffuse small cleaved cell lymphoma. B cell origin of the lymphomas was established in 6 patients. Patients were treated with chemotherapy and 6 had in addition localized radiation therapy. Four patients with stage IV DLCL expired within 1.25 yrs of the lymphoma diagnosis, but 2 with stage II DLCL are alive in complete remission (CR) 8 and 25 yrs, respectively, after treatment. The patient with gastric lymphoma has been in CR for 4 yrs. The one with plasmacytoid lymphoma died within 1 yr of diagnosis and the other 4 lymphomas with intermediate and low grade lymphomas are alive 1-10 yrs after diagnosis. Three of these asbestos workers exhibited evidence of deteriorating pulmonary function following combination chemotherapy in which bleomycin was included. By contrast, 5 patients receiving chemotherapy without bleomycin showed no progression of pulmonary symptomatology. Thus, bleomycin use in individuals with lymphoma and a history of asbestos exposure should be questioned.

15 SPECIFIC CHROMOSOME ABERRATIONS IN NON-HODGKIN'S LYMPHOMA AND LYMPHOID LEUKEMIA J. D. Rowley, Section of Hematology/Oncology University of Chicago, Chicago, Illinois 60637, USA

The association of specific chromosome aberrations with particular morphologic subtypes of leukemia and lymphoma has been one of the remarkable discoveries of the last decade. Although this has been most clearly demonstrated in the acute myeloid leukemias, recent studies have confirmed the relevance of this paradigm to the acute lymphoid leukemias and to the non-Hodgkin's lymphomas as well.

The best known example is the presence in Burkitt's lymphoma (BL) of a translocation involving chromosomes 8 and 14 [t(8;14)(q24;q32)] or two related variant translocations involving No. 8 and either Nos. 2 or 22. The unifying feature of these translocations is the juxtaposition of a protooncogene, *MYC*, at 8q24 and one of the immunoglobulin genes, heavy chain, (IgH) kappa or lambda light chain, located on chromosomes 14, 2, or 22, respectively. Although the t(8;14) is common in BL, it is also seen in some non-BL small noncleaved cell lymphomas and in some diffuse large cell lymphomas. Present evidence suggests that the breakpoints relative to *MYC* may be different in the Burkitt's and non Burkitt's lymphomas with a t(8;14).

One of the most common lymphomas is follicular small cleaved cell lymphoma, approximately 60% of which have a translocation involving chromosomes 14 and 18 [t(14;18)(q32;q21)]. Some patients with a t(14;18) acquire a t(8;14) as the disease evolves to a more malignant phenotype. This 14;18 translocation junction has been cloned; the break in No. 14 is in the IgH gene and in No. 18 as in a gene called *BCL-2*. The use of DNA probes for *BCL-2* has revealed some heterogeneity in the breakpoint in chromosome 18. This DNA probe is especially useful because the translocation can be detected in tissues with too few dividing cells for chromosome studies.

Similar analyses in T cell leukemias have revealed a translocation comparable to that in BL. This translocation also involves Nos. 8 and 14; the break in No. 8 is at the end of the *MYC* gene and in No. 14 is in the alpha chain of the T-cell receptor (*TCR-α*) at 14q11. Other structural rearrangements involving 14q11 also interrupt *TCR-α*. Thus in the translocations in BL and in T cell leukemia, *MYC* is involved in both with similar breakpoints; the specificity regarding the translocation is provided by the cell specific gene, namely the immunoglobulin genes in B cells and *TCR-α* in T cells. The genetic analysis of other rearrangements will provide new insights into the biological and clinical significance of these specific chromosome abnormalities.

16 HODGKIN'S DISEASE: THE MILAN CANCER INSTITUTE EXPERIENCE WITH MOPP AND ABVD. G. Bonadonna, Istituto Nazionale Tumori, 20133 Milano, Italy.

The first randomized study comparing MOPP vs ABVD was activated in 1973 and the final results indicated that the two regimens had similar effectiveness. In 1974, we started two different programs. In patients with stage IIB-III (A + B), three cycles of either MOPP or ABVD preceded and followed extensive radiotherapy (RT, subtotal or total nodal irradiation according to disease presentation). The 7-year results indicated that ABVD was superior to MOPP in terms of complete remission rate (CR: 92% vs 81%, $P < 0.02$), freedom from progression (FFP: 81% vs 63%, $P < 0.002$) and overall survival (77% vs 68%, $P = 0.03$). Moreover, cardiopulmonary studies failed to document significant laboratory differences between the two treatment groups, while iatrogenic morbidity occurred only in patients subjected to MOPP. The second study, carried out in patients with stage IV disease, was aimed at overcoming the problem of drug resistant cells. MOPP and ABVD were alternated every month (MM/AA) and compared to MOPP alone. In the absence of progressive lymphoma, treatment was continued for 12 cycles. The CR rate was 89% following MM/AA and 74% after MOPP. The eight-year results showed that both FFP (MM/AA 65%, MOPP 36%, $P < 0.005$) and relapse-free survival (73% vs 45%, $P < 0.01$) were superior after the alternating regimen. Also tumor mortality was lower following MM/AA (16%) compared to MOPP (36%). Based on the above mentioned findings and to test the assumption that a more close alternation of effective drugs could kill a larger fraction of tumor cells, in July 1982 we started a new study in patients with stage II, III and IV randomly testing two different alternating sequences. MM/AA was compared with MA/MA (i.e. half cycle of MOPP and ABVD within one month). Either regimen was administered to CR plus two consolidation cycles (minimum 6 cycles) with the intent to spare toxicity due to prolonged and presumably unnecessary treatment. Involved field RT was given only to the area(s) of bulky lymphoma. A total of 209 patients with a minimum follow-up of 12 months are presently evaluable. The very preliminary results show that either sequence is able to achieve CR in 91% of patients (MM/AA 91%, MA/MA 90%). So far, 22% of CRs have already failed. The most critical factor both in achievement and maintenance of CR remains the number of involved nodal sites (< 3 sites: CR 97%, relapse 18%; > 3 sites: CR 84%, relapse 28%). At the time of present analysis, tumor mortality accounted for 3% in either treatment group. Present results suggest that the proper role and extent of RT as well as the duration of chemotherapy need to be verified in an appropriately designed clinical trial.

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17 HODGKIN'S DISEASE: NCI TRIALS ADDRESSING THE REMAINING CHALLENGES. R.C. Young, D.L. Longo, E. Glatstein, S. Hubbard, P. Duffey, and V.T. DeVita, Jr., NCI, NIH, Bethesda, MD.

Although major progress has been made in curative treatment of all stages of Hodgkin's disease (HD), major questions remain in several crucial areas. These involve optimal management of early stage disease, advanced disease, and patients with massive mediastinal involvement as well as the late complications of HD treatment.

While Stage I & II patients are generally effectively managed with irradiation therapy 20-40% of these ultimately relapse and salvage chemotherapy rescues about half at an increased risk of secondary leukemia. Furthermore, state-of-the-art radiation therapy is not always available and there is significant variation in technical expertise which can compromise optimal results. As a result, the NCI has undertaken a randomized comparison of irradiation therapy and MOPP chemotherapy for treatment of central IA-IIIA₁ HD. Eighty-nine patients have been randomized. CR's for both groups are high (95-100%) but relapses are more common after radiation therapy. Disease-free, long term survival, and comparative toxicities will be the crucial endpoints of this trial.

Massive mediastinal HD represents a significant management problem and radiation alone generally achieves 5-year disease-free survivals of 45%. As a result, combined modality approaches have been generally preferred. The current NCI trial includes all stages of HD with a mediastinal mass > 33% of the chest diameter. Patients have radiologic simulation of mediastinal disease followed by 3 full cycles of MOPP-ABVD followed by irradiation therapy. Forty-five massive mediastinal patients have been treated. Eighty-four percent achieved CR and with a median follow-up of >34 months, 16% have relapsed.

Although major progress in the treatment of advanced HD has been made, nearly half of patients still die prematurely. The 20% who are primary induction failures generally live less than 1 year. The use of alternating non-cross resistant combinations has been successful in some trials and the NCI has attempted to improve our results with MOPP alone using an alternating sequence regimen MOPP-CABS (CCNU, adriamycin, bleomycin and streptozotocin). One hundred and two patients with Stages IIIA-IVB have been randomized. CR's are 90% and 86% respectively. With a median follow-up of >41 months, 23-26% have relapsed and deaths are equally distributed between the two therapies. While results are excellent in both arms (overall survival = 68% at 6 yrs), there is no evidence of additional benefit from this alternating sequence approach.

Acute non-lymphocytic leukemia has been one of the most disturbing late complications of curative HD treatment. Long term follow-up (median 15.3 yrs) of MOPP-treated HD patients at the NCI indicates an overall low risk with chemotherapy alone (2% at 10 yrs) and for combined modality therapy a peak risk between 3 and 9 years; after 11 years risk appears to fall to that of a normal population.

18 THE CONTINUING CHALLENGE OF HODGKIN'S DISEASE. S. A. Rosenberg, Division of Oncology, Stanford University, Stanford, CA, USA

The results of treatment for patients with Hodgkin's disease in several major medical centers indicate that approximately 75% of individuals can be cured with their initial therapy. These excellent results achieved by modern radiotherapy, chemotherapy or combined modality therapy might suggest the "problem" of Hodgkin's disease has been solved. Experienced clinicians and clinical investigators know this is not true. Major problems remain and their solutions are needed.

Management programs are costly and morbid. With increased followup duration, long term morbidity is being documented. Data is accumulating that non-hematologic neoplasms and cardiovascular deaths are increased. The role of laparotomy and splenectomy continues to be challenged and needs re-definition. New prognostic factors are needed to select patients for minimal treatment programs for the most favorable and maximal treatment programs for the least favorable. Debate continues as to which chemotherapy regimen is the most effective and least toxic.

These questions will be presented and data presented, if available, to put these problems in their proper prospective.

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19 HODGKIN'S DISEASE: IS PARA-AORTIC IRRADIATION NECESSARY IN ALL CASES OF SURGICALLY STAGED SUPRADIAPHRAGMATIC DISEASE? N.J.S. Voss, R.N. Fahey, Division of Radiation Oncology, Cancer Control Agency of British Columbia, 600 West 10th Ave., Vancouver, B.C., Canada

During the period 1978-1985, 91 consecutive patients with surgically staged supradiaphragmatic Hodgkin's disease, PSIA and PSIIA, (mediastinal mass <1/3 chest diameter or absent, age \geq 16 years) were treated with irradiation alone at the Cancer Control Agency of B.C. Those patients without mediastinal disease (extrathoracic only), did not receive para-aortic irradiation (short mantle). For those patients with mediastinal disease (+/- extrathoracic), treatment fields were extended to include L2 (long mantle).

One stage IA and 9 stage IIA patients relapsed. All have entered 2nd remission and have remained in remission. Two patients died; one due to acute leukemia after salvage chemotherapy, the second of non-Hodgkin's lymphoma without recurrence of Hodgkin's disease. Relapse patterns will be analysed.

There have been no deaths due to Hodgkin's disease. For those patients without mediastinal involvement, para-aortic irradiation is not indicated, and may safely be omitted.

RESULTS WERE AS FOLLOWS:		SURVIVAL	RELAPSE FREE SURVIVAL
PSIA + PSIIA	91 pts.	89 (97.8%)	81 (89%)
PSIA	27 pts.	27 (100%)	26 (96.3%)
PSIIA	64 pts.	62 (96.9%)	55 (85.9%)

ANALYSIS BY DISEASE LOCATION

EXTRATHORACIC ONLY (SHORT MANTLE)	46 pts.	46 (100%)	44 (95.7%)
PSIA	24 pts.	24 (100%)	23 (95.8%)
PSIIA	22 pts.	22 (100%)	21 (95.5%)
MEDIASTINAL +/- EXTRA (LONG MANTLE)	45 pts.	43 (95.6%)	37 (82.2%)
PSIA	3 pts.	3 (100%)	3 (100%)
PSIIA	42 pts.	40 (95.2%)	34 (81%)

20 RADIO THERAPY (RT) VS CHEMOTHERAPY (CHT) IN PATIENTS WITH EARLY STAGE HODGKIN'S DISEASE (H.D.) (PATH.ST. II AND IIA) - REPORT AFTER 4.5 YEARS OF FOLLOW-UP.

G.P. Biti, L. Cionini, G. Cimino, A.P. Anselmo, G.P. Bellesi, A. Bosi, R. Maurizi Enrici, V. Mungai, P. Ponticelli, P.L. Rossi Ferrini, C. Biagini, F. Mandelli, G. De Giuli. - CNR Radiother.Chemother. Coop.Group for H.D., Depart. of Radiother. and Haematol. of Florence and Rome.

One of the main problem of the therapeutical approach to early stage H.D. is to identify the treatment modality most effective in terms of cure rate, whilst reducing iatrogenic damage. Treatment strategies including both Radiotherapy and Chemotherapy increased cure rate but iatrogenic damage (including acute non-lymphatic leukaemia) was increased as well. De Vita and coworkers showed (1976-78) that cure was possible even in advanced stages with MOPP polichemotherapy. A randomized study was therefore stated in 1979 at our Institutions, comparing MOPP (6 courses) with Radiotherapy ("mantle" + paraortic nodes fields) in patients with early stage H.D. (Path.ST. I and IIA). The aim of the study was to assess whether MOPP (a treatment less expensive and more easily available) is at least as effective as Radiotherapy (in terms of cure rate), with less iatrogenic damage.

MATERIAL: from August 1979 to December 1982 89 consecutively treated patients with laparotomy documented early stage H.D. entered the study. All patients were randomly allocated to receive: mantle + lumbar bar RT (45 pts) or MOPP (6 courses) Chemotherapy (44 pts). There is no difference between patients in the two arms as far as sex, age, histology, number and size of initially involved areas, or values of blood samples are concerned.

RESULTS: complete remission (CR) was achieved in 45/45 pts in RT group, and in only 40/44 pts in the CHT group. Considering CR rates according to prognostic factors, only mediastinal involvement significantly affected the achievement of CR in CHT group ($p < 0.01$). Including non responding (NR) patients (4) overall survival (OS) and disease free survival (DFS), at 66 months, were respectively 84.1% and 65.9% in CHT group, 93.5% and 74% in RT group. Excluding NR patients OS and DFS were respectively 87.8% and 72.7% in CHT group and 93.5% and 74% in RT group. No statistical difference has been found in the two groups of patients considering the prognostic factors (bulky disease, number of involved areas etc.). Seven out of 11 pts in the CHT group relapsed in previously affected areas, whereas only 3 out of 11 of RT group did so. Survival probability of relapsing patients at 52 months resulted to be 76% for the patients in RT group and 45% in the MOPP group. Treatment related complication for the CHT and RT groups were: azoospermia (100% and 0% respectively); amenorrhea (50% and 10% respectively). Treatment-related acute non lymphoid leukaemia up to date, did never occur. In conclusion, our results suggest that Chemotherapy is not an effective substitute for Radiotherapy in early stage H.D.

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- 21** THE CLINICAL STAGES I AND II HODGKIN'S DISEASE : THE E.O.R.T.C LYMPHOMA GROUP EXPERIENCE OVER TWO DECADES. TOWARDS COMPREHENSIVE MANAGEMENT TAILORED TO PROGNOSTIC FACTORS. M. Tubiana, P. Carde, M. Henry-Amar, J.M.V. Burgers, M. Hayat, E. van der Schueren, E.M. Noordijk, A. Tanguy, B. de Pauw, J.M. Cosset, R. Somers, Institut Gustave-Roussy, 94800 Villejuif, France.

From 1964 (H1) to 1986 (H6), the EORTC lymphoma group conducted four consecutive controlled clinical trials on clinical stages I and II Hodgkin's disease in which over 1 600 patients were entered. From the onset the main aim of these trials was to identify the subsets of patients who can be safely treated by a limited field radiotherapy (RT). Therefore several prognostic factors were prospectively registered. In the H2 trial (1972-1976), the histologic subtype was the only variable taken into account for the therapeutic strategy and the prognostic significance of the staging laparotomy findings was assessed. In the H5 trial (1976-1982), patients were subdivided into 2 subgroups according to 4 prognostic indicators (systemic symptoms and erythrocyte sedimentation rate -ESR-, age, histologic subtype and mediastinal involvement); in the favorable group, patients were submitted to a staging laparotomy (lap); lap- pts were randomized between mantle field RT and mantle field plus para-aortic RT; the relapse free survival (RFS) and total survival (TS) were identical in the 2 arms. In the unfavorable group, patients were not laparotomy staged as an aggressive treatment was felt justified, pts were randomized between total nodal irradiation or combination of 6 MOPP and mantle field RT; the RFS and TS were significantly higher in the latter group but in patients below the age of 40, the results of the 2 treatments were equivalent.

Once the multivariate analysis of the data had shown that the number of involved lymphatic areas had a paramount prognostic impact, this indicator was taken into account in the H6 trial in which the pts were subdivided into 2 subgroups according to 2 prognostic factors (systemic symptoms and ESR, number of involved areas). In the favorable group, pts were randomized between work-up with or w.o staging lap. In the arm w.o lap, pts were treated by RT only. In the arm with lap, lap- pts were treated by RT only, lap+ pts received RT + combination chemotherapy. The preliminary data suggest that the incidence of relapse is slightly higher in the arm w.o laparotomy.

From H1 to H6 trials, the proportion of pts having received CT during the course of the disease gradually decreased; the H6 data suggest that a further reduction in the management aggressiveness is conceptually possible. On the basis of the prognostic factors identified, one can delineate three subsets of pts and modulate the aggressivity of the initial treatment according to these subsets.

- 22** RESULTS OF THE HD1 AND HD3 TRIALS OF THE GERMAN HODGKIN'S DISEASE STUDY GROUP. V. Diehl, M. Pfreundschuh, U. Ruhl, M. Löffler, K. Smith, H. Gerhartz, E. Hiller. Med. Univ. Klinik I, D-5000 Cologne 41, F.R.G.

Between 7/83 and 9/86 575 untreated patients with Hodgkin's disease (HD) were staged clinically (including CT-scans of chest/abdomen, sonograms and lymphangiograms) and (except in CS IIIB/IV) surgically (laparotomy with splenectomy). In 78/248 (32%) patients who underwent diagnostic laparotomy with splenectomy infradiaphragmal disease was detected which had not been detected by CT scans, sonograms or lymphangiograms. So far, 331 untreated patients qualified for the HD1 and HD3 protocols. Patients in stages I-IIIa with the risk factors large mediastinal mass, extranodal disease and/or massive splenic involvement were entered into the HD1 protocol and patients in stages CS/PS IIIB/IV into the HD3 protocol.

In HD1 patients received chemotherapy consisting of 2x COPP/ABVD and were then randomized to receive additional radiotherapy of 40 Gy EF or 20 Gy EF. 51/65 evaluable patients (78%) achieved complete remission (CR). The survival of HD1 patients in stages I-IIIa with risk factors is as good as the survival of patients in stages I-IIa without risk factors who received only RT.

In HD3 (IIIB/IV) patients received chemotherapy with 3x COPP/ABVD. Patients in CR were randomized to receive consolidation therapy by one additional cycle of COPP/ABVD or by radiotherapy (20 Gy IF), while those in non-CR received salvage therapy. Salvage therapy consisted of radiotherapy in the case of persistent nodal disease or chemotherapy with 4x CEVD (CCNU 80 mg/m² i.v. d1; Etoposid 120 mg/m² p.o. d1-5, d22-26; Vindesin 3 mg/m² i.v. d1,d23; Dexamethasone 1.5 mg/m² p.o. d1-26). 58/93 (62%) evaluable patients achieved CR after 3x COPP/ABVD. This is significantly better (p<0.01) than the 31% CR rate observed in IIIB/IV patients who were treated in a pilot study with 6x COPP. After salvage therapy the overall CR rate was 71%. The progression-free survival of patients who received consolidation therapy was significantly (p<0.01) better than the survival of patients who refused consolidation therapy after achieving CR.

Recruitment of patients continues and updated results of the HD1 and HD3 trials will be presented. Supported by BMFT 01ZP550A

23 MOPP VS ALTERNATING MOPP/ABVD IN ADVANCED HODGKIN'S DISEASE (HD).

R. Somers, M. Henry-Amar on behalf of the EORTC Lymphoma Cooperative Group and the Groupe Pierre et Marie Curie. The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Between June 1981 and November 1986, 190 patients with stages IIIB-IV HD have been included in a protocol which compared 8 courses of MOPP with an alternating regimen in which 2 courses of MOPP were alternated with 2 courses of ABVD to a total of 8 courses. Additional radiotherapy (RT) was given to patients with initial masses larger than 5 cm or not in complete remission (CR) after 4 courses. An other aim of this study was to evaluate if it is possible to predict the response to treatment according to results observed after the first 2 or 4 courses of chemotherapy (CT). A stepwise logistic regression was used in an attempt to define the prognostic factors of CR at the end of CT. The covariates used in the model were age, sex, B-symptoms, sedimentation rate, clinical stage, presentation of the disease, tumor volume and clinical response after 2 or 4 courses of CT. Data were updated on January 1, 1987.

Data presented concern the 164 patients who completed initial treatment (86%). There were 81 patients in the MOPP group and 83 in the MOPP/ABVD group. After 8 courses of CT, patients in CR were 56% in the MOPP group and 55% in the MOPP/ABVD group; patients in partial remission (PR) were 22% and 34% respectively. Addition of RT increased the proportion of CR to 66% in the MOPP group and to 75% in the MOPP/ABVD group. Of the 45 patients in PR at the end of CT, 24/31 (77%) were in CR after RT. At 3 yrs, there was no statistical difference in relapse free survival (RFS) (72% vs 77%) nor in overall survival (80% vs 86%) between the 2 arms. From logistic regression analysis, only remission status after 4 courses of CT was predictive of final CR at the end of CT: 95% of the 64 patients in CR after 4 courses were still in CR at the end of CT as compared to 30% of the 100 patients not in early CR ($p < 0.001$). Moreover, CR after 4 courses was related to a better 3-yr RFS: 86% vs 64% ($p < 0.01$), and a better 3-yr survival: 83% vs 70% ($p < 0.02$) as compared to patients with a late CR.

In conclusion, comparison of 8 courses of MOPP with an alternation of 2 courses of MOPP with 2 courses of ABVD to a total of 8 courses showed no difference in CR rate, 3-yr RFS and survival. There was an increase in the overall proportion of patients in CR (55% to 70%) due to additional RT after completion of CT. Early CR after 4 courses of CT had a significant influence on the 3-yr RFS and on survival. Thus, the results suggest that patients not in CR after 4 courses of CT should be submitted to immediate consolidation therapy.

24 IMPROVED SURVIVAL WITH SEQUENTIAL BLEO-MOPP FOLLOWED BY ABVD FOR ADVANCED HODGKIN'S DISEASE (HD): 7-YEAR RESULTS. J. Glick, A. Tsiatis, P. Rubin, J. Bennett, for the Eastern Cooperative Oncology Group (ECOG). Philadelphia, PA 19104, Boston, MA 02115, and Rochester, NY 14642.

Between June, 1976 and Sept., 1981, 232 evaluable patients (pts) with previously untreated HD, Stages IIIB, IVA and IVB were entered on an ECOG trial that treated all pts with a fixed 6-cycle induction program of low-dose Bleomycin-MOPP. Complete and partial responders were then randomized to consolidation with either ABVD x 3 cycles (87 pts) or to XRT = low-dose radiotherapy (1500-2000 rad) to all sites of pretreatment involvement except bone marrow (85 pts) per Prosnitz (Cancer 37:2826, 1976). 60 pts in CR or PR after induction were not randomized into consolidation (primarily because of patient refusal or rapid relapse from PR), but have been followed for relapse-free and overall survival.

The CR rate with Bleo-MOPP induction was 59%, and the PR rate was 38%. However, 63% of these PR converted to restaged CR with consolidation (62% CR conversion on ABVD, 64% on XRT). Thus, the overall CR rate at the end of consolidation was 75%. No prognostic factor predicted for a significantly higher CR rate, either during induction with Bleo-MOPP or during consolidation with ABVD or XRT. There were no imbalances in prognostic factors between pts on the ABVD and XRT arms. With a median followup for all pts of 70 mos. the updated 7-yr results are as follows:

	ABVD %	XRT %	P
Complete Response	75	75	NS
Freedom from Progression (FFP)	62	45	0.07
CR Duration	63	48	0.12
Survival of CR's	87	65	0.05
Overall Survival	85	65	0.05

No significant differences were found in CR duration between those pts achieving a CR on induction and those pts converting to CR during consolidation. FFP, survival of CR's, and overall survival were significantly longer for pts receiving ABVD versus those receiving XRT consolidation. The major benefit of ABVD was seen in pts with histology other than nodular sclerosis: significantly longer CR duration ($p = 0.008$) and overall survival ($p = 0.03$) on the ABVD arm when compared to XRT. Overall survival for all 232 pts (including early deaths and pts who refused consolidation therapy) is 71%. Hematologic toxicity was significantly greater with XRT, while emesis was significantly increased on ABVD.

The 6-yr overall survival of 85% with sequential Bleo-MOPP--> ABVD in this ECOG trial compares favorably with the 76% survival on Bonadonna's alternating monthly MOPP/ABVD (Annals Int Med, 1986).

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- 25** RANDOMISED STUDY OF LOPP (LEUKERAN, ONCOVIN, PROCARBAZINE, PREDNISONE) AND LOPP ALTERNATING WITH EVAP (ETOPOSIDE, VELBE, ADRIAMYCIN, PREDNISONE) IN ADVANCED HODGKIN'S DISEASE - PRELIMINARY RESULTS. B. W. Hancock*, B. Vaughan Hudson**, G. Vaughan Hudson** for the British National Lymphoma Investigation (BNLI)
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** The Middlesex Hospital, London, W1N 8AA, U.K.

Previous BNLI studies in advanced Hodgkin's disease have shown: (1) MOPP is more effective than MOP (Prednisone omitted), (2) addition of Bleomycin to MOPP is of no benefit. (3) Maintenance with CCNU, vinblastine and Bleomycin is of no benefit and (4) LOPP is as effective yet much less toxic than MOPP (reviewed by Haybittle et al., Lancet, 1985, i, 967-972). In this current BNLI multi-centre study, started in June, 1983, patients are randomised to LOPP or LOPP alternating with EVAP. Over 300 patients had been entered; 167 are included in this preliminary analysis. The distribution of poor prognostic factors favours the LOPP/EVAP arm and overall survival is the same for both arms (10 deaths, 1 septicaemic, for LOPP and 13, 3 septicaemic, for LOPP/EVAP). The complete remission (CR) rate is better for LOPP/EVAP than for LOPP (70 and 58% respectively); the improved CR is particularly a feature in patients with poor initial prognostic factors. Apart from an increased infection rate associated with more severe and unpredictable myelosuppression and invariable alopecia the LOPP/EVAP regimen has been well tolerated by most patients.

- 26** PREDICTIVE VALUE OF EARLY RESPONSE TO MOPP IN "HIGH-RISK" STAGE II AND III HODGKIN'S DISEASE (HD).
A. Levis*, U. Vitolo*, G. Cametti*, M. Bertini*, M. Canta*, A. Urgesi*, U. Monetti*, A. Ciocca Vasino* and L. Resegotti*.
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From 1977 to 1984, a series of 82 patients (pts) with "high risk" stage II and III HD (B symptoms, or bulk mediastinal mass, or "E" lung disease) were staged without laparotomy and planned to be treated with combined modality treatment: 6 MOPP plus radiotherapy (subTNI in stage II and TNI in stage III). All patients were given at least the first 3 courses of MOPP and the status of response to therapy (CR=Complete Remission, PR=Partial Remission, F=Failure) at that time was called Early Response to Chemotherapy (ERC). The rate of nitrogen mustard and procarbazine delivery (MRD) during the first 3 cycles of MOPP was also evaluated.

At the completion of the therapy patients were restaged and the final response was assessed: 67 (81.7%) pts entered CR. 55.2% of the complete responders achieved CR in the first 3 courses of MOPP. 9-year survival and 9-year disease free survival (DSF) rates of the pts achieving CR were 75.7% and 78% respectively. Survival was significantly better for the pts who achieved CR in the first three cycles of chemotherapy than for the pts who entered CR at a later stage of therapy: 9-year survival 89.7% vs 60.9% (p=0.00). 9-year DFS rates were different according to the status of response after the first 3 courses of MOPP: CR 89.7%, PR 69.4%, F 30%. The differences were statistically significant among each curve (CR vs PR p=0.03, CR vs F p=0.0, PR vs F p=0.0). No other prognostic factor (stage, B symptoms, histology, bulk mediastinal mass, "E" lung disease, age) adversely affected DSF.

Prognostic value of ERC was confirmed in a multivariate regression analysis, even after correction by MRD. These data suggest that the rapidity of response to chemotherapy could be an important prognostic factor in high-risk stage II and III Hodgkin's disease.

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27 THE SIGNIFICANCE OF RESIDUAL MEDIASTINAL WIDENING FOLLOWING TREATMENT FOR HODGKIN'S DISEASE.

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Hodgkin's disease commonly involves the mediastinum and bulk disease at this site is thought to be of adverse prognostic significance, at least in stages I and II. Following treatment, persistent widening of the mediastinum as visualised on the postero-anterior chest radiograph is a common finding but, short of biopsy, it is impossible to differentiate between scar tissue and residual active disease.

We have studied 110 patients presenting to the Manchester Lymphoma Group with mediastinal Hodgkin's disease. In all cases the post-treatment chest radiograph was reviewed and the mediastinum defined as normal, or showing minimal, non-bulky or bulky abnormality (mediastinum width equal or greater than 1/3 of the trans-thoracic diameter at T5-6). With a median follow up of 70 months (range 9-166 months) there were 3/40 (3%) relapses in those with a normal mediastinum; 7/44 (16%) relapses in those with a minimal abnormality and 6/26 (23%) relapses in those with a more marked mediastinal abnormality. Cox multivariate analysis was then performed to take account of age, sex, stage, histology and presence of mediastinal bulk at presentation in each of four treatment groups; radiotherapy alone, chemotherapy alone, radiotherapy and adjuvant chemotherapy, chemotherapy followed by radiotherapy to the mediastinum. Those patients receiving chemotherapy had conventional MVPP to a minimum of six courses (minimum of four courses in the adjuvant group).

In patients treated by chemotherapy alone, persistent mediastinal widening was found to predict for relapse ($p = 0.039$). No other predictors for relapse were identified in this or any of the other treatment groups. These results indicate that residual mediastinal widening is of prognostic significance following treatment by chemotherapy alone and we conclude that radiotherapy should be given to patients with any degree of mediastinal abnormality at the completion of chemotherapy for Hodgkin's disease.

28 SECONDARY MALIGNANCIES AFTER HODGKIN'S DISEASE IN THE NETHERLANDS CANCER INSTITUTE. R. Somers, F.E. van Leeuwen, B.G. Taal, B. Coster, S.J. Huisman. The Netherlands Cancer Institute, Amsterdam, The Netherlands.

A retrospective analysis was carried out regarding the risk of second cancers (SD) in patients with Hodgkin's disease (HD) registered at the Netherlands Cancer Institute between 1966 and 1983 ($n=744$). In this group of patients 16 cases of leukemia (15 ANLL), 6 cases of myelodysplasia (MDS), 9 cases of Non Hodgkin lymphoma (NHL), 33 cases of solid tumours (of which 14 lung tumours) were observed. The median interval between the diagnosis of HD and the occurrence of secondary leukemias, NHL and solid tumours was 5.7 y., 13.3 y. and 6.8 y., respectively. NHL was located in the digestive tract in 6 patients; immunologically it was of B-cell type in the 6 cases tested; with a CHVP regimen 4 cases survived more than one year (17, 17+, 69+, 70+ months).

The cumulative proportions of SC after 10 and 15 years for leukemia (+ MDS), NHL and lung cancer were 5,1 and 6,3; 0,7 and 5,9; 3,0 and 6,2% respectively. A person-years type of analysis was carried out using the cancer incidence rates of the SOOZ cancer registry in the south eastern part of the Netherlands.

	Leukemia		Non Hodgkin lymphomas		Solid tumours	
	RR(O/E)	95% CI	RR(O/E)	95% CI	RR(O/E)	95% CI
RT only	5.6	(0.14-309)	6.7	(0.17- 37)	2.6	(1.5-4.1)
CT only	150	(31.0-439)	0	(0-369)	1.7	(.04-9.4)
Init. RT+CT	114	(49.3-225)	66.7	(18.2-171)	2.2	(.71-5.1)
Salv. RT+CT	44.4	(12.1-114)	57.1	(15.6-146)	2.7	(1.2-5.4)
ALL	45.7	(26.1-74.2)	31.0	(14.2-58.9)	2.5	(1.7-3.5)

A Cox model analysis performed on leukemias and MDS showed an increasing risk with increasing intensity of chemotherapy and with increasing age (> 40 year).

It can be concluded that the median interval between diagnosis of HD and secondary NHL is longer than that for leukemia and solid tumours. The risk of leukemia can be largely attributed to the intensity of chemotherapy, that of solid tumours to radiotherapy, whereas for NHL combined modality treatment was shown to be a risk factor.

ABSTRACTS - Third International Conference on Malignant Lymphoma, Lugano

- 29** MAC (MORPHOLOGY-ANTIBODY-CHROMOSOME) METHOD IN THE CHARACTERIZATION OF THE CELLS IN LYMPHOMAS. S. Knuutila, Department of Medical Genetics, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland

The novel MAC method allows the parallel study of the karyotype, surface markers and some morphologic features of the same mitotic cell. This presentation gives outlines of the MAC method. In addition, results acquired from lymphomas will be presented.

Some very general conclusions can be drawn from these results.

- 1) The abnormal karyotypes confined to one monoclonal population only.
- 2) Within this clone there may be cells with an abnormal karyotype and cells with a normal karyotype.
- 3) In Hodgkin's disease only Reed-Sternberg cells present an abnormal karyotype thus indicating that these cells are neoplastic, whereas small T and B cells have a normal karyotype indicating that in this case lymphocytes are reactive, non-malignant cells
- 4) In some cases the MAC method can help the pathologist to diagnose a malignant lymphoproliferative disease.

- 30** MONOCLONAL ANTIBODIES IN THE DIAGNOSIS OF MALIGNANT LYMPHOMAS H. Stein, J. Gerdes, R. Schwarting, G. Tippelmann, Institute of Pathology, Klinikum Steglitz, Freie Universität Berlin, D 1000 Berlin 45, FRG

To elucidate the identity of Hodgkin (H) and Sternberg-Reed (SR) cells of Hodgkin's disease (HD) and to throw light on the relationship between HD, NHL and malignant histiocytosis, we immunostained a large number of malignant lymphomas with monoclonal antibodies directed at lineage-specific, differentiation-stage-characteristic or proliferation-associated antigens and included antibodies we obtained by using the HD-derived cell line L428 for immunization and a selection procedure on frozen HD biopsy tissue samples. The results of these investigations provide evidence: a) that H and SR cells are not histiocytic but lymphocytic in origin; b) that H and SR cells represent - in relation to differentiation - activated lymphoid cells of either T or B cell type; c) that nearly all cases of malignant histiocytosis are derived from activated lymphoid cells of T (more commonly) or B cell type; d) that peripheral pleomorphic T cell lymphomas contain a mixture of non-activated and activated T cells; e) that HD seems to differ from NHL in that the former releases cytokines which induce the admixture of various reactive cell types, whereas NHL do not secrete cytokines in effective quantities.

31 REARRANGEMENTS OF ANTIGEN RECEPTOR GENE DNA IN MALIGNANT LYMPHOMA. J. Sklar, Department of Pathology, Stanford University, Stanford, California 94305.

Rearrangement of DNA sequences occur within neoplastic lymphocytes at the genetic loci of antigen receptor genes (genes for immunoglobulins and T cell receptor molecules). In general, rearrangements of immunoglobulin genes occur within B cells and T cell receptor genes within T cells. Two kinds of rearrangements are detected within neoplastic lymphocytes: intragenic rearrangements involving the assembly of gene segments into active antigen receptor genes; and intergenic rearrangements, most often consisting of chromosomal translocations. The former type of rearrangement occurs in normal developing lymphocytes, and sometimes in neoplastic lymphocytes, while the latter type of rearrangement is found only in neoplastic lymphocytes. Intragenic rearrangements can be analyzed by the Southern blot hybridization procedure and provide specific markers for clonal populations of lymphocytes. Detection of these markers can be useful for diagnosing lymphoma, since neoplasia is a clonal process, but detection of intragenic rearrangements is not entirely specific for neoplasia. Other useful applications of intragenic rearrangements include determination of tumor cell lineage and identification of multiple clones within single patients. In contrast, intergenic rearrangements are highly specific for neoplasia and frequently for specific subtypes of lymphoma as well. DNA sequences surrounding the breakpoints of several chromosomal translocations associated with various forms of lymphoma have been molecularly cloned and analyzed at a nucleotide level. Hybridization probes constructed from DNA near chromosomal breakpoints are potentially useful diagnostic reagents, but application of these probes must contend with the fact that such breakpoints are heterogeneous at the DNA level. Molecular analyses of chromosomal breakpoints suggest that intergenic rearrangements often results from abnormal attempts at intragenic rearrangement. Frequently intergenic rearrangements juxtapose genes next to DNA of antigen receptors. Transcription of these genes is often modified by their placement at these sites. Products of these genes presumably affect the development and progression of lymphoma and may in the future be valuable molecules in the diagnosis and the treatment of malignant lymphoma.

32 Ig GENE REARRANGEMENT IN NON-HODGKIN'S LYMPHOMA. R. Rudders, S. Dhillon and T. Krontiris, Tufts-New England Medical Center, Boston, MA 02111

We have examined the initial diagnostic lymph node biopsy from 68 consecutive patients with B cell non-Hodgkin's lymphoma for genetic markers of malignant transformation. DNA prepared from each biopsy was hybridized with probes for the J_H, C_μ, C_κ and C_λ regions of the Ig genes. In 31/54 instances, SmIg, the standard marker for monoclonal B cell malignancy, with either undetected or light chain restriction was not clearly present. However, in 24 of these 25 tumor DNAs there was an easily detected rearrangement in at least one of the Ig genes. In follicular lymphoma which often given ambiguous immunophenotypic results, monoclonal gene rearrangements were detected in 16 of 18 tumor DNAs yet monoclonal surface Ig was detected in only 8 of these cases. Of note was the observation that 23/54 tumors contained an Ig heavy chain rearrangement that was restricted to a single allele. Two rearrangements with or without a residual germ line band was seen in 16 additional cases. Although there were eight tumors that were potentially oligoclonal by immunophenotypic criteria, in only 3 was there support for oligoclonality by gene rearrangement criteria and only a single tumor displayed more than 2 rearranged heavy chain bands. These data indicate that: 1) Ig gene rearrangement is highly sensitive and should replace immunophenotypic criteria in defining monoclonality in all histologic types of B cell lymphoma; 2) B cell tumors have a striking degree of rearrangement of a single Ig heavy chain allele suggesting a model in normal B cells that may differ from the current murine model; 3) Continuing rearrangement or oligoclonality is an uncommon event in both low and high grade lymphoma.

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33 DETECTION OF LYMPHOMA CELLS IN PERIPHERAL BLOOD AND BONE MARROW BY DNA HYBRIDISATION. M. Brada, H. Molgaard, J.P. Sloane, J. Treleaven, A. Horwich, M.J. Peckham.

Aim:

To detect circulating lymphoma cells in patients with active non-Hodgkin's lymphoma (NHL) by immunoglobulin gene or T cell receptor gene rearrangement studies.

Method:

We studied peripheral blood from 50 patients and bone marrow from 12 patients with active B and T cell NHL. DNA extracted from mononuclear cell layer was analysed by hybridisation techniques with immunoglobulin gene (Ig) and T cell receptor gene (TCR) probes. (J_H and $J_{\beta 2}$ respectively).

Results:

Nineteen of fifty patients (38%) with active B and T cell NHL had circulating clones of cells detected by immunoglobulin or T cell receptor gene rearrangement. Lymphoma tissue and peripheral blood were studied simultaneously in 22 patients, 9 of which had a circulating clone of cells in peripheral blood. In 7 patients the gene rearrangement in lymphoma tissue and peripheral blood was identical and in two patients the gene rearrangements were different in the two samples. Fifty-four per cent of patients with advanced disease (CSIII & IV) and 46% of patients with low grade lymphoma had circulating lymphoma cells compared to 18% with CSI & II disease and 31% with intermediate and high grade lymphoma. Only four patients had lymphoma cells seen on peripheral blood smear.

The presence of circulating lymphoma cells correlated with conventional assessment of bone marrow involvement, although circulating clones were detected in 30% (12/40) of patients with apparently normal bone marrow. In addition the comparison of DNA hybridisation and conventional bone marrow assessment shows a false negative rate for histology of 20% (2/10).

Conclusion:

Ig and TCR gene rearrangement studies are a sensitive method of detection of minimal disease in peripheral blood and bone marrow, and provide a tool for the understanding of the biology of lymphoma.

34 CORRELATION BETWEEN CELL SURFACE ANTIGEN EXPRESSION, AND IMMUNOGLOBULIN (Ig) AND T-CELL RECEPTOR β CHAIN GENE REARRANGEMENT IN LYMPHOPROLIFERATIVE DISORDERS. Ph. Gaulard, T. Henni, Z. Henni, J.P. Marolleau, D. Rocha, C. Haioun, M. Divine, J.P. Farcet, Y. Pinaudeau, M. Goossens, F. Reyes - CHU HENRI MONDOR 94010 CRETEIL, FRANCE.

53 samples representing lymphoproliferative disorders were examined for cell surface antigen expression using monoclonal antibodies against light and heavy Ig chains, B and T-cell differentiation and HLA-DR and Tac antigens, and for rearrangement of the heavy chain Ig (J_H) and the β chain of T-cell antigen receptor (Tcr β) genes. 42 cases were histologically classified according to the Working Formulation (WF); 34 expressed B-cell differentiation antigens, 31 of which had monotypic Ig. A clonal Ig gene rearrangement was found in all cases but one; in addition, 1 non monotypic proliferation exhibited an oligoclonal pattern of rearrangement. 7 cases showed a mature (CD_{3+}) T-cell phenotype with a lack of expression of several T-cell antigens in 4 cases. In another case, CD_4 was the only T-cell antigen expressed. In these 8 cases, a monoclonal pattern of rearrangement of Tcr β was present. 11 cases could not be classified according to the WF. In 9 cases (1 mycosis fungoïde, 3 lymphomas with intermediate sized cells, 2 angio-immunoblastic lymphadenopathy (AIL), 1 lymphomatoid granulomatosis (LG), 1 polymorphic reticulosis (PR), 1 lymphomatoid papulosis (LP)), a predominant mature T-cell population was present, which was abnormal by the loss of expression of several T-cell differentiation antigens in 5 cases. A unique rearrangement of Tcr β was found in these cases, except in one case of AIL which was in germ-line configuration. In addition in 2 lymph nodes with partial infiltration, the diagnosis of B-cell neoplasm could only be ascertained by the demonstration of a clonal Ig gene rearrangement.

These findings indicate that genomic studies are not necessary for the diagnosis of B-cell lymphomas with a monotypic Ig expression. In contrast, they allow the detection of a clonal population and determination of cell lineage in the other lymphoproliferative disorders. These include PR, LG, AIL, LP and cases with partial tumoral infiltration. Moreover the correlation found between the presence of a clonal Tcr β rearrangement and the expression of an abnormal cell phenotype, suggest that the latter should represent a useful tool for the diagnosis of peripheral T-cell lymphomas.

35 The p24 Antigen Recognized by CD9 Monoclonal Antibodies is Related to a Subunit of the Cytoadhesion Receptor: Biochemical Comparison and DNA-Mediated Gene Transfer. Stephen C. Peiper, A. Thomas Look, Lisa K. Jennings, Costan W. Berard, and Charles J. Sherr. Departments of Pathology and Laboratory Medicine, Tumor Cell Biology, and Hematology-Oncology, St. Jude Children's Research Hospital and University of Tennessee Center for Health Sciences, Memphis, Tennessee, U.S.A.

Monoclonal antibodies of the CD9 cluster group recognize a 24-kDa protein, p24, which is expressed by virtually all B cell lymphomas and leukemias, platelets, and other normal and malignant cells. This cell surface protein has covalently attached lipid, an unusual post-translational modification. Platelet aggregation is induced after binding either CD9 monoclonal antibodies or the GTB-18 monoclonal antibody raised against the 23-kDa β chain of platelet glycoprotein (gp) I β . The reactivity of these antibodies was compared to determine whether they recognize similar proteins. Proteins of identical electrophoretic mobility were immunoprecipitated by the SJ-9A4 CD9 and GTB-18 monoclonal antibodies from lysates of platelets labeled by lactoperoxidase-catalyzed cell surface radioiodination. Similarly, the two antibodies immunoprecipitated co-migrating proteins from lysates of Nalm-1 human pre-B acute lymphoblastic leukemia cells metabolically labeled with [35 S]methionine. The metabolically labeled proteins immunoprecipitated with each antibody from Nalm-1 cells were subjected to limited proteolysis with V-8 protease from *Staphylococcus aureus* and with chymotrypsin and yielded identical peptide fragments. Taken together, these studies indicate that p24 is functionally, immunologically, and structurally related to the β chain of platelet glycoprotein I β .

We have employed a strategy of DNA-mediated gene transfer and fluorescence-activated cell sorting to molecularly characterize p24 at a genetic level. DNA from Nalm-1 cells was cotransfected with a plasmid containing the v-fms oncogene into the NIH-3T3 murine fibroblast cell line. Cells incorporating exogenous DNA were selected for expression of human p24 by fluorescence-activated cell sorting after staining with SJ-9A4. A line of primary transformants expressing p24 was obtained, which reacted with both CD9 and GTB-18 monoclonal antibodies. These transformants will be useful in the biochemical and molecular characterization of p24 and in determining its relationship to gpI β .

36 PROGNOSTIC VALUE OF NUCLEIC ACID FLOW CYTOMETRY (FCM) IN DIFFUSE LARGE CELL LYMPHOMA. P. McLaughlin, B. Osborne, D. Johnston, J. Butler, J. Sullivan-Halley, P. Jennings, F. Cabanillas, and B. Barlogie. U.T. M.D. Anderson Hospital and Tumor Institute, Houston, Texas, U.S.A.

Between 1978-1985, 55 previously untreated patients (pts) with diffuse large cell lymphoma (DLCL) had acridine orange DNA and RNA FCM studies of fresh biopsied involved tissue. Pts received intensive combination therapy (CHOP-based), and complete remission was attained in 74%. DNA and RNA indices measured malignant cell nucleic acid content relative to normal lymphocytes. Pretreatment DNA and RNA features were correlated with survival and relapse free survival (RFS):

MEDIAN MONTHS OF SURVIVAL AND RFS

	DNA Index		RNA Index			S Phase (%)		
	1	$\neq 1$	≤ 1	1-1.8	>1.8	≤ 10	10-18	>18
Survival	39+	13	18	54+	12	20	11	54+
RFS	39+	11	6	54+	8	18	8	54+

Significantly better outcome was noted for intermediate RNA content (47% of pts), high S phase (33% of pts), and diploid (45%) DLCL's. Most previous analyses of pts with all types of lymphoma have shown that higher %S correlates with increasing histologic grade and consequent shorter survival. We now find that within one histologic category, DLCL, a high %S identifies pts with the highest potential for cure following intensive combination chemotherapy. Likewise, intermediate RNA content appears to correlate with long survival. Other prognostic features, including stage, serum LDH, and tumor bulk were found not to be highly correlated with FCM features. Thus, within a specific histologic category, FCM provides prognostic information that appears to be independent of other known prognostic factors. FCM analysis can identify those pts likely to be cured with conventional therapies, and those pts with adverse prognostic features who might benefit from innovative treatment schedules or regimens.

- 37** RISK-ADAPTED CHEMOTHERAPY, INVOLVED FIELD IRRADIATION WITH REDUCED DOSES AND SELECTIVE SPLENECTOMY IN CHILDHOOD HODGKIN'S DISEASE: UPDATE OF THE GERMAN MULTICENTER STUDY DAL-HD-82. G. Schellong¹, J.H. Brämwig¹, E.W. Schwarze², M. Wannenmacher³ (for the German Therapy Study Group DAL-HD-82)
¹University Children's Hospital Münster, ²Institute of Pathology, University of Kiel, ³Department of Radiotherapy, University of Freiburg, FRG

It was the aim of the cooperative therapy study HD-82 for children with Hodgkin's disease to reduce chemo- and radiotherapy in the framework of a combined modality treatment, and to investigate prospectively a strategy for selective splenectomy previously developed (Cancer 57, 1986, 2049 - 2056). Between Dec. 1981 and Dec. 1984, 207 patients (131 boys and 76 girls) below 16 years of age from 53 centers were enrolled in this study.

In 174 out of 207 patients (84.1 %) the intraoperative criteria for selective splenectomy were applied. 69 out of 174 patients (39.7 %) had a splenectomy. 50 of the removed 69 spleens (72 %) had histologically proven HD. These results are comparable to the 36 % and 73 % predicted from the retrospective analysis in the HD-78 study.

According to the stage of disease 3 different treatment groups with 2 x OPPA (stage I/IIA), 2 x OPPA and 2 x COPP (stage IIB/IIIA) and 2 x OPPA and 4 x COPP chemotherapy¹ (stage IIB/IV) were formed. Radiotherapy was given as involved field irradiation with a total dose of 35, 30 or 25 Gy in each respective group. Until December 1986, 3 out of 203 protocol patients died from intercurrent disease and 5 patients relapsed (follow-up period 24 to 60 months, median 42 months). The probability for disease-free survival after 5 years is 96 % for the total group and 99 %, 96 % and 89 % for the three treatment groups including 100, 53 and 50 patients. The projected overall survival rate after 5 years is 98 %.

Conclusions: 1. A stage dependent chemotherapy with 2, 4 or 6 cycles OPPA/COPP is highly sufficient to eradicate occult microfoci, so that only involved field irradiation is needed. 2. There is a high probability of preventing local recurrence with radiation doses of 35, 30 or 25 Gy in combination with the applied chemotherapy. 3. The decisional model for selective splenectomy has proved to be very successful in prospective application.

1) O = vincristine, PP = procarbazine and prednisone, A = adriamycin, C = cyclophosphamide

- 38** PRIMARY CHEMOTHERAPY (CT) AND LOW-DOSE RADIATION (RT) IN INVOLVED FIELDS (IF) IN CHILDHOOD HODGKIN'S DISEASE (HD). Results of a joint cooperative study by the French Society of Pediatric Oncology (SFOP) and Hopital Saint Louis, Paris.

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In an attempt to reduce side effects of therapy in children with HD, a first study was led in Villejuif (1975-1980) in 60 clinically staged (CS) patients (pts). It showed that 1) laparotomy can be avoided when primary CT is given to all pts, 2) 3 cycles of MOFP are as efficient as 6 cycles in remission induction, 3) RT can be limited to initially involved areas (Eur Paediatr Hematol Oncol 2:67, 1985).

In a further step of desescalating therapy and with the background of the previous study, a national study was started in 1982 aiming at reducing both toxicity of chemotherapy and sequelae of radiotherapy in clinically staged children. 1) in CS IA-IIA: 4 ABVD are compared to 2 MOFP-2ABVD; 2) in CS IB-IIB-III: all pts receive alternating cycles 3 MOFP-3 ABVD; 3) After evaluation of response to CT, good responders (at least 70 % tumor regression) are given 20 Grays (Gys) to initially involved areas in CS IA-IIA, and to IF + lombospenic field in CS IB-IIB-III-IV. Bad responders receive 40 Gys.

From January 1982 to december 1986, 159 pts were included (86 IA-IIA, 22 IB-IIB, 29 III, 21 IV). Out of them 130 completed therapy and are evaluable. Median F.U. is 28 m. 122 were good responders to chemotherapy: 90 achieved complete remission and 32 > 70 % remission. They were given 20 Gys RT. 8 were considered as bad responders to chemotherapy: 4 reached CR with 40 Gys and 4 (4 CS IVB) had progressive disease.

Six pts relapsed: 4 in unirradiated areas and only 2 in irradiated nodes (3 nodal relapses, in 2 pts, among 283 irradiated nodal areas), which supports the effectiveness of low-dose RT after effective CT. No difference appears this far between the 2 randomized arms. At 4 years, overall actuarial survival is 95 % and actuarial disease-free survival is 89 %. Longer follow-up is needed to confirm these results.

39 HODGKIN'S DISEASE IN CHILDHOOD: THE EXPERIENCE OF THE ITALIAN ASSOCIATION OF PEDIATRIC HEMATOLOGY AND ONCOLOGY WITH PROTOCOL AIEOP-MH '83. V.Vecchi, R.Burnelli, D.Rosati, P.Tamaro, A.Amici, G.C.izzi, N.Bontempi, A.De Pascale, P.Mingozzi, A.Pession - Italian Pediatric Cooperative Group.

In order to minimize long-term side effects, without compromising treatment efficacy, the Italian Association of Pediatric Hematology and Oncology (AIEOP) proposed a protocol, AIEOP-MH'83, for pts with Hodgkin's Disease (HD). The chemoradiotherapeutic program was based upon low dose of radiotherapy (RT) and short chemotherapy (CT) in early stages and alternative non-cross-resistant drug combinations in advanced stages. No staging laparotomy was performed.

As of October 1986, 110 pts were registered by the 22 participating Institutions. Six pts were too early to evaluation; the characteristics of the 104 remaining pts were as follows: 61 males, 43 females, median age 9.7 yrs (range 3-15 yrs). Histological patterns were: nodular sclerosis (NS): 56 cases; lymphocytic predominance (LP): 9; mixed cellularity (MC): 38; lymphocytic depletion (LD): 1. According to stage distribution, 17 pts were I A, 11 B, 36 II A, 9 II B, 15 III A, 14 III B, 3 IV A, 9 IV B.

Patient population was subdivided into 3 therapeutic groups according to clinical stage: **Group A**, consisting of 38 pts in stage I, II A with M/T < 0.33, was treated with 3 courses of ABVD + involved field RT. **Group B**, consisting of 40 pts in stage I₂A, I B, II A with M/T > 0.33, II B and III A, was given 6 alternating cycles of MOPP/ABVD + extended field RT. **Group C**, formed by 26 pts in stage III B and IV was treated as group B plus 4 further alternating courses of MOPP/ABVD as maintenance therapy. RT dosage, 2000-2500 cGy, was according to age: < or > 6 years.

Response to initial treatment, evaluated in pts who completed CT was as follows: 34/37 (91.8%) pts treated with 3 courses of ABVD achieved a "good remission", defined as complete disappearance (28 pts) or at least reduction of tumor \geq 70% ("good partial": 6 pts). 49/56 (87.5%) pts treated with 6 courses of MOPP/ABVD were complete (40 pts) or good partial (9 pts) responders. The overall good response rate to initial CT of 93 evaluable pts was 89%. Regarding the effectiveness of RT after initial CT, preliminary results showed that in group A RT determined an increased good remission rate, while in group B and C it did not appear to improve the good response rate; but further data and a longer follow-up are needed to confirm these issues.

The overall actuarial survival and Freedom from Progression (FFP) rates after a median observation time of 17 mos are 91.9 \pm 3.3% and 87 \pm 4.9% respectively. Actuarial FFP rates of group A, B and C are 93.7 \pm 6%, 93.1 \pm 4.7%, 61 \pm 18.7% respectively after a median observation time of 22+, 17+ and 13+ mos.

Only 1 pt (stage V B LD HD) failed to achieve remission and died. One stage III A MC pt with immunodeficiency disease died in CCR because of fatal infection. 3 pts (stage II B and 2 stage IV B with NS) relapsed during CT, 7, 5 and 10 mos from diagnosis and died. 3 pts (stage I A with MC, II A with M/T > 0.33 MC and stage III B MC) showed a recurrence of disease when off-therapy, after 23, 12 and 24 mos from diagnosis respectively: stage II A pt relapsed in an irradiated area and died; the other two pts are alive in II CR. The last relapsed pt was a stage III B NS HD whose recurrence happened during RT and, at present time, he is alive in II CR after autologous bone marrow transplantation.

In terms of efficacy this chemoradiotherapeutic regimen seems to be similar to other current studies, and if this good outcome will be associated with minimal and acceptable long-term side effects, the main endpoints of our protocol will be achieved.

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40 CONTINUAL PROGRESS IN THE MANAGEMENT OF CHILDREN WITH HODGKIN'S DISEASE USING COMBINED MODALITY THERAPY. S.S. Donaldson, M.P. Link, Department of Therapeutic Radiology and Department of Pediatrics, Division of Hematology-Oncology, Stanford University Medical Center and the Children's Hospital at Stanford, Stanford California 94305. USA

For more than 25 years, combined modality therapy using a modified approach of low doses of irradiation and multiagent chemotherapy have been systemically employed at Stanford University Medical Center for children with Hodgkin's disease. This therapy was designed to decrease the recognized morbidity known to accompany high dose, extended field irradiation (XRT) when administered to children.

Our first protocol involved 55 children managed uniformly with surgical staging, low dose XRT and 6 cycles of MOPP chemotherapy. Their stages included: I (3), II (19), III (22), IV (6) with ages of 14 years or less, in whom growth and development was the major concern. 53 of the 55 underwent laparotomy. XRT doses to involved fields ranged between 15-25 Gy, depending on bone age. Actuarial survival and freedom from relapse are 89% and 90% respectively, with median follow up of 7-1/2 years, while the local control rate is 97%. Major bone and soft tissue impairment has not occurred; all children have heights \pm 1 to 1-1/2 standard deviations from the mean. However, absolute azoospermia is present in 4/4 boys tested 3-10 years following MOPP, amenorrhea developed in one girl with pelvic disease, and secondary acute leukemias have occurred in 3 children (actuarial risk of 11% at 10 yrs-S.E. 7%)

Thus, our second protocol was designed to further refine therapy by selective use of surgical staging, using 6 cycles of ABVD/MOPP alternating around 15 Gy involved field XRT. Boosts of XRT to total of 25 Gy are given to those who fail to achieve a complete response after 2 cycles of chemotherapy. 33 children have entered this study with stages: II (9), III (15), IV (9). Staging laparotomy and splenectomy have been performed in 25 of 35 children (76%). The 8 exceptions include 7 with stage IV and 1 with stage III disease. Of the 25, 15 (60% of the group) had subdiaphragmatic disease detected by staging laparotomy and thus a change in the definition and extent of the involved field of radiation. The actuarial survival and freedom from relapse is 94% and 92% with median follow up at 2 years. No complications have been observed. However, ultimate evaluation of fertility and secondary tumor induction will require longer term follow up.

Optimal management of children with Hodgkin's disease requires individualization of management, with therapy selected as a function of stage and tumor burden so to enhance likelihood of cure while minimizing late effects.